STANDARDIZATION OF PROCESS FOR PREPARATION OF MALT FROM HORSE GRAM

by

Mr. Anandakumara E
(Reg. No. 014/301)

A thesis
Submitted in Partial Fulfilment of the Requirements
For the Degree of

MASTER OF SCIENCE

in

FOOD TECHNOLOGY

DEPARTMENT OF FOOD SCIENCE AND TECHNOLOGY
POST GRADUATE INSTITUTE,
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1. INTRODUCTION

Food legumes are second most valuable plant source for human and animal nutrition (Bhatt and Karim, 2009) and recognized as second most important group of crops after cereals which have been a vital ingredient of balanced human diet (Bhadana *et al*., 2013).

In the developing countries, primarily a handful of conventional legumes dominating the production and market chains and playing crucial role in eradicating protein malnutrition still, some of the underutilized indigenous legumes like, horse gram has great significance in the nutritional security of rural, tribal and underprivileged masses (Tontisirin, 2014). Horse gram is one of the highly nutritious vegetable pulse crop with ethno-medicinal values in India, which is commonly known as *Kulattha* (Sanskrit), *Kurti-kalai* (Bengali), *Kollu* (Tamil), *Ullavallu* (Telugu), *Muthira* (Malyalam), *Gahot* means which destroys stone in initial stage (Pati and Bhattacharjee, 2013).

Horse Gram is scientifically known as *Macrotyloma uniflorus*. It also goes by the name *Dolichos uniflorus* due to a lot of confusion in the *Dolichos* category the right name for the horse gram scientifically is *Macrotyloma uniflorum*. It belongs to family *Fabaceae.* According to USDA (United States Department of Agriculture) database both the name's *Macrotyloma uniflorum* and *Dolichos uniflorus* mean the same. Horse gram was probably domesticated in India where its cultivation known since prehistoric times.

Horse gram make a significant contribution to the diet of the rural households particularly, during drought, famine and dry season besides, in many cases these are the life-savers for millions of resource poor people in the regions where ensuring food and nutritional security is one of the significant problems. Presently, attention towards underutilized legumes is increasing for finding new alternate protein sources to meet the ever increasing demand for vegetable protein (Pugalenthi *et al*., 2005). This neglected and under-valorised

crop has great untapped potential to support smallholder rural farming communities by providing income, food and nutritional security as well as sustaining the genetic resources needed to address present and future environmental challenges.

Horse gram is a potential grain legume having excellent nutritional and remedial properties with better climate resilience to adopt harsh environmental conditions (Kumar, 2006). It is one of the most important unexploited food legume being grown in almost all over the world including temperate and subtropical regions encompassing the countries in East and North-East Africa, Asian countries particularly, India, China, Philippines, Bhutan, Pakistan, Sri Lanka and Queensland in Australia (Durga, 2012; Krishna, 2010).

Horse gram is an important food and feed crop traditionally grown in arid regions of the developing world and often considered as minor/ neglected/ underexploited/ poor man's pulse. Its innate climate resilience suggests its scope as a suitable alternative in the present climate change era. It is a treasure house of various therapeutic, bioactive compounds along with excellent nutritional quality makes it a wholesome food that should be added to diet on a regular basis. The health benefits of horse gram are being recognized in the western world recently, but have been known for its ability to prevent and cure various diseases by Indian "*Ayurvedic*" system since centuries. Furthermore, there are still great possibilities exist for this legume to be explored for its chemoprofile, pharmacology, biological evaluation, toxicological consequence, innate health-promoting aspects and many undiscovered phytochemicals as well as there is need to promote and support the initiatives that make the most use of this indigenous underutilized legume to address food and nutritional security issues (Bhartiya *et al.*, 2015).

The horse gram is 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 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 in addition to its nutritive value.

Horse gram is a nutritious food legume. It is rich in protein, iron, calcium and polyphones. Horse grams that fail to meet food grade standard can be used as livestock feed, because of their high protein content and lack of digestive inhibitors. It has the greatest potential for the utilization of nutraceuticals, forage and food for malnourished and drought prone areas of the world and the presence of flavonoids, terpenoids, glycosides, tannins, steroids, and saponins etc has an impact on antibacterial, antifungal and antioxidant activity.

Horse gram is famous for its medicinal use because different parts of the plants are used for the treatment of heart disease, asthma, bronchitis, urinary discharges and for treatment of kidney stone. Horse gram water is prescribed for treating jaundice. Horse gram helps in lowering cholesterol levels and plays a role in antioxidation.

Presently, attention towards underutilized legumes is increasing for finding new alternate protein sources to meet the ever increasing demand for vegetable protein (Pugalenthi *et al*., 2005). Generally, the pulses are used in the form of whole seeds or splits (Dhal). However, horse gram is mainly utilized in the form of whole and sprouts. Since the sprouting is a time consuming process, sprout from horse gram will be of immense use.

The whole seeds of horse gram are generally used in preparation of Usali, Chutney, and Bassaru in Karnataka and Andra Pradesh. However, it is consumed as a whole seed, as sprouts, or as whole meal in India, especially in southern Indian states.

Malted grain is used to make beer, whisky, malted shakes, malt vinegar, confections and some baked goods, such as malt and rich tea biscuits. Malted grain that has been ground into a coarse meal is known as sweet meal. Various legumes are malted, though barley is the most common. A high protein form of malted barley is often a label-listed ingredient in blended flours typically used in the manufacture of yeast breads and other baked goods.

Objectives

1. To standardize the process for preparation of malt from horse gram.
2. To study the quality of horse gram malt and dried horse gram malt.
3. To study the sensory evaluation of rehydrated horse gram malt.
4. To study the effect of storage on keeping quality of dried horse gram malt.

2. REVIEW OF LITERATURE

2.1 Importance of legumes in human nutrition

The major importance of legumes lies in their actual and potential value as a source of plant protein for human nutrition. In some parts of India, legumes constitute 30-50% of the total diet. Although the per capita availability of legumes is about 50g/day, the daily intake in various regions of India ranges from 14 to 140g per capita per day (Salunkhe, 1982). The digestibility of cereal seed protein is generally 75-90% whereas that of raw and cooked legume seed is 15-80% and 50-90% respectively (Eggum and Beames, 1983).

The use of dry seeds of horse gram as human food is limited due to its poor cooking quality, presence of high level of enzyme inhibitors and heamagglutinin activities (Ray 1969). The seeds are rich in tannins and polyphenols compared to the other legumes (Kadam and Salunkhe, 1985).

2.2 Nutritional composition

Horse gram seed is low in fat and is excellent sources of protein, dietary fibre, a variety of micronutrients and phytochemicals (Sreerama *et al*., 2012) still it has remained an underutilized food legume, consumed only by the farming communities of inaccessible areas and low-income groups (Aiyer, 1990).

Horse gram seed contains carbohydrate (57.2%), protein (22%), dietary fibre (5.3%), fat (0.50%), calcium (287mg), phosphorous (311mg), iron (6.77 mg) and calories (321 Kcal) (Gopalan *et al*., 1999) as well as vitamins like thiamine (0.4mg), riboflavin (0.2mg) and niacin (1.5mg) per 100g of dry matter (Bolbhat and Dhumal, 2012).

Horse gram has been recognized as potential source of protein and other nutrients (Sreerama *et al*., 2012). It has high nutritional value equivalent to other commonly grown pulse crops in all aspects and also an excellent source of iron, molybdenum and calcium (Prasad *et al*., 2010; Tuteja, 2008;

Bhokre, 2012). Grain legumes are an important source of nutrients and renowned as poor man's meat especially in developing countries (Hayat *et al*., 2014).

2.3 Carbohydrate

In whole and dehulled horse gram seeds, carbohydrate content ranged from 51.9-60.9% and 56.8-66.4% respectively (Sudha *et al*., 1995). Carbohydrate of raw horse gram seeds comprises 36±1.17g starch per 100g dry matter in which approximately 85% digestible, 14.47% resistant and 3.38% resistant starch associated to insoluble dietary fibres (Bravo *et al*., 1999). Carbohydrate includes starch, monosaccharides, oligosaccharides and other polysaccharides (Ekanayake *et al*., 2000).

Horse gram seed contains 6.38% total soluble sugars (Bravo *et al*., 1999) of which 55–65% constitute flatulence producing raffinose family oligosaccharides, stachyose and verbascose (Machaiah and Pednekar, 2002) and processing such as soaking, cooking and sprouting may bring about changes in the levels of oligosaccharides. Carbohydrate content ranges from 50-60% in commonly consumed pulses (Bains and Brar, 2005). In the legume seeds, starch is the major source of available carbohydrate and most abundant (22–45%) along with oligosaccharides (1.8-18%) and dietary fibre (4.3-25%) (Hoover and Zhou, 2003; Ofuya and Akhidue, 2005).

Horse gram has high non-digestible carbohydrate content which cause lower glucose release into the blood stream with potential beneficial effects in the dietary management of diabetes and this resistant starch is regarded as a prebiotic among the new generation of dietary fibres (Samanta *et al*., 2011).

2.4 Crude protein

Horse gram is one of the cheapest sources of protein for both human beings and animals (Katiyar, 1984). Horse gram seed contains 22–24% protein, which is comparable to commonly consumed pulses like chickpea, pigeonpea,

green gram and black gram however, due to varietal difference large variability observed in protein content ranging from 18.5–31.16% (Begum *et al*., 1977; Murthy, 1980). Horse gram protein comprises higher lysine content than pigeon pea and chickpea making it a good complement to a cereal based diet (Venkatesha, 1999; Prasad *et al*., 2010) whereas, in horse gram methionine is the major limiting amino acid and threonine and tryptophan are the other minor limiting amino acids (Khader and Venkat Rao, 1986).

The dehulled horse gram seeds exhibit higher protein content (18.4–25.5%) than the whole (17.9–25.3%) (Sudha *et al*., 1995). A wild species of horse gram (*Macrotyloma sargarhwalensis*) contains 38.37±1.03% crude protein. The true seed protein (34.88%) content of this wild horse gram reported about two times higher than the other commonly grown horse gram lines (Yadav *et al*., 2004).

2.5 Dietary fibre

Horse gram seeds contains more insoluble dietary fibre (Kawale *et al*., 2005) required for normal lower intestinal function in humans (Anderson *et al*., 1994). In most grain legumes consumed as pulses by humans, the fibre content ranges from 8.0-27.5% with soluble fibre in the range 3.3 –13.8% (Guillon and Champ, 2002).

Adequate dietary fibre is essential for proper functioning of the gut and has also been related to risk reduction for a number of chronic diseases including heart disease, certain cancers and diabetes. Fibre includes pectin, gum, mucilage, cellulose, hemicelluloses and lignin (Khogare, 2012).

Horse gram seeds contains 28.8% total dietary fibres, mainly insoluble dietary fibre 27.82% and soluble dietary fibre 1.13% with (Khatoon and Prakash, 2004) whereas horse gram flour contains 16.3% total dietary fibre (Sreerama *et al*., 2012).

In horse gram crude fibre is only one-seventh to one-half of total dietary fibre (Trowell, 1976) and the high content of dietary fibre in horse gram flours might be helpful in terms of maintaining positive effects on intestine and colon physiology, besides other homoeostatic and therapeutic functions in human nutrition (Sreerama *et al*., 2012). Pulse derived fibre have been shown to alter energy expenditure, substrate trafficking and fat oxidation as well as visceral adipose deposition (Ramen *et al*., 2013).

2.6 Fat

In an optimum balance, the essential fatty acids linoleic acid with α-linolenic acid may slow the onset of Parkinson's and Alzheimer's diseases and these fatty acids are important for healthy cell membrane formation and functional development of the brain and nervous system, therefore, increase intake of legumes can be beneficial to human health (Morris *et al*., 2013; Ryan *et al*., 2007). Further, horse gram lipids have anti-ulcer activity due to presence of phytosterol esters (Berger *et al*., 2004) which imparts protective and healing effect on acute gastric ulceration produced by alcohol (Jayraj *et al*., 2000).

Dehulled horse gram seeds exhibit higher crude fat content (0.81–2.11%) than the whole (0.70–2.06%) seeds (Sudha *et al*., 1995) and the fat content of horse gram seeds ranges from 0.6–2.6% (Sreerama *et al*., 2010).

Horse gram seeds are a good source of essential fatty acids and contains 27.5% saturated fatty acids (21.97% palmitic, 2.85% arachidic, 2.32% stearic acid and 0.36% myristic), 72.49% unsaturated fatty acids (42.78% linoleic, 16.15% oleic and 13.56% linolenic acid) and among unsaturated fatty acids linoleic acid is useful for the treatment of diabetes (Mishra and Pathan, 2011).

2.7 Moisture and ash content

In whole and dehulled horse gram seeds, moisture content is found 11.55% and 9.73%, whereas ash content ranges from 3.0-3.8% and 2.7–3.4%, respectively (Sudha *et al*., 1995) and moisture content in horse gram seeds is about 11.39% (Gopala Krishna *et al*., 1997). However, in horse gram moisture content in seeds depends on 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 optimum range for safe storage in pulses (Mohan *et al*., 2011).

Horse gram contains high mineral content and also used as leafy vegetable and its leaves contain relatively very high content of minerals as compared to other common vegetables (Mandle *et al*., 2012).

In horse gram accessions, mean concentrations of macro minerals (Ca, K, Mg, P and S) ranges from 1.3-14mg and micro minerals (Cu, Fe, Mn, Ni and Zn) ranges from 1.0-95.0µg per gram dry weight (Morris *et al*., 2013). It is a fairly rich source of calcium which is 238mg in whole seed and 223mg in dehulled seed per 100g seed (Sudha *et al*., 1995). In raw horse gram, the calcium and iron content ranges from 244-312mg and 5.89 -7.44mg per 100 g of seed, respectively with invitro bioaccessibility of 22.50 -38.50mg of calcium and 0.26 -0.85mg of iron per 100g of seeds. Germination, cooking and roasting significantly increased the invitro bioaccessibility of calcium and iron (Khatun *et al*., 2013).

Change in the vitamin C content and antioxidant capacity of the raw and the sprouted horse gram applied a 300, 400 and 500 MPa pressure for 15 min at room temperature. In the raw seeds no vitamin C content could be detected, whereas the horse gram sprouts contained considerable amount of vitamin C. The antioxidant capacity in the germinated seeds increased by around 58-67%. The high pressure treatment modified somewhat the vitamin C content and also the antioxidant capacity and beyond a pressure of 500 MPa the decrease was

significant. Although the treatment of the sprouts at high pressure resulted in a high (15-17mg/100g) vitamin C content and also the antioxidant capacity was by around 26-59 per cent higher than for the non-sprouted horse gram the highpressure treatment had only a slight effect on the quality of the freshly consumed sprouts (Doblado *et al*., 2007).

2.8 Antinutritional factors

Changes in phytate phosphorus and minerals during germination and cooking of horse gram and reported that the phytate phosphorus in horse gram sees accounted for 57% of the total phosphorus. During germination, there was a continuous decrease in the proportion of phytate phosphorus. Cooking decreased phytate phosphorus in germinated seeds. No significant changes were noticed in magnesium and iron, in horse gram (Borhade *et al.,* 1984).

Horse gram seeds have higher trypsin inhibitors and hemagglutinin activities and polyphenols than moth bean seeds. Dehusking, germination, cooking, and roasting have been shown to produce beneficial effects on nutritional quality of both the legumes. A soak solution (1.5% $NaHCO_3$ + 0.5% Na_2CO_3 + 0.75% citric acid) treatment has been shown to reduce cooking time and improve protein quality (Kadam and Salunkhe, 1985).

Conventional processing methods such as dehusking, germination, cooking, and roasting have been shown to produce beneficial effects by decreasing the content of undesirable components which results in enhanced acceptability and nutritional quality in addition to optimal utilization of horse gram as human food (Kadam and Salunkhe, 1985).

The effects of cooking and processing on protein quality of horse gram. Steaming (5-40 min), autoclaving (5-60 min at 10 lb pressure), puffing (10 min on hot sand), roasting and dehusking of horse gram. A significant improvement in protein quality in horse gram was shown with dehusking,

roasting and puffing. The apparent digestibility of it improved on dehusking and puffing (Khader and Venkat Rao, 1986).

In horse gram, antinutrients like phytates, tannins and oxalic acid reduce the availability of iron. Oxalic acid combines with calcium and iron to form an insoluble salt and then both the essential elements become unavailable for absorption. During seed germination phytin is reduced and availability of Ca and Fe increases (Sudha *et al*., 1995).

In horse gram proximate nutrients, calcium, and some antinutrients in 16 varieties of whole horse gram and their dehulled seeds were estimated. A significant portion of the antinutrients studied were removed by dehulling. Findings indicate that dehulling could remove a large portion of oxalic acid and tannins (Sudha *et al*., 1995).

Impact of germination and dehulling on nutrients, antinutrients, invitro iron and calcium bioavailability and invitro starch and protein digestibility of some legume seeds, and they concluded that, germination caused significant increase in protein, thiamin, invitro iron and calcium bioavailability and invitro starch and protein digestibility contents of all the legume samples. Further increase in mentioned parameters was observed after dehulling the germinated legumes. Phytic acid and tannin were reduced by 18-21% and 20-38%, respectively, on germination and more reduction was observed in dehulled over germinated samples (Raihaneh and Jamuna, 2006).

In horse gram during seed germination, phytate is hydrolyzed by endogenous phytase and other phosphatases to release phosphate, inositol and various micronutrients. Several antinutritional factors like trypsin inhibitors (94.0 U/g flour), phytic acid (0.66%), polyphenols (1.6%), hemagglutin (2.6 U/g) and oligosaccharides (5.8%) are present in horse gram seeds. The activities like dehulliing, cooking, sprouting, roasting and germination reduce

the level of these factors and help for improving its digestibility (Bohn *et al*., 2008).

Pulses contain several antinutritional factors that reduce the bioavailability of nutrients (Jain *et al*., 2009). As far as the presence of antinutritional factors are concerned, some of the commonly considered antinutritional compounds like phytic acid, phenols, tannins are now being considered as potential antioxidants having health promoting effects. The phytic acid has now been shown to possess rich antioxidant, anticarcinogenic and hypoglycaemic activities (Bhatt and Karim, 2009).

The utilization of horse gram as human food is restricted due to presence of high level of enzyme inhibitors, haemagglutinin activities, oligosaccharides, tannins, polyphenols and phytic acid compared to the other legumes which can be reduced by processing (Dhumal and Bolbhat, 2012).

Effect of sprouting on trypsin inhibitors of cowpea and they concluded that sprouting effect on the antinutritional factor trypsin in cowpea and it was discovered that there was a progressive reduction in the trypsin inhibitors the germination period increases. The reduction was four fold of the original content (Malomo *et al*., 2011). Horse gram flour contains trypsin inhibitors activity (92.46±18 U/g), phytic acid (10.2±0.4 mg/g), polyphenols (14.3±0.4 GA/g) and oligosaccharides (26.8 mg/g) (Sreerama *et al*., 2012).

2.9 Remedial properties

Traditional texts describes its use as traditional medicine for curing kidney stones, asthma, bronchitis, leucoderma, urinary discharges, heart diseases and piles (Ghani,1998; Yadava and Vyas, 1994). In seed extract of horse gram water soluble, non-tannin and non-protein crystallization inhibitors are reported and a marked decrease in anticalcifying activity observed with the post-harvest storage of seeds (Peshin and Singla, 1995).

Horse gram has anthelmintic activity which can be used as dietary food for infants to eradicate worms and it has supposed to have unique property of dissolving kidney stones, therefore, in many parts of the country it is given to prevent or cure urinary stones (Philip *et al*., 2009).

It is considered as *Garmi dal* and preferred during the winter months by rural communities (Khanal *et al*., 2009). Horse gram seed are rich source of dietary antioxidants (Siddhuraju and Manian, 2007) as well as has antidiabetic effect (Gupta *et al*., 2011).

Horse gram is prescribed for persons suffering from jaundice, water retention, as part of a weight loss diet, iron deficiencies and also helpful for maintaining body temperature in the winter season (Ramesh *et al*., 2011). Horse gram used as medicine to treat hiccups, worms and in the treatment of bacterial and fungal infections (Kawsar *et al*., 2008). It has functional ingredients against hypercholesterolemia and obesity (Kumar *et al*., 2013).

Horse gram has long history as traditional medicine to cure many diseases, still it is neglected for its remedial potential. The seed of horse gram are useful for the cure of piles, hiccup, abdominal lump, bronchial asthma, in causing and regulating perspiration and horse gram seed powder is useful in stopping excessive perspiration (Pati and Bhattacharjee, 2013).

The extract of horse gram exerts a hypolipidaemic and hypoglycaemic actions (Senthil, 2009) and has also been found beneficial in urinary troubles, acid peptic disorder (gastritis), constipation, sunburn, kidney stone, female diseases (leucorrhoea, menstrual troubles, bleeding during pregnancy, post partum excessive discharges), colic caused by wind, piles, rheumatism, hemorrhagic disease, intestinal worms (Pati and Bhattacharjee, 2013).

2.10 Horse gram as functional food

The seeds, sprouts or whole meal of horse gram is used by large populations in rural areas (Kadam and Salunkhe, 1985). In some parts of India, leaves of horse gram are also used as vegetable (Mandle *et al*., 2012) and leaves contain additional health enhancing traits such as anthocyanins which are potent antioxidants by acting as free radical scavengers and shown to be antiinflammatory (Morris, 2008). Non-toxic extracts from aerial parts of horse gram justifying its ethno-botanical use (Kawsar *et al*., 2008).

Horse gram flour found to have favorable functional properties like higher water absorption capacity (148.1±3.4 mg/100g), emulsion activity (58.1±0.5%) and emulsion stability (52.0±1.6%) as compared to chickpea flour which suggest its scope to be exploited in the preparation and development of food products such as bakery products, soups and snacks as well as may be used to produce composite flours as partial substitutes of chickpea flour in confectionery and other traditional food products (Sreerama *et al*., 2012).

Horse gram has great potential both as food suggested by experiences worldwide. Its nutritious composition, medicinal properties and indomitable pest resistance makes it a rich yet cheap source of food, fuel supplement and green manure (Bhardwaj *et al*., 2013).

Horse gram is low cost pulse with high protein and acceptable cooking quality and has the potential to formulate products (Hiramath *et al*., 2001). In addition, horse gram flour has good functional properties with swelling capacity (1.43±0.01ml), water solubility index (7.56±0.10%), oil absorption capacity (80.76±0.03%), water absorption capacity (142.14±0.10g/100g) and swelling index (0.46±0.15%), hence can be used as functional foods for nutrition and food formulation (Marimuthu and Krishnamoorthi, 2013).

Raw horse gram seed is a rich source of antioxidant activities which are concentrated more in the seed coat of the seeds and consumption of food items

prepared with unprocessed raw horse gram seeds may have more health benefits for hyperglycaemic individuals (Tiwari *et al*., 2013).

Horse gram seeds and sprouts of horse gram are excellent examples of functional food as it has role in lowering the risk of various diseases and exerting health promoting effects in addition to its nutritive value (Ramesh *et al*., 2011). However, the metabolic changes during sprouting affect the bioavailability, palatability and digestibility of essential nutrients (Masood *et al*., 2014).

The inception of neutraceutical concept and health consciousness among masses has increased the utilization of potential antioxidants from legumes including horse gram as it reduces the risk of intestinal diseases, diabetes, coronary heart disease, prevention of dental caries etc. due to presence of bioactive compounds (Prasad and Singh, 2014).

Horse gram seed proteins exhibit free radical scavenging capacities which can be used as a food supplement, natural antioxidant and useful as therapeutics for health benefits of human (Petchiammal and Hopper, 2014). Horse gram flour is rich in protein, calcium and dietary fibre, after simple processing like soaking and drying or roasting eliminates the antinutritional content hence suitably processed horse gram flour could be used in the preparation of various food products (Thirukkumar and Sindumathi, 2014).

The seeds of two varieties of horse gram ‘AK-21’ and ‘AK-42’ were assessed for their functional properties using five common indigenous products kasar, laddoo, mathri, biscuits and khakra. The hydration capacity at 6 hr for both varieties was 0.03g /seed and at 12, 18 and 24 hr similar i.e. 0.04g/seed (Jain *et al*., 2012).

2.11Preservation of sprouts (Malt)

Boyee (1988) performed study on harvesting and drying of peas and beans. The research to improve drying of peas and beans included determining physical characteristics, bulk density and 1000 seed weight of range of varieties since these physical properties affects resistance to heat flow of bed and moisture diffusion from single seed. Physical dimensions of number of varieties can be used to determine characteristics which enables prediction of farm drying system performance e.g. resistance to air flow, drying rate constant, equilibrium moisture content and seed quality as a function of drying air temperature and RH.

Anarase (1992) studied the dehydration of chick pea by conventional tray drying of variety PG-5 and PG-12. Three levels of drying air temperature (60, 70 and 80 °C) were used. Bulk density of samples of both varieties was decreased after dehydration. Bulk density of samples of both varieties decreased more after dehydration at 80 ºC than at 70 and 60 °C and particle density and 1000 grain weight also decrease with dehydration.

Pisal (1997) conducted studies on preparation and preservation of instant sprouts of moth bean. The steeping of moth bean seeds for 8 hr in plain water and sprouting for 12 hr followed by blanching in plain water for 3 min and sun drying were found to be best condition for preparation of good quality instant sprouts.

Mankotia *et al.*, (2003) studied on effect of soaking sprouting and cooking on physicochemical properties of moth beans and they concluded that, sprouting may cause significant changes in proximate composition of grains.

Pawar (2010) studied the effect of soaking water temperature and period of germination on nutritional quality of moth bean. Nutritional parameters like IVPD, IVSD and ascorbic acid in raw moth bean seeds were 67.78 %, 29.27 g/2hr and 5.80 mg/100 g respectively. The IVPD, IVSD and ascorbic

acid was recorded at 30 ºC and 48 hr germination period was 89.64 %, 84.17 g/2hr and 36.46 mg/100 g respectively.

Geetha *et al.,* (2014) studied on microwave drying of sprouted horse gram mathematical modeling of drying kinetics and they concluded that, effect of microwave power output on certain parameters such as moisture content, moisture ratio, drying rate, drying time and effective moisture diffusivity. As the microwave output power increased, drying time decreased significantly.

Yewale and Jejurkar (2015) reported that the effect of drying methods (shade drying, sun drying and mechanical drying) and drying temperature (50, 60, 70 and 80 ºC) on physical properties and chemical composition of sprouted moth bean. The engineering properties fresh sprouted moth bean seeds viz., bulk density, true density, and 1000 grain weight were 0.419g/cc, 1.351g/cc, 61.12g, respectively which was after drying found varied between 0.373 to 0.427g/cc, 1.135 to 1.481 g/cc and 25.74 to 22.13g, respectively. The chemical constituents of fresh sprouted moth bean viz., moisture content, IVPD, IVSD and ascorbic acid were 62.6 % (w.b.), 85%, 80 g/2hr and 34.67mg/100 g, respectively, which was after dehydration under various treatments found varied between 13.14% (w.b.) to 5.97% (w.b.) 81.75 to 88.26%, 78.08 to 85.06 g/2hr and 26.87 to 34.41mg/100g, respectively.

Vitthalrao and Sharad (2016) studied that sprouting exert significant influence on the antioxidant activity in selected pulses and reported that soaking and sprouting process of pulses increases significantly on the antioxidant activity, total phenolics, totoal flavonoids and ascorbic acid content in pulses.

3. MATERIALS AND METHODS

The present investigation on standardization of process for preparation of malt from horse gram was conducted at the Department of Food Science and Technology, Mahatma Phule Krishi Vidyapeeth, Rahuri during the year 2014-2015.

3.1 Materials

3.1.1 Horse gram

Fresh horse gram (local variety) was procured from Rahuri (Plate 1). The seeds were cleaned and stored in polythene bag for the research purpose.

3.1.2 Chemicals

Most of the chemicals used in this investigation were of analytical grade. They were obtained from M/s British Drug House, Mumbai, Sarabhai Chemicals, Baroda and E. Mark, (India), Mumbai.

3.1.3 Equipments

Incubator, cabinet drier (Plate 2) and sealing machine were used from the department of Food Science and Technology.

3.1.4 Packaging materials

The packaging materials are HDPE (25 micron) pouches were procured from Rahuri.

3.2 Methodology: Experimental details

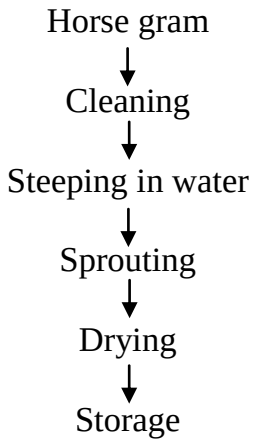

Fig 1. Flow chart for preparation of malt from horse gram

a) Standardization of steeping, malting time and malting temperature

Sr .No	Steeping time (hr)	Malting time (hr)	Malting temperature (°C)
T_1	4	18	25
T_2	4	24	30
T_3	4	30	35
T_4	8	18	25
T_5	8	24	30
T_6	8	30	35
T_7	12	18	25
T_8	12	24	30
T_9	12	30	25

b) Treatment details

1. Steeping (for 4, 8 and 12 hr)

A_1- Plain water

A_2- Plain water + 0.1% KMS

A_3- Plain water + 0.5% Na_2HCO_3

2. Drying

C_1-Cabinet drying at 60°C ± 2°C

3.3. Physicochemical parameters

Size of the seeds (length and thickness) was determined with an electronic digital caliper. Seeds were randomly selected and the results were registered as the average of 10 seeds, hydration and swelling capacities, hydration and swelling indexes and cooking time were determined by the techniques used by Bishnoi and Khetarpaul (1993).

3.4 Functional properties of horse gram

3.4.1 Hydration capacity

Raw whole grain sample weighing 100g were counted and transferred to a measuring cylinder and 100ml water was added. The cylinders were covered with aluminum foil and left over night at room temperature. Next day seeds were drained, superfluous water removed with filter paper and swollen seeds reweighed. Hydration capacity per seed and swelling capacity per seed was determined by using the following formula (Jain *et al*., 2012):

$$\text{Hydration capacity} = \frac{\text{Weight of soaked seed - Weight of seed before soaking}}{\text{No. of seeds (N)}}$$

3.4.2 Hydration index

Hydration index was calculated using the formula

$$\text{Hydration index} = \frac{\text{Hydration capacity per seed}}{\text{Weight of one seed (g)}}$$

3.4.3 Swelling capacity

Raw whole grains weighing 100g were measured, counted and volume was noted and soaked overnight. The volume of the soaked seeds was noted in a graduated cylinder.

Swelling capacity per seed was calculated as

$$\text{Swelling capacity} = \frac{\text{Volume after soaking - Volume before soaking}}{\text{No. of seeds (N)}}$$

3.4.4 Swelling index

The swelling index was calculated as below:

$$\text{Swelling index} = \frac{\text{Swelling capacity per seed}}{\text{Volume of one seed (ml)}}$$

3.5 Chemical analysis of horse gram and horse gram malt

3.5.1 Moisture

The initial moisture content was determined by using standard method of A.O.A.C. (1990) procedure as follows.

A required number of aluminum dishes were heated, cooled and weighed. About 10g of sample of horse gram and malted horse gram was weighed accurately and dried in oven from 60 °C to 105 °C within 5 hr. After cooling in desiccators, it was reweighed. The drying was repeated for one more hour, cooled as above and reweighed until constant weight was obtained. The mean loss in weight was taken as moisture content and reported as per cent. The per cent moisture content was calculated by using equation.

$$M = \frac{W_1 - W_2}{W_1} \times 100$$

Where,

M = moisture content in per cent

W_1 = weight of wet sample (g)

W_2 = weight of dry sample (g).

3.5.2 Ascorbic acid

Ascorbic acid content was estimated by using 2, 6-dichlorophenol indophenol dye as reported by Ranganna (1986).

Reagents

1. Metaphosphoric acid (3%): In 100ml distilled water 3g metaphosphoric acid was dissolved.
2. Dye solution: Sodium salt of 2, 6-dichlorophenol indophenol dye (50mg) was dissolved in about 150ml of hot distilled water contained water containing 42mg sodium carbonate. The mixture was cooled and

diluted with distilled water to make up to volume of 250ml. The dye solution was prepared fresh for every analysis.

3. Standard ascorbic acid solution (0.1mg/ml): L-ascorbic acid (100mg) was dissolved in 3 per cent metaphosphoric acid and the volume was made to 100ml of this stock solution 10ml was diluted with metaphosphoric acid to 100ml.
4. Standardization of dye: Standard ascorbic acid solution (5ml) was mixed with 3.00 per cent metaphosphoric acid. It was titrated with dye solution until faint pink colour appeared and persisted for about 15 seconds.

The dye factor was calculated as

$$\text{Dye factor} = \frac{0.5}{\text{Titre}}$$

Procedure

An aliquot (10ml) was pipette out in a conical flask and titrated against with 2, 6 dichlorophenol indophenol dye and a burette reading at pink colour development for 15 seconds was recorded. Ascorbic acid content in the sample was calculated in mg/100 g as per the formula.

$$\text{Ascorbic acid (mg /100 g)} = \frac{(\text{Titre-blank}) \times \text{Dye factor} \times \text{Volume of extract}}{\text{Extract taken for titration (ml)} \times \text{Volume of sample taken (ml)}} \times 100$$

3.5.3 Protein

The protein content was determined by Microkjedhal method (A.O.A.C., 1990).

Reagents

1. Concentrated sulphuric acid (sp.gr. 1.84, N-free)
2. Catalyst mixture – Potassium sulphate (99g), mercuric oxide (4.1g) and copper sulphate (0.89 g) were weighed and mixed thoroughly.

3. Sodium hydroxide (50 per cent, w/v) – Sodium hydroxide pellets (50g) and sodium thiosulphate (5g) were dissolved in distilled water separately, mixed and the volume was made to 100ml.
4. Boric acid (4 per cent w/v) – Four grams boric acid was dissolved in distilled water separately, mixed and the volume was made up to 100ml.
5. Hydrochloric acid (0.02 N) – 0.177 ml of hydrochloric acid (sp.gr. 1.18, 35 per cent) was dissolved in distilled water and the volume was made to 100ml.
6. Hydrogen peroxide (30 per cent, v/v).
7. Mixed indicator – This was prepared by dissolving bromocreasol green (0.1g) and methyl red (0.1g) in 100ml. of 95 per cent (v/w) ethyl alcohol separately. Bromocreasol green solution (10parts) and methyl red solution (2parts) were mixed together and transferred to a bottle provided with stopper.

Procedure

Powdered sample of the horse gram flour (200mg) was accurately weighed and transferred carefully to a digestion flask, to which catalyst mixture (1g) was added and thoroughly mixed with the sample, concentrated sulphuric acid (5ml) and hydrogen peroxide (5ml) were carefully added and the sample was digested in a digestion chamber. Initially the flask were heated slowly for 10 to 15 min and then the temperature was raised gradually 50˚C that the content boiled briskly. The digestion was continued until the sample became clear and colorless. The flasks were then cooled and minimum quantity of water was added to dissolve the solid in the flasks. After cooling the contents were transferred to volumetric flasks. The digestion flasks were washed 3 to 4 times and all the washings were transferred to volumetric flasks and the volume was made to 50ml.

Boric acid solution (10ml) was pipette into a 150ml beaker and 6 to 8 drops of mixed indicator solution were added to it. The beaker was placed under condenser of distillation assembly. Care was taken to ensure that the tip of the condenser dipped below the surface of the solution. Five mililitre digest was pipette into the distillation flask and mixed with 5ml sodium hydroxide solution (50 percent w/v). The distillation was continued to collect all the ammonia. The confirmation of distillation was made with the help of a litmus paper. The distillate was then titrated with standard hydrochloric acid solution. Before distillation, the color of boric acid plus indicator was pinkish red, which changes to blue green during distillation, and finally to pinkish red at the end of titration with standard hydrochloric acid solution. Simultaneously, blank titration was carried out each time. The per centage of nitrogen content was calculated from the volume of standard hydrochloric acid required for titration. The protein content was calculated by multiplying the nitrogen content by factor of 5.7 (A.O.A.C., 1990).

$$\% N = \frac{(S - B) \times N \times 14.007}{\text{Weight of sample (g)}} \times \frac{\text{Volume made}}{\text{Volume taken (ml)}} \times 100$$

Where,

S = ml of HCl required for sample titration,

B = ml of HCl required for blank titration,

N = Normality of HCl (0.02 N)

% Crude protein = % N × 5.7

3.5.4 Total sugars

Total sugars were determined by method of Lane and Eynon (1923) as reported by Ranganna (1986) as follows.

Procedure

In a 100ml volumetric flask, ten ml sample was taken. To this 2ml concentrated HCl was added and the flask was kept in hot water bath at 70-80°C for 30 min. After cooling, the hydrolysate was neutralized by adding a pinch of sodium carbonate till formation of effervesces stopped. The volume of neutralized hydrolysate was made to 100ml with distilled water. The total sugars in the neutralized hydrolysate were determined in the same way as described under reducing sugars.

$$\text{Total sugars (\%)} = \frac{\text{Factor x Volume made up}}{\text{Titer x Weight of sample taken}} \text{ x } 100$$

3.5.5 Iron

The iron was determined by using standard method of A.O.A.C., (1990) procedure as follows.

Reagents

1. Phenanthroline (0.1 % w/v) – 100mg of 1, 10 phenanthroline monohydrate was dissolved in water at 80˚C, cooled and diluted to 100ml.
2. Hydroxylamine hydrochloride (10%)
3. Acetate buffer (8.3g of anhydrous sodium acetate was dissolved in water, 12ml glacial acetate acid was added to it and diluted to 100ml).
4. Standard iron solution (100 µg/ml – Ferrous ammonium sulphate (0.7022 g) was dissolved in water, 2 drops conc. HCl were added and diluted to 100ml).

Procedure

A. Standard curve

Standard iron solution (0-4 ml) was pipette in 25ml volumetric flask and the volume was adjusted to 5ml. 1 ml of hydroxylamine hydrochloride was added, mixed and kept for 5 min. 5ml acetate buffer and 1ml phenanthroline reagent were added to it and the volume was made to 25ml with distilled water and mixed. The color intensity was read at 515 nm.

B. Iron in sample

Five gram sample was burnt in furnace and the acid extract was prepared as earlier 5ml of the extract was withdrawn and kept for color development. The intensity of the color was measured on spectonic – 20 spectrophotometer.

$$\text{Iron (mg \%)} = \frac{\text{Reading (OD)}}{\text{(mg/100g) Extract used for color development}} \times \frac{\text{Vol. of acid extract prepared}}{\text{Weight of sample}} \times 100$$

3.5.6 Phytic acid

Phytic acid was assayed as ferric-phytate by the extraction method of wheeler and ferrel (1971).

Reagents

1. Trichloroacetic acid (TCA) 5%: Five gram of TCA was dissolved in glass distilled water and volume made up to 100 ml.
2. Potassium thiocyonate 29%: Twenty nine gram of potassium thiocyonate was dissolved in glass distilled water and volume made up to 100 ml.
3. Ferric chloride 0.25%: Twenty five mg ferric chloride was dissolved in 100ml 5% TCA.
4. Phytic acid : Fifty milligram of sodium salt of phytic acid was dissolved in 100ml 5% TCA.

Procedure

Two hundread miligram sample was weighed into a centrifuge tube and extracted with 5% TCA at 60°C and centrifuged at 5000 rpm for15 min. the precipitate was washed twice with 5% TCA and centrifuged. The supernatants were pooled and volume made up to 50ml. an aliquot (20ml) was pipette into a digestion tube and 5ml of 0.25% $Fecl_3$ 6 H_2O (Ferric chloride) was added. The tube was heated in a block digester at 95°C for 45 min. The contents were cooled and volume made up to 75ml. The available ferric ion after reaction was determined by reaction with potassium thiocyonate which developed a blood-red colour compound. The absorbance was read at 485 nm against a reagent blank to determine the volume of ferric chloride solution which will represent 1mg phytic acid a standard curve of absorbance against standard sodium salt of phytic acid. Phytate content was calculated from the iron concentration by assuming a constant Fe:P molecular ratio 4:6 the precipitate.

$$\text{Phytate P mg (100mg sample)} = \frac{\mu g\ Fe \times 115}{\text{Weight of sample (g)}}$$

3.5.7 Trypsin inhibitors

a) Extraction of inhibitors

The flour was extracted with distilled water (1:20 w/v) for 2 hr at room temperature by using mechanical shaker. The extract was filtered and filtrate was used for assay after appropriate dilution to produce 40 to 60 per cent inhibition. The inhibitor was added after adding buffer to the enzyme and after waiting for 2 to 3 min for reaction. For blank, the enzyme was inactivated by acetic acid.

b)Trypsin inhibitors assay

Trypsin activity was determined by the method of Erlanger *et al.*, (1961) with some modification.

The substrate was prepared by dissolving 21.8mg Na-benzoyl-DL-arginine-P-nitroanilide (BAPNA) in 1ml of dimethyl-sulfoxide and adding pre-warmed buffer (0.05M Tris-HCl, pH 8.2 containing 0.02 M $CaCl_2$) to a total volume of 100ml. this solution was placed in a water bath 37˚C. Then 0.15ml enzyme solution (1 mg trypsin/ml 10^{-3}) was mixed with 0.15ml of 0.1 M sodium acetate buffer pH 4.9 in a test tube at 37˚C and reaction was started by adding 2ml substrate solution. The reaction was adding 1ml acetic acid. The quantity of p-nitroanilide liberated by enzyme action was then estimated at 410 nm on spectroniv 20. One unit of tryspin activity was one mole of p-nitroanilide released per min.

Trypsin activity activity (TIA) of the mixture of diluted extract and trypsin and was expressed in terms of trypsin inhibitors unit (TIU) per gram sample. One trypsin inhibitory unit (TIA) was defined as one unit of tryspin activity inhibited.

$$TI(U/g) = \frac{[(\Delta A253/min)\ blank - (\Delta A253/min)\ sample] \times Dilution\ factor}{(\Delta A253/min)\ blank \times \mu g\ inhibitor\ reaction\ mixture}$$

3.5.8. Total phenolics

Extract preparation

Ten grams of each powdered seed samples were shaken separately in ethanol for 72 hr on an orbital shaker at room temperature. Extracts were filtered using a Buckner funnel and Whatman No 1 filter paper. Each filtrate was concentrated to dryness under reduced pressure at 40^{o}C using a rotary evaporator. Each extract was resuspended in methanol to make 50 mg/ml stock solution

Procedure

The total phenolic content in the seed extracts were determined with the Folin Ciocalteu's reagent. The reaction mixture contained: 200µl of diluted rice bran extract, 800µl of freshly prepared diluted Folin Ciocalteu reagent and 2ml of 7.5% sodium carbonate. The final mixture was diluted to 7ml with deionized water. Mixtures were kept in dark at ambient conditions for 2 hr to complete the reaction. The absorbance of seed extracts was measured at 765 nm using a spectrophotometer.

Total phenol content was expressed as gallic acid equivalents (GAE).

$$T = C \times V / M$$

Where,

T is the total phenolic content in mg/g of the extracts as GAE,

C is the concentration of gallic

V is the volume of the extract solution in ml and

M is the weight of the extract in g.

3.5.9. Total tannins

Colorimetric estimation of tannins was performed based on the measurement of blue colour formed by the reduction of phosphotungstomolybdic acid by tannin like compounds in alkaline solution. A known amount of extract was mixed with 5.0 ml of Folin-Denis reagent (FD) and Na_2CO_3 solution and made up to 100 ml, mixed well and absorbance was read at 760 nm after 30 min using spectrophotometer. Total tannin content expressed as mg tannic acid equivalent /100g of sample (TAE).

$$\text{Total tannin content (TAE/100g)} = \frac{\text{Weight of ethanol fraction in gm}}{\text{Weight of the sample taken in gm}}$$

3.5.10 Total flavanoids

Total flavonoid content was determined using aluminium chloride ($AlCl_3$) according to a known method. The seed extract (0.1ml) was added to 0.3ml distilled water followed by 5% $NaNO_2$ (0.03 ml). After 5 min at 25°C, $AlCl_3$ (0.03ml, 10%) was added. After further 5 min, the reaction mixture was treated with 0.2ml of 1 M NaOH. Finally, the reaction mixture was diluted to 1ml with water and the spectrophotometer. Total flavanoid content was calculated as quercetin equivalents (QE). Absorbance was measured at 510 nm using a spectrophotometer. Total flavanoid content was calculated as quercetin equivalents (QE).

$$\text{Flavonoids (QE/100g)} = \frac{[\text{Flavonoids}](\mu g/ml) \times \text{Total volume of ethonolic extract (ml)}}{\text{Mass of extract } (\mu g)}$$

3.6 Organoleptic evaluation of horse gram malt

Organoleptic evaluation of horse gram malt was carried out according to method of Amerine *et al*., (1965) on a 9 point Hedonic scale (Appendix-I). The average scores of the seven semi-trained judges for different quality characteristics *viz.* colour flavour, taste and overall acceptability were recorded.

3.7 Storage study

Best one among treatments was selected for further 3 months storage in HDPE (25 micron) pouches and for which organoleptic and physicochemical quality parameters mentioned will be analyzed at 30 days regular interval.

3.8 Microbial analysis of dried horse gram malt

Microbial count was recorded as colony forming units (CFU). The nutrient agar (NA) was prepared and used as growth medium. Petri dishes were incubated at 37 ± 0.5°C for 48 hr for counting the microbial colonies. A colony

counter with magnifying lens was used for counting the colonies. Total count was taken along with pinpoint colonies.

3.9 Statistical analysis

The experimental data was analyzed for the statistical significance of treatment Completely Randomized Design (CRD) according to the procedure given by Nigam and Gupta (1979).

4. RESULTS AND DISCUSSION

The horse gram seeds were used for preparation of malts using various pretreatments. The results of the research work are presented and discussed in this chapter as below.

4.1Physical properties of horse gram

The shape, colour, length, weight, thickness, bulk density, true density and angle of repose of horse gram seeds are shown in Table 1. The horse gram was flattned in shape, average length 5.55 (mm), thickness 2.05 (mm), bulk density 0.818 (g/cc), true density 1.3 (g/cc) and it has a colour of light red, brown, grey and black in colour. The small size and thickness of the seeds might be attributed to drought resistant grain crops being hardy in nature, have a thick endosperm as observed in other millets. These results were concurred with the results of Jain *et al*., (2012).

4.2 Proximate composition of horse gram

4.2.1 Moisture

The moisture content in raw horse gram is 10.20 per cent. The moisture content in horse gram was 11.55 per cent (Sudha *et al*., 1995), 9 to 12 per cent (Mohan *et al*., 2011) and 8 per cent (Sreerama *et al.,* 2012).

4.2.2 Protein

The first major component in horse gram is protein. The protein content in raw horse gram was 21.65 per cent. The protein content in horse gram was ranged from 17.9 to 25.3 per cent (Sudha *et al*., 1995), 22 per cent (Gopalan *et al*., 1999), 23 per cent (Venkatesha, 1999), 21.50 to 23.50 per cent (Sreerama *et al.,* 2012) and 21.6 to 23.6 per cent (Saroj Kumar Prasad and Manoj Kumar Singh, 2015).

Table 1. Physical properties of horse gram

Parameter	Horse gram
Shape	Flattned
Colour	Light red, brown, grey and black
Average length (mm)	5.550
Average weight (g/100 seeds)	3.320
Thickness (mm)	2.050
Bulk density (g/cc)	0.818
True density (g/cc)	1.340
Angle of repose (°)	36.90

Table 2. Proximate composition of horse gram

Nutrients	Amount
Moisture (%)	10.20
Protein (%)	21.65
Total sugars (%)	6.900
Iron (mg/100g)	7.100
Ascorbic acid (mg/100g)	2.300
Trypsin inhibitors (TIU/g)	94.00
Phytic acid (mg/g)	10.20
Total phenolics (GAE/100g)	1.500
Tannins (TAE/100g)	0.103
Total flavonoids (QE/100g)	0.032

4.2.3 Total sugars

The total sugars content in raw horse gram is 6.90 per cent. The total sugars content in horse gram was 7.00 per cent (Kasat, 1988), 7.10 per cent (Pisal, 1997), 6.8 per cent (Bravo *et al*., 1999) and 6.38 per cent (Saroj Kumar Prasad and Manoj Kumar Singh, 2015).

4.2.4 Iron

The iron content in raw horse gram is 7.10 mg/100g. The iron content in horse gram was ranged from 6.33 to 7.52 mg/100g (Morris *et al*., 2013) and 5.89 to 7.44 mg/100g (Khatun *et al*., 2013).

4.2.5 Ascorbic acid

The ascorbic acid in raw horse gram is 2.30 mg/100g. The ascorbic acid content in horse gram was 2.40 mg/100g (Kasat, 1988) and 2.45 mg/100g (Ghorpade, 1984).

4.2.6 Trypsin inhibitors

The trypsin inhibitors in raw horse gram is 94.00 U/g. The trypsin inhibitors content in horse gram was ranged from 92.18 to 92.62 U/g (Jain *et al*., 2009) and 98.40 to 98.56 U/g (Sreerama *et al*., 2010).

4.2.7 Phytic acid

The phytic acid in raw horse gram is 10.20 mg/g. The phytic acid content in horse gram was ranged from 10.20 to 10.25 mg/g (Sreerama *et al*., 2012) and 8.42 to 8.81 mg/g (Saroj Kumar Prasad and Manoj Kumar Singh, 2015).

4.2.8 Total phenolics

The total phenolics in raw horse gram is 1.50 GAE/100g. The total phenolics content in horse gram seeds was ranged from 1.47 to 1.51GAE/100g (Sreerama *et al*., 2012) and 1.511 to1.522 GAE/100g (Marimuthu and Krishnamoorthi, 2013).

4.2.9 Total tannins

The total tannins in raw horse gram is 0.103 TAE/100g. The total tannin content in horse gram was ranged from 0.100 to 0.105 TAE/g (Sreerama *et al*., 2012) and 0.104 to 0.107 TAE/100g (Marimuthu and Krishnamoorthi, 2013).

4.2.10 Total flavonoids

The total flavonoids in raw horse gram is 0.032 QE/100g. The total flavonoids content in horse gram was ranged from 0.038 to 0.065 QE/100g (Marimuthu and Krishnamoorthi, 2013).

4.3 Functional properties of horse gram seeds

4.3.1 Hydration and swelling capacity

The hydration and swelling capacities of horse gram were presented in Table 3. The hydration capacity at 4 hr was comparable with that of velvet bean. However, swelling capacity of horse gram was lower as compared to velvet bean as reported by (Gurumoorthi and Janardhanan, 2008). Lower values for swelling capacity reflected that the seeds possessed very hard and impermeable seed coat and they were not hydrated easily.

The hydration and swelling index of horse gram were presented in Table 4. The hydration index was higher while its swelling index was lower. Both hydration and swelling indexes increased in a linear manner up to 12 hr. These results indicate that the maximum hydration and swelling of horse gram seeds had occurred at 12 hr. Moreover, the beans which absorb more water are highly preferred by the consumers as well as processors. The lower hydration and swelling capacity of horse gram seeds as compared to other beans and peas clearly indicated their hard to cook phenomenon. These results were cognate with the result of Jain *et al*., (2012).

Table 3. Hydration and swelling capacity

Steeping (hr)	Hydration capacity (g/seed)	Swelling capacity (ml/seed)
4	0.03	0.04
8	0.04	0.07
12	0.04	0.06

Number of seeds (N) =10

Table 4. Hydration and swelling indices

Index	Hours	Horse gram
Hydration	4	1.013
	8	1.237
	12	1.258
Swelling	4	1.192
	8	1.638
	12	1.674

Number of seeds (N) =10

4.4 Standardization of steeping and malting time

The data on effect of steeping and malting time from various treatments are presented in the Table 5. The treatments were steeped in 4, 8 and 12 hr containing 0.1 % KMS and 0.5% Na_2HCO_3 followed by 18, 24 and 30 hr malting time and malting temperature 25, 30 and 35˚C respectively. It indicated that the treatment i.e., 12 hr steeping, 24 hr malting time and 30˚C malting temperature was superior as compare to other treatments.

4.5 Characteristics of horse gram malt

The data on effect of characteristics of horse gram malt were presented in Table 6. It indicated that appropriate steeping time was 12 hr containing 0.1% KMS and malting period was 24 hr at 30˚C was superior and best as compared to other treatments. The appropriate steeping time, malting time and temperature were decided on the basis of malting (%) and length of acrospires as presented in the Table 6.

4.6 Organoleptic evaluation of rehydrated horse gram malt

The treatment combinations were A_1, A_2 and A_3 i.e., plain water steeping, plain water containing 0.1% KMS (A_2) and plain water containing 0.5% Na_2HCO_3 (A_3) and other combinations were selected for the experiment as shown in Table 7. The treatment combinations $A_1T_9C_1$, $A_2T_8C_1$, $A_3T_8C_1$, $A_2T_9C_1$ and $A_3T_9C_1$ were superior to other treatments as far as colour components was considered while $A_2T_8C_1$ is superior in overall acceptability as shown in Table 7. Treatment combination $A_2T_8C_1$ was the most superior to others with respect to malting (%), length of acrospires, colour and overall acceptability.

Table 5. Standardization of steeping period and malting period

Steeping + Malting time (hr)	Mean*	Remarks
4+18	4.15	Insufficient malting
4+24	4.30	Insufficient malting
4+30	4.50	Insufficient malting
8+18	5.70	Uneven and insufficient malting
8+24	6.30	Uneven and insufficient malting
8+30	6.80	Length of acrospires is very short
12+18	7.80	Length of acrospires is very short
12+24	8.20	Appropriate and best malting
12+30	8.80	Little excessive
SE(±)	0.07	
CD (0.05)	0.20	
CV%	2.24	

*Score out of 9

Table 6. Characteristics of horse gram malt

Steeping + Malting time (hr)	Malt (%)	Length of acrospires (cm)
4+18	10.75	0.55
4+24	14.00	0.59
4+30	20.00	0.71
8+18	46.50	0.80
8+24	62.00	0.91
8+30	71.25	1.10
12+18	81.25	1.21
12+24	86.25	1.32
12+30	92.50	1.50
SE(±)	0.870	0.05
CD@5%	2.540	0.15
CV%	3.250	10.74

Table 7. Organoleptic evaluation of rehydrated horse gram malt

Treatments	Colour	Flavour	Texture	Overall acceptability
$A_1T_9C_1$	7.75	7.25	7.25	7.35
$A_2T_8C_1$	7.75	7.75	8.50	7.95
$A_3T_8\ C_1$	7.25	7.00	7.25	7.10
$A_2T_9\ C_1$	7.50	7.00	7.25	7.18
$A_3T_9\ C_1$	7.50	7.00	7.00	7.08
SE(±)	0.26	0.24	0.46	1.84
CD (0.05)	0.80	0.72	1.41	5.54
CV%	0.07	0.06	0.12	0.66

Note: Out of 27 treatments best 5 treatments were selected on the basis of visual appearances and placed for organoleptic evaluation in that best one treatment selected and kept for further storage study.

Where,

A_1- Plain water

A_2- Plain water + 0.1% KMS

A_3- Plain water + 0.5% Na_2HCO_3

C_1-Cabinet drying at 60˚C ± 2˚C

	Steeping time (hr)	Malting time (hr)	Malting temperature (˚C)
T_8	12	24	30
T_9	12	30	25

4.7 Changes in weight and recovery of dried horse gram malt

The data on changes in weight at various stages of preparation of dried horse gram malt are shown in Table 8. After steeping the seeds in water, there was increase in weight to the extent of about two times of the initial weight and on malting slightly decreased. The recovery of horse gram malts after drying was to the extent of 90%. The drying ratio was 1:0.90 and rehydration ratio was 1:0.98. These results were similar with the results of horse gram (Kasat, 1988) and moth bean (Pisal, 1997).

4.8 Rehydration of dried horse gram malt

The time taken for complete rehydration of horse gram malt was about 3 hr in cold water, but rehydration in boiling water to the desired level took place 5 min are shown in Table 9. Thus rehydration in dried horse gram malt in boiling water was better and quicker than in cold water. These results were similar with the results of horse gram (Kasat , 1988) and moth bean (Pisal, 1997).

Table 8. Changes in weight and recovery of dried horse gram malt

Particulars	Weight (g)
Initial weight	100.00
Steeping	206.00
Weight after malting	184.00
Recovery of malted horse gram	89.870
Drying ratio	1:0.90
Rehydration ratio	1:0.98

Table 9. Rate of rehydration of horse gram malt

Particulars	Weight (g)
Initial weight	100
Weight after 1 min	150
Weight after 2 min	180
Weight after 3 min	205
Weight after 4 min	205

4.9 Chemical composition of raw horse gram seeds, horse gram malt, dried horse gram malt and rehydrated horse gram malt

4.9.1 Moisture

The data on moisture content of raw horse gram seeds, horse gram malt (Plate 3), dried horse gram malt and rehydrated horse gram malt are presented in Table 10. The moisture content of raw horse gram seeds and dried horse gram malt ranged from 10.20 to 10.10 per cent. The decrease in moisture content after drying might be due to higher drying temperature. However, the moisture content of horse gram malt and rehydrated horse gram malt ranged from 59.40 to 55.95 per cent. The increase in moisture content after malting and rehydration malt might be due to steeping and cooking.

4.9.2 Protein

The data on protein content of raw horse gram seeds, horse gram malt (Plate 3), dried horse gram malt and rehydrated horse gram malt are presented in Table 10. The protein content of raw horse gram seeds and dried horse gram malt ranged from 21.65 to 22.88 per cent. The increase in protein content after malting might be due to synthesis of enzymes protein and reduced phytic acid content in malting (Vanderstoep, 1981). However, the protein content of horse gram malt and rehydrated horse gram malt ranged from 11.60 to 12.40 per cent. The decrease in protein content after malting and rehydration might be due to regaining of moisture content during steeping and cooking.

4.9.3 Total sugars

The data on total sugars content of raw horse gram seeds, horse gram malt (Plate 3), dried horse gram malt and rehydrated horse gram malts are presented in Table 10. The total sugars content raw horse gram seeds and dried horse gram malt ranged from 6.90 to 7.30 per cent. However, the total sugars content of horse gram malt and rehydrated horse gram malt ranged from 3.10 to 3.40 per cent. The increased in total sugars content after malting might be due

to complex sugar are converted are into simple sugar (Chavan and Kadam, 1989). Furthermore, the decrease in total sugar content after rehydration might be due to regaining of moisture content during steeping, cooking and some of the are sugar are soluble in water.

4.9.4 Ascorbic acid

The data on ascorbic acid content of raw horse gram seeds, horse gram malt (Plate 3), dried horse gram malt and rehydrated horse gram malt are presented in Table 10. The ascorbic content of raw horse gram seeds and dried horse gram malt ranges from 2.30 to 9.50 mg/100g. However, the ascorbic acid of horse gram malts and rehydrated horse gram malt ranges from 10.03 to 9.20 mg/100g. The significant increase in ascorbic acid content after malting might be due to several enzyme become active and bring about profound changes in the ascorbic acid content (Masood *et al*., 2014). Furthermore, the decrease in ascorbic content after rehydration might be due to higher temperature during drying and cooking.

4.9.5 Trypsin inhibitors

The data on trypsin inhibitors content of raw horse gram seeds, horse gram malt (Plate 3), dried horse gram malt and rehydrated horse gram malt are presented in Table 10. The trypsin inhibitors content of raw horse gram seeds and dried horse gram malt ranges from 94.00 to 55.55 U/g. The decrease in trypsin inhibitors content after malting might due to possibility that the trypsin inhibitors could be utilized as an energy source during the early stages of malting (Malomo *et al*., 2011). However, the trypsin inhibitors content of horse gram malt and rehydrated horse gram malt ranges from 50.6 to 6.06 U/g. The gradual decreases in trypsin inhibitors after rehydration might be due to higher cooking temperature.

4.9.6 Phytic acid

The data on phytic acid content of raw horse gram seeds, horse gram malt (Plate 3), dried horse gram malt and rehydrated horse gram malt are presented in Table 10. The phytic acid content of raw horse gram seeds and dried horse gram malt ranges from 10.20 to 3.20 mg/g. The decrease in phytic acid in storage attributed to an increase of phytase activities i.e., enzyme would make a solubilization of phytates and would release soluble protein and minerals (Borade *et al*., 1984). However, the phytic acid content of horse gram malt and rehydrated horse gram malt ranges from 3.40 to 2.05 mg/g. The gradual decreases in phytic acid after malting and rehydration might be due to steeping, malting and cooking.

Table 10. Chemical composition of raw horse gram, horse gram malt, dried horse gram malt and rehydrated horse gram malt

Particulars	Moisture (%)	Protein (%)	Total sugars (%)	Ascorbic acid (mg/100)	Trypsin inhibitors (TIU/g)	Phytic acid (mg/g)
Raw	10.20	21.65	6.90	2.30	94.00	10.20
Malted	59.40	11.60	3.10	10.03	50.60	3.400
Dried	10.10	22.88	7.30	9.50	55.55	3.200
Rehydrated	55.95	12.40	3.40	9.20	6.060	2.050
SE(±)	0.007	0.050	0.07	0.008	0.030	0.060
CD @5%	0.021	0.170	0.21	0.024	0.110	0.180
CV (%)	0.041	0.640	2.73	0.200	0.130	2.600

4.10 Changes in chemical composition of dried horse gram malts during storage

4.10.1 Moisture

The data on changes in moisture content of dried horse gram malt during in storage were presented in Table 11. The moisture content of dried horse gram malt was reduced from 10.20 to 10.00 per cent. The reduced moisture content in storage might be due to the higher temperature condition of ambient condition.

4.10.2 Ascorbic acid

The data on changes in ascorbic acid content of dried horse gram malt during in storage were presented in Table 11. The ascorbic acid content of dried horse gram malt was increased from 9.50 to 9.66 mg/100g. The increased ascorbic acid in storage might be due to several enzymes become active and bring changes in ascorbic acid content (Masood *et al*., 2014) and increases in the ascorbic acid level considered to be consequence of the reactivation of ascorbic acid biosynthesis undergone in the seeds during germination (Mao *et al*., 2005).

4.10.3 Protein

The data on changes in protein content of dried horse gram malt during in storage were presented in Table 11. The protein content of dried horse gram malt was reduced from 22.88 to 22.00 per cent. The reduced protein content in storage might be due to an increase in proteolytic activity during malting it leads to hydrolysis of protein resulting releasing of amino acids (Chavan and Kadam, 1989).

4.10.4 Total sugars

The data on changes in total sugars content of dried horse gram malt during in storage were presented in Table 11. The total sugars content of dried horse gram malt was increased from 7.30 to 7.50 per cent. The increased total

sugars content in storage might be due to complex sugars converted into simple sugars and reduced moisture content (Chavan and Kadam, 1989).

4.10.5 Iron

The data on changes in iron content of dried horse gram malt during in storage were presented in Table 11. The iron content of dried horse gram malt was increased from 7.40 to 7.51 mg/100g. The increased iron content in storage might be due to the reduced in phytic acid content in malting (Kadam and Salunkhe, 1985).

4.10.6 Trypsin inhibitors

The data on changes in trypsin inhibitors content of dried horse gram malt during in storage were presented in Table 11. The trypsin inhibitors content of dried horse gram malt was reduced from 50.55 to 50.42 U/g. The decrease trypsin inhibitors content in storage might be due to the trypsin enzymes metabolism (Malomo *et al*., 2011).

4.10.7 Phytic acid

The data on changes in phytic acid content of dried horse gram malt during in storage were presented in Table 11. The phytic acid content of dried horse gram malt was decreased from 3.20 to 3.06 mg/g. The decrease in phytic acid in storage attributed to an increase of phytase activities i.e., enzyme would make a solubilization of phytates and would release soluble protein and minerals (Borade *et al*., 1984).

Table 11. Changes in chemical composition of dried horse gram malts during storage

Storage period (days)	Moisture (%)	Ascorbic Acid (mg/100g)	Protein (%)	Total sugars (%)	Iron (mg/100g)	Trypsin inhibitors (TIU/g)	Phytic Acid (mg/g)
0	10.20	9.500	22.88	7.300	7.400	50.55	3.20
30	10.15	9.600	22.45	7.350	7.450	50.49	3.15
60	10.10	9.630	22.30	7.450	7.480	50.45	3.11
90	10.00	9.660	22.00	7.500	7.510	50.42	3.06
SE(±)	0.007	0.007	0.007	0.007	0.007	0.007	0.007
CD @5%	0.021	0.021	0.021	0.021	0.021	0.020	0.021
CV (%)	0.130	0.147	0.063	0.191	0.189	0.030	0.451

4.11 Changes in phytochemicals of dried horse gram malt during storage

4.11.1 Total phenolics

The data on changes in total phenolics content of raw horse gram, malted horse gram malt and changes during in storage of total phenolics content were presented in Table 12. The total phenolics content ranged from 1.50 to 1.44 GAE/100g. The decrease in total phenolics content during malting and in storage might be due to the binding of polyphenols with other organic substances such as carbohydrate or protein (Saharan *et al.*, 2002). Apart from that, during the period of soaking prior to germination, the enzyme polyphenol oxidase may be activated, resulting in degradation and consequent losses of polyphenols (Saxena *et al.*, 2003; Khandelwal *et al*., 2010).

4.11.2 Total tannins

The data on changes in total tannins content of raw horse gram, horse gram malt and changes during in storage of total tannins content were presented in Table 12.The total tannins content ranged from 0.103 to 0.94 TAE/100g. The reduction in tannin content during malting and in storage might be due to the result of formation of hydrophobic association of tannins with seed proteins and enzymes. In addition, loss of tannins during germination also may be due to the leaching of tannins into the water (Shimelis and Rakshit, 2007) and binding of polyphenols with other organic substances such as carbohydrate or protein (Saharan *et al.*, 2002).

4.11.3 Total flavonoids

The data on changes in total flavonoids content of raw horse gram seeds, horse gram malt and changes during in storage of total flavonoids content were presented in Table 12. The total flavonoids content ranged from 0.032 to 0.027 QE/100g. The decreased total flavonoids during malting and in storage might be due to the soaking period prior to germination, the enzyme polyphenol

oxidase may be activated, resulting in degradation and consequent losses of polyphenols (Saxena *et al.*, 2003; Khandelwal *et al*., 2010).

4.12 Changes in microbial count of dried horse gram malt during storage

The microbial studies of dried horse gram shown in below. Microbial studies were carried out at initially and after 90 days of storage. The low microbial count recorded at the end of storage. Packaging in HDPE (50 micron) pouches recorded significantly lower bacterial count during storage.

Treatment	**Standard plate count (-x10^{3}cfu/ml)**	
	Initial	**Final**
A_1T_8C	0	2

Where,

A_2- Plain water + 0.1% KMS

C-Cabinet drying at 60˚C ± 2˚C

	Steeping time (hr)	Malting time (hr)	Malting temperature (˚C)
T_8	12	24	30

4.13 Effect of storage on quality of dried horse gram malt

The dried horse gram malt was stored in HDPE (25 micron) pouches were shown in Plate 4. The samples stored for three months and dried malted horse gram malt was assessed for chemical analysis for interval of 30 days. The effect of storage on chemical composition of dried horse gram was presented in Table 11&12. These results were cognate with results of horse gram (Kasat, 1988) and moth bean (Pisal,1997).

Table 12. Changes in composition of phytochemicals in dried horse gram malt during storage

Phytochemicals	Raw horse gram	Fresh malt	0 days	30 days	60 days	90 days
Total phenolics (GAE/100g)	1.500	1.490	1.480	1.460	1.450	1.440
Total tannis (TAE/100g)	0.103	0.980	0.970	0.960	0.950	0.940
Total flavonoids (QE/100g)	0.032	0.027	0.026	0.025	0.024	0.027
SE(±)	0.003	0.005	0.005	0.005	0.005	0.005
CD @5%	0.011	0.015	0.015	0.015	0.015	0.015
CV (%)	1.747	1.605	1.619	1.640	1.654	1.618

5. SUMMARY AND CONCLUSIONS

The present research work carried on standardization of process for preparation of malt from horse gram was undertaken to standardize various pretreatments and their combinations, to improve quality of malt and study the effects of storage on keeping quality of dried horse gram malt. In order to improve the quality of malt from horse gram, the seeds were subjected to different pretreatments. The results are summarized as below:

1. The horse gram seeds were steeped for 4, 8 and 12 hr containing 0.1% KMS and 0.5% Na_2HCO_3 fallowed by malting period 18, 24 and 30 hr and malting temperature 25, 30 and 35°C respectively. The horse gram seeds were steeped in plain water containing 0.1% KMS and 0.5% Na_2HCO_3 for 4 and 8 hr malting period with malting temperature 25 and 30°C these treatments are insufficient and uneven malting. However, the treatments were steeped in plain water containing 0.1% KMS for 12 hr and malting period for 24 hr with malting temperature at 30°C is appropriate, best and superior as compare other treatments with respect to malting (%) and length of acrospires.
2. It was observed that horse gram seeds were steeped in plain water 12 hr containing 0.1% KMS with malting for 24 hr and malting temperature at 30°C fallowed by drying is done at 60°C in a cabinet drier but it will took less rehydration time as compare to other treatments. However, this treatment was superior as compare to other treatments with respect to malting characteristics and organoleptic parameters such as percentage of malted grains, length of acrospires colour, texture, flavor and cooking time.
3. Horse gram seeds contains 10.20% moisture, 21.65% proteins, 6.9% total sugars, 7.1% iron, 2.3mg/100 gm ascorbic acid, 94 U/g trypsin

inhibitors, 10.2 mg/g phytic acid, 1.5 GAE/100g total phenolics, 0.13 TAE/100g tannins and 0.032 QE/100g total flavonoids. The steeping of horse gram seeds for 12 hr in plain water and malting for 24 hr followed by 30°C malting temperature and mechanical drying at 60°C were found to be the best conditions for preparation of good quality dried malt.

4. The chemical analysis of raw seeds and horse gram malts showed that there was a negligible change in protein content on malting but the protein become easily digestible because of breakdown of complex protein into simple form. After malting, total sugars, iron and ascorbic acid slightly increased. The protein, total sugar and ascorbic acid in rehydrated malts lower as compare to that of malts due to regaining of moisture content. However, trypsin inhibitors and phytic acid was decreased after malting might be due to the to possibility that the trypsin inhibitors and phytic acid could be utilized as an energy source during the early stages of malting. However, the phytochemical content of dried horse gram slightly decreases during malting and in storage due to the leaching of tannins into the water and binding of polyphenols with other organic substances such as carbohydrate or protein.
5. The drying ratio of dried horse gram malt was 1:0.90. The recovery of dried horse gram malt was 90% of initial weight of horse gram seeds. Dried horse gram malts on rehydration gave good quality of malts. The appropriate rehydration period was 20 min in boiling water and 3 hr in cold water. The rehydration ratio was 1:0.98.
6. The dried malts were stored in HDPE (25 micron) pouches for period of three months. There was a slight change in the nutritional composition during storage for 90 days. The simple convenient packaging like HDPE (25 micron) pouches can be used for storage of dried horse gram malt.

7. After assessing the nutritional and rehydration properties of dried horse gram malt, it can be concluded that marketable horse gram malt can be prepared by following appropriate technology of steeping in water for 12 hr containing 0.1% KMS for 24 hr malting period and at 30˚C in incubator fallowed by drying at 60˚C in cabinet drier .
8. Dried malt can be used for vegetable purpose after rehydration form and it can also used for bakery industry in powder form. The malt had high nutritional valve as compared to raw horse gram.

Conclusion

From the above discussion it can be concluded that a good quality malt can be prepared by steeping of horse gram seeds for 12 hr containing 0.1% KMS with 24 hr malting period and at 30˚C in incubator fallowed by drying at 60˚C in cabinet drier without affecting their overall quality. The storage studies, indicated that dried horse gram malt could be stored up to 90 days at ambient conditions without affecting sensory quality.

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***** Originals not seen.

8. VITA

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of

MASTER OF SCIENCE

in

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2016

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