Optimization of Malting Conditions of Sorghum (*Sorghum bicolor*)

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(*Sorghum bicolor*)

by

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Optimization of Malting Conditions of Sorghum (*Sorghum bicolor*)

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Approval Letter

This *dissertation* entitled *Optimization of Malting Conditions of Sorghum (Sorghum bicolor)* presented by Pankaj Dahal has been accepted as the partial fulfillment of the requirement for the B. Tech. degree in Food Technology

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Abstract

A study was carried out to known about the effects of malting on physical, chemical and functional properties of sorghum. Design expert (Central composite rotatable design) was used to design the experiment for different temperature (20-30°C), relative humidity (70-95%) and time (3-6 days) for germination period for all three factors. Grains were steeped for 24 h at 28°C, at last grains were dipped in KMS solution for 10 min to prevent the mold growth during germination. After steeping the grains were germinated for pre-determined malting condition. Then, the germinated grains were dried in cabinet dryer at 50°C for 24 h to obtain desired moisture content. The dried malt samples were then taken for analysis. Analysis of physical, chemical and functional properties of both grain and malt samples were performed and malting condition was optimized on the basis of reducing sugar, starch content and amylase activity.

The 1000 kernel wt., bulk density and particle density were found to decrease after malting from 29.91 g to 18.52 g, 82.33 kg/HL to 66 kg/HL and 1.23 g/ml to 0.94 g/ml respectively. While porosity of grain varies after malting from 0.26 to 0.35. Protein, starch, crude fiber, crude fat and moisture content were found to decrease after malting from 12.8% to 10.05%, 69.77% to 51.16%, 1.13% to 0.95%, 4.71% to 2.25% and 10.37% to 6.93% respectively. While Ash content, reducing sugar and amylase activity were increased after malting from 1.45% to 2.96%, 1.04% to 5.14% and 0.00193 mg/g sample/15 min to 41.33 mg/g sample/ 15 min. The water absorption capacity, foam stability and swelling power increased after malting from 1.22 g/g sample to 1.80 g/g sample, 10.39% to 18% and 0.87 g/g sample to 3.08 g/g sample respectively while gelatinization concentration decreased after malting from 12% to 6%. Optimization of malting condition was carried out on the basis of amylase activity, reducing sugar and starch content by keeping maximum reducing sugar and amylase activity and minimum starch content. The optimum malting condition was found as 20°C, 95% RH for 5 days.

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List of Abbreviations

Abbreviation	Full form
ANOVA	Analysis of Variance
CCRD	Central composite rotatable design
DP	Diastatic Power
FAN	Free Amino Nitrogen
FAO	Food and Agriculture Organization
KMS	Potassium metabisulphite
NRC	National Research Council
NSI	Nitrogen Solubility Index
RH	Relative Humidity
RSM	Response Surface Methodology
WAC	Water Absorption Capacity

Part I

Introduction

1.1 General introduction

Sorghum [*Sorghum bicolor* (L) Moench] is the fifth most important cereal crop in the world; however, it has a wide range of other applications that are being explored with worldwide interest in renewable resources (Dahlberg *et al.*, 2011). Having been domesticated for a variety of useful products and cultivated in a broad range of environments, sorghum exhibits a great range of phenotypic diversity. Around the world, sorghum is grown for the production of dense grain panicles (for food, feed, and/or energy), tall, thick sweet stalks (for food, feed, and/or energy), and various forage types (for feed and fuel) (Kimber *et al.*, 2013). The sorghum kernel varies in color from white through shades of red and pale yellow to deep purple-brown. The most common colors are white, bronze and brown. Kernels are generally spherical but vary in size and shape. The caryopsis can be rounded and bluntly pointed, 4 to 8 mm in diameter. The grain is partially covered with glumes (FAO, 1995).

Large grains with carotene and xanthophyll increases the nutritive value of sorghum according to FAO (1995). The crop is rich in minerals but with bioavailability vary from less than 1% for some forms of iron to greater than 90% for sodium and potassium. The reasons for this are varied and complex, since many factors interact to determine the ultimate bioavailability of a nutrient. Like other grains, sorghum protein is generally low in the essential amino acids such as lysine and therionine. Sorghum, like legume and oil seed meals has some limitations, due to the presence of antinutritional factors, such as trypsin and amylase inhibitors, phytic acid, and tannins. These compounds are known to interfere with protein, carbohydrates and mineral metabolism (Mohammed *et al.*, 2011).

Malting is a biotechnological technique which involves the controlled germination of a cereal grain which aims at activating enzyme systems that catalyze the hydrolysis of polymerized reserved food materials, notably, proteins, starches and cell-wall substances, thus, extracting fermentable materials (Gautam *et al.*, 2015). The goal of malting in brewing is to transform the food reserves of the grain, such that they can be dissolved and extracted by hot water during the mashing stage to produce wort, which is an aqueous solution of fermentable carbohydrates and soluble protein. The extent of transformation or modification

of the grain during malting is influenced by the sprouting conditions to which the grain is exposed. It has been established that malting conditions such as steeping schedule, duration of germination, drying temperature (kilning) and sorghum cultivar influence the quality characteristics of malt (Djameh *et al.*, 2015).

Sorghum has been malted for centuries and is used for the production of baby food and traditional alcoholic and nonalcoholic beverages. Both α- and β-amylases are needed to hydrolyze starch and produce fermentable sugars in these processes. However, improvements and standardization of malting procedures and of malt evaluation techniques need to be made. Sorghum is commercially processed by pneumatic and floor malting methods in southern Africa. Malting conditions (moisture, temperature, time) are better controlled during pneumatic malting; the malt obtained is more uniform and better quality than that obtained from floor malting (Beta *et al.*, 1995).

1.2 Statement of the problem

The main objective of malting is to promote synthesis of hydrolytic enzymes which will solubilize large macromolecules into low molecular weight compounds of desired characteristics. The malting process involves germination and growth resulting in the development of enzymes which degrade the reserved food materials in the endosperm of the grain. Malting induces important beneficial biochemical changes in sorghum grains especially. Indeed, soaking/steeping generates grain softening and increases water availability. The enzymes produced during germination lead to the hydrolysis of starch and proteins with release of sugars and amino acids directly available (Milala and Addy, 2014).

Sorghum malting for brewing process had been carried out for several years in many countries. But scientific research on the malting at different temperature and relative humidity for various period of time has not been carried out yet. So, a detailed study on change in chemical composition and the amylase activity due to malting of sorghum at different temperature and relative humidity should be done in order to optimize the malting process of sorghum.

1.3 Objectives

1.3.1 General objective

The general objective is to optimize the malting conditions of sorghum.

1.3.2 Specific objectives

The specific objectives were to:

i. To perform the malting of sorghum at different condition.
ii. To study the physical properties, functional properties and chemical composition at different condition.
iii. To optimize the malting process in accordance to the reducing sugar, starch content and α-amylase activity.

1.4 Significance of the study

Sorghum is one of the gluten free product and contain less protein as compared to barley. A major objective of malting is to promote the development of hydrolytic enzymes, which are not present in the non-germinated grain (Taylor and Belton, 2002). The amount of reducing sugar increases in the malted flour sorghum varieties. Sorghum in vitro digestion studies show that malting caused an improvement in protein digestibility and other protein quality characteristics, including percentage of protein, nitrogen solubility index and content of the first limiting amino acid and lysine (Mella, 2011).

Other benefits of the malting process include increased vitamin C content, phosphorus availability, and synthesis of lysine and tryptophan. Also during malting, both starch and protein are partially degraded allowing for better digestibility. Furthermore, amylases are elaborated and as a result, the viscosity of gelled starch decreases (Mella, 2011).

1.5 Limitations

i. Only one variety of sorghum *(Sorghum bicolor* var. L. Moench*)* was used for malting.

ii. Relative humidity was maintained in desiccator using sulphuric acid solution which may less effective than humidity chamber.

Part II

Literature review

2.1 Sorghum

Globally speaking, sorghum is the dietary staple of more than 500 million people in more than 30 countries. Only rice, wheat, maize, and potatoes surpass it in the quantity eaten. For all that, however, it produces merely a fraction of what it could. Indeed, if the 20th century has been the century of wheat, rice, and maize, the 21st could become the century of sorghum (*Sorghum bicolor*) (NRC, 1996). It belongs to family Poaceae family, tribe Andropogoneae, subtribe Sorghinae, genus *Sorghum* (Smith and Frederiksen, 2000).

2.1.1 Origin and historical background

Sorghum was originated in north east Africa. Domestication of sorghum started in Ethiopia and Egypt around 3000 years back. It progenitor races of durra, guinea and caffra were found to grow wild in different parts of Eastern, Western and Central Africa. In India, it might have been introduced through early sailors well before the Christian era but not earlier to 1500 BC. It was introduced to America and Australia about 125 years back, and Mediterranean/Middle Eastern countries started sorghum cultivation 300 years back (Joshi, 2015).

Sorghum (Holcus) *saccharatum* (Halapense andropogon), a variety of *S. vulgare*, or of the Durrha, is the sugarcane of Northern China and by the Zulu Kaffirs called Imphee. The Japanese cultivate it only for the sugar and the alcohol, and for the purpose it is now cultivated in the United States (Collier, 1884).

The common Sorghum (vulgare) is the principle grain food in Africa. It is used to make bread, or eaten as musb. It is the principle nutriment in many parts of India, where it is called Jovari, and in the dry regions of Arabia, in Syria, where it has been cultivated since time immemorial. Sorghum bicolor is cultivated in Abyssinia at 8000 feet above the sea (Collier, 1884).

Sorghum has not yet attracted the same amount of research as the other major cereals, wheat, rice and corn. However, with rapidly increasing interest in its food and industrial properties, cultivation of this crop, ranking fourth amongst cereals in world production, is

likely to expand. In addition to their important sources of animal fodder and building materials (Nesbitt, 2012).

2.1.2 Production of sorghum

The total production of sorghum in the world in 1990 was 58 million tonnes, a decrease from 60 million tonnes in the year 1989 and 62 million tonnes in 1988. A decrease in yield from 1340 kg/ha in 1989 to 1312 kg/ha in 1990 was reported, while the area remained around 44 million hectares in both years. Table 2.1 provides data on area, yield and production of sorghum in various regions of the world (FAO, 1995). In 2007, the world planted 43.8 million hectares of sorghum, with over 80% of the area devoted to the crop being found in Africa and Asia (FAO, 2008).

An estimation of the world-wide tonnage produced in 2007-2008 is shown in Table 2.1.

Table 2.1 Area, yield and production of sorghum by region

Countries	Production (tones x 1000)	% of total
United states	12,827	20
Nigeria	10,000	16
India	7,780	12
Mexico	6,100	10
Sudan	4,500	7
Ethiopia	3,230	5
Argentina	2,900	5
Australia	2,691	4
China	1,900	3
Burkina faso	1,800	3
Brazil	1,700	3
Other countries	6,800	12
Total	62,308	100

Source: U.S. Grain Council (2008)

The five largest producers of sorghum in the world are the United States (25%), India (21 .5%), Mexico (almost 11%), China (9%) and Nigeria (almost 7%). Together these five countries account for 73% of total world production (FAO, 1995).

2.1.3 Structure of the sorghum grain

Examination of a microscopic view of a section through a mature sorghum kernel helps to divide the kernel between the outer seed cover or pericarp, the embryo or germ, and the endosperm (Wall and Blessin, 1969). The structure of sorghum is shown in Fig. 2.1.

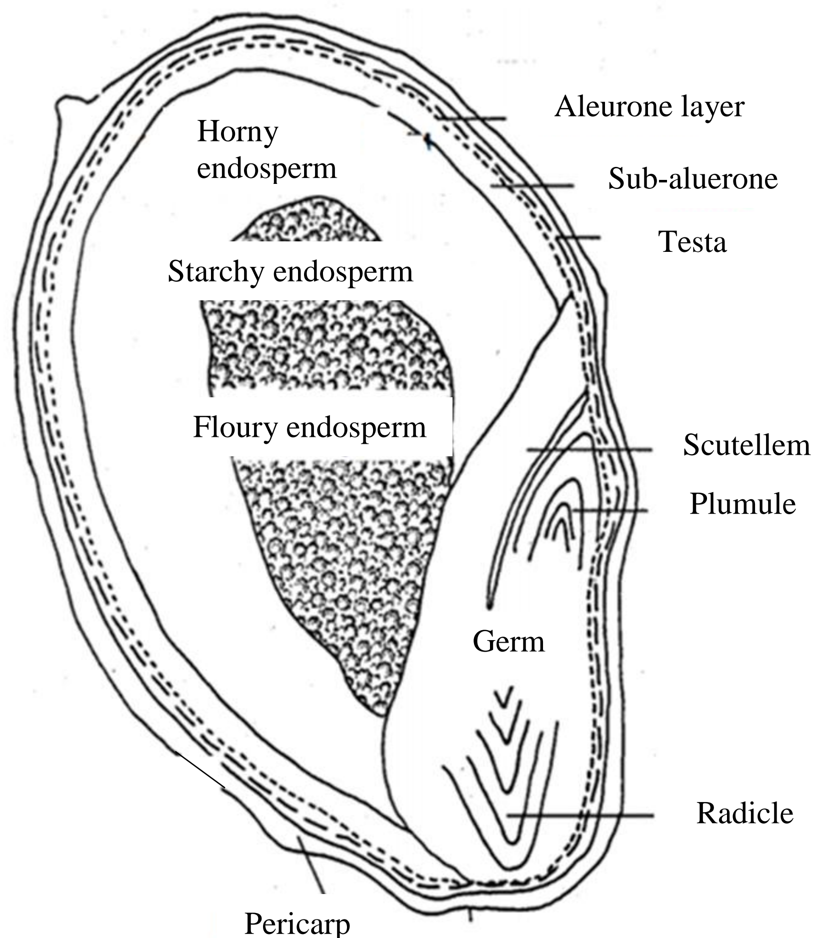

Fig. 2.1 Structure of Sorghum grain .

Source: Sautier and O'Deye (1989)

2.1.3.1 Pericarp

The pericarp constitutes 4.3% to 8.7% of the sorghum caryopsis (Waniska and Rooney, 2000). It has a thickness of 8 to 160 μm varying within individual mature caryopses (Earp,

McDonough, and Rooney, 2004).It is subdivided into three tissues: epicarp, mesocarp and endocarp. The epicarp is covered with a thin layer of wax and is usually pigmented. The sorghum mesocarp contains starch granules, a characteristic unique to sorghum (Serna-Saldivar *et al.*, 1994). The tube cells, which are part of the pericarp, conduct water during germination while, the cross cells form a layer that impedes moisture loss. The pericarp contains approximately 5% to 8% of the grain protein (Waniska and Rooney, 2000).

2.1.3.2 Testa

Some sorghum cultivars have pigmented sub-coat (testa) located between the pericarp and the endosperm as shown in Fig. 2.1 (Earp, *et al.*, 2004) . The pigmented testa contains tannins (proanthocyanidins) (Waniska and Rooney, 2000). Tannins protect the grain against insects, birds and fungal attack but condensed tannins are associated with nutritional disadvantages and reduced food quality (Serna-Saldivar and Rooney, 1995). The nutritional disadvantages of sorghum tannins lie primarily in their ability to form poorly digestible complexes with dietary protein (Butler *et al.*, 1984).

2.1.3.3 Aluerone layer

The endosperm consists of an outer single-cell layer of aleurone tissue. Aleurone cells are rich in oil, protein, and ash (Wall and Blessin, 1970).

2.1.3.4 Endosperm

The endosperm constitutes 82% to 87% of the sorghum grain (Waniska and Rooney, 2000). It is composed of peripheral, and floury and corneous (horny, vitreous, glassy) areas as shown in Fig. 2.1 (Serna-Saldivar *et al.*, 1994). The peripheral region has several layers of dense cells containing more protein bodies and smaller starch granules than the corneous area. The peripheral and corneous areas affect processing and nutrient digestibility (Waniska and Rooney, 2000). In a review of the composition of the sorghum endosperm cells, Taylor *et al.* (2006) noted that both the floury and corneous endosperm cells are composed of starch granules, protein matrix, protein bodies and the cell walls are predominated by water insoluble glucuronoarabinoxylans (GAX). The endosperm contains approximately 81% of sorghum protein (Waniska and Rooney, 2000). In normal sorghum cultivars, most of the proteins in the endosperm are prolamin (soluble in alcohol-water mixtures) as well as some

limited amounts of glutelins (soluble in dilute acid and dilute alkali) (Taylor and Schussler, 1986).

To understand the phenomenon of sorghum endosperm hardness and factors responsible for it, Shull *et al.* (1990) observed the differences in developing sorghums of varying hardness. They found that corneous sorghum endosperm had more and evenly distributed proteins. Similarly, in a review of biochemical basis and implications of hardness and grain strength in sorghum and maize, Chandrashekar and Mazhar (1999) noted that the protein bodies in the corneous endosperm contained more γ-prolamin, which seemed to be cross-linked by disulphide bonds, than in soft grains. These authors suggested that the amounts of α- and γ- prolamin relative to the total prolamin content may be essential for corneous texture, in which these prolamin are usually higher in hard grains than in soft grains. Furthermore, Ioerger *et al.* (2007) investigated the role of cross-linking of sorghum storage proteins (kafirins) into larger polymeric groups in influencing grain hardness. They used a number of protein analytical techniques to study the protein composition of isolated corneous and floury endosperm. These authors found that corneous endosperm had a greater level of kafirin crosslinking than did floury endosperm and that the cross-linking produced a larger molecular weight distribution than in the floury endosperm. These workers also reiterated that the γ-kafirins in the corneous endosperm may have the most obvious relationships to indicators of kafirin cross-linking in the corneous endosperm (Ioerger *et al.*, 2007).

Reviewing the traditional food applications of sorghum, Murty and Kumar (1995) reported that sorghum endosperm texture determines the food making properties of sorghum. However, there are differing reports on the preferences for traditional sorghum foods based on the endosperm hardness. For example, Bello *et al.* (1990) and Da *et al.* (1982)found that to (a West African thick porridge) prepared using corneous endosperm sorghum produced desirable firmer texture than floury endosperm sorghum. On the other hand, Fliedel (1995), working on tuwo and Aboubacar *et al.* (1999) using tuwo (a sorghum porridge consumed in Niger) did not find any correlation between thick porridge firmness and endosperm texture. However, there is a consensus that corneous endosperm sorghum is not suitable for preparation of fermented and unfermented flatbreads as it produces undesirable stiffer bread (Rooney *et al.*, 1988; Yetneberk *et al.*, 2004).

2.1.3.5 Germ

The germ is the living part of the sorghum grain. It consists of two main parts: embryonic axis and scutellum as shown in Fig. 2.1. The embryonic axis contains the new plant. During germination and development the radicle forms the primary roots while the plumule forms the shoot (Evers and Millar, 2002). The scutellum is the cotyledon and has reserve nutrients: moderate quantity of oil, protein, enzymes, and minerals, doubling up as a link between endosperm and germ (Waniska and Rooney, 2000). The germ contains approximately 15% of the protein in sorghum. It is rich in albumin (water-soluble) and globulins (soluble in dilute salt solution) which are rich in lysine and other essential amino acids (Taylor and Schussler, 1986).

2.1.4 Utilization of sorghum

The traditional method of consumption as a food grain staple (roti, porridge, or mixed with rice) continue to dominate sorghum use for some time, particularly in India, Pakistan, and Burma. But more importantly, sorghum use (and its perception) as a source of feed for livestock and poultry has developed rapidly during recent times. Presently, a little less than 20% of the sorghum produced in Asia goes for animal feed (Kelley *et al.*, 1992).

Grain sorghum has long been a potential source of industrial raw material. The grits obtained from the endosperm can be used in brewing, just as corn grits and broken rice are now used. Until methods of milling that permitted a satisfactory separation of the germ from the endosperm were developed in 1947, sorghum grits were too high in oil for the best use in fermentation industries. Most of the oil of the grain is in the germ. The oil is suitable for salad oils. The starch from grain sorghum can be used for food products, adhesives, and sizing for paper and fabrics (Martin and Macmasters, 1950-1951).

2.1.4.1 Human food

While total food consumption of all cereals has risen considerably during the past 35 years, world food consumption of sorghum has remained stagnant, mainly because, although nutritionally sorghum compares well with other grains, it is regarded in many countries as an inferior grain. Per caput consumption of sorghum is high in countries or areas where climate does not allow the economic production of other cereals and where per caput incomes are relatively low. These include especially the countries bordering the southern

fringes of the Sahara, including Ethiopia and Somalia, where the national average per caput consumption of sorghum can reach up to 100 kg per year. Other countries with significant per caput consumption include Botswana, Lesotho, Yemen and certain provinces in China and states in India. In most other countries, food consumption of sorghum is relatively small or negligible compared to that of other cereals (FAO, 1989).

More than 95% of total food use of sorghum occurs in countries of Africa and Asia. In Africa, human consumption accounts for almost three-quarters of total utilization and sorghum represents a large portion of the total calorie intake in many countries. For example, in Burkina Faso about 45% of the total annual calorie intake from cereals comes from sorghum, although its share has declined from 55% in the early 1960s. China and India account for about 90 % of total food use in Asia (FAO, 1989).

Available data from Africa indicate that despite an increase in total food use between the early 1960s and the mid-1980s, the average per caput consumption declined from 20 to 15 kg per year. Decreases were concentrated in Kenya, Mozambique, Nigeria and Somalia but occurred also in Botswana, Ethiopia, Lesotho and Zimbabwe. In Asia, both total and per caput food use of sorghum declined (FAO, 1989).

This decline in per caput consumption in many countries was due in part to shifts in consumer habits brought about by a number of factors: the rapid rate of urbanization, the time and energy required to prepare food based on sorghum, inadequate domestic structure, poor marketing facilities and processing techniques, unstable supplies and relative unavailability of sorghum products, including flour, compared with other foodstuffs. Changes in consumption habits were concentrated in urban areas. Per caput food consumption of sorghum in rural producing areas remained considerably higher than in the towns. In addition, national policies in a number of countries had a negative influence on sorghum utilization as food. For instance, large imports of cheap wheat and rice and policies to subsidize production of those crops in some countries had considerable negative impact on the production of sorghum (FAO, 1989).

2.1.4.2 Alcohol distilleries

Although the quantity of sorghum grain presently used by the alcohol sector is comparatively low, it seems to be the most "enthusiastic" user of the crop as an industrial raw material. With recent changes in government policies on licensing alcohol production and trade, the

use of grains to produce potable alcohol is being promoted, thereby providing an opportunity for sorghum to gain greater acceptability as a raw material in the industry (Kleih *et al.*, 2007).

There are few complaints about sorghum, although some distillers indicated a preference for varieties with a higher starch content and less protein. Distilleries had no objection to using severely blackened grain as long as the starch content was acceptable (Kleih *et al.*, 2007).

In general, like most other industrial users, distilleries purchase rainy-season sorghum through traders or brokers in main producing centers. Though there were few complaints about this system, some distillers felt that brokers sometimes abused their position to "control" the market. In this context, contract farming may be an option providing better linkages between producers and industrial users (Kleih *et al.*, 2007).

2.1.4.3 Animal feed sector

While discussing sorghum utilization for animal feed in India, one has to distinguish between poultry and dairy production. Although the latter has a solid foundation in the co-operative sector, the poultry industry appears to be more dynamic. According to poultry producers and feed millers, very little sorghum was used in poultry feed in 1998/99 due to the availability of maize and its price advantage. Nevertheless, it was acknowledged that in the past, when maize was expensive, sorghum had been used at an inclusion rate of up to 10% in the case of broilers and up to 15% in the case of layers. The demand for sorghum in poultry feed largely depends on the price of maize, which is the energy source preferred by poultry producers. According to industry sources, to make sorghum competitive, its price should be 20 - 30% lower than that of maize (Kleih *et al.*, 2007).

2.1.4.4 Starch industries

Some of the India's main starch manufacturers, who are primarily based in Ahmedabad, have used up to 50000MT of sorghum in the past when maize was in short supply. Starch producers have even undertaken their own research into sorghum-based starch manufacturing technologies, and their conclusion was that sorghum was not a preferred raw material and would only be used if there were no alternatives (Kleih *et al.*, 2007).

2.1.4.5 Other industries

Although beer brewers are aware of sorghum-based beer production in Africa, they prefer barley malt as the principal raw material. In addition, broken rice or flaked maize are used as adjuncts. However, one brewery (i.e., Hindustan Breweries in Mumbai) expressed interest in undertaking trials using sorghum as an adjunct (Kleih *et al.*, 2007).

With the exception of a small market for speciality breads in urban centers, sorghum is not accepted as a raw material for industrial food processing. Wheat flour or maize starch are the preferred ingredients. Composite flours do not currently appear to be an option in bread baking or biscuit manufacturing (Kleih *et al.*, 2007).

Export of sorghum does not appear to be an option for the time being. Moreover, Indian sorghum at present is not globally competitive and export quotas for coarse grains are usually taken up by maize (Kleih *et al.*, 2007).

2.1.5 Physical properties

Sorghum is a naked kernel, free from hull. In terms of size and shape, sorghum varieties differ widely. The average dimensions of a sorghum caryopsis (grain) are length 4 mm, width 2 mm and weigh about 25 to 35 mg (Haussmann *et al.*, 1999). The shape varies from obovoid to ellipsoid with 1000 kernel weight varying from 20 to 80 g (Serna-Saldivar and Rooney, 1995). The mean particle and bulk density of sorghum grain obtained were 1.02 g/cm^3 and 568.5 g/cm^3 respectively. The particle density of sorghum grain decreased with increasing moisture within the moisture range of 8.89-16.50% wb (Simonyan *et al.*, 2007). The value of sphericity, 1000 kernel wt., bulk density, particle density (specific gravity), and porosity as 0.67, 32.41 g, 69.9 kg/HL, 1.18 g/cm^3 and 40.80% of sorghum grain (Ndirika and Mohammed, 2005).

Botanically, the sorghum kernel is dry, indehiscent, single seeded fruit. The caryopsis is composed of three major portions: the outer covering (pericarp), the storage tissue (endosperm), and the germ (Johnson and Peterson, 1974).

2.1.6 Chemical composition of sorghum

The sorghum grain has the composition as that of the corn which are as follows.

2.1.6.1 Protein

A typical mature sorghum seed of one of the common hybrids might contain about 15% protein, of which around half would be prolamines, or alcohol soluble proteins, about a third would be glutelin type proteins, 7-9% would be globulins, and the remainder, usually near 5-6%, would be albumin. The tissues differ in their percentage contents of protein, and in the types of proteins which make up the total. There is very little prolamine in the germ and hull, while they predominate in the endosperm. The aleurone layer is rich in albumin and globulins. A major factor affecting the amino acid composition of the proteins is the cultivar, variety and hybrid. Fertilization use and location of the field affect both the content of protein and the amino acid composition (Matz, 1991).

2.1.6.2 Carbohydrate

Carbohydrates other than starch are present only in small amounts. Both waxy and regular types average 1.20% sugars composed of approximately 0.85% sucrose, 0.09% glucose, 0.09% D-fructose and 0.11% raffinose. Sweet variety contain about 2.8% of these sugars (Matz, 1991).

Starch content of sorghum ranges from 61 to 74%. Gelatinization temperature of sorghum starch is quite high compared to those from other cereals. Most sorghum starches start to gelatinize at c. 70-75°C and reach maximum gelatinization far above these temperatures until about 90-97°C (Verbruggen, 1996).

On a 1 kg basis, sorghum starch contain 200–300 g of amylose and 700–800 g of amylopectin, with waxy varieties containing 0–150 g of amylose and 850–1000 g of amylopectin (Li *et al.*, 2015).

2.1.6.3 Lipids

Average oil content of the whole grain is 3.6%, with oil contents of the endosperm, germ, and bran, 0.6, 28.1, and 4.9%, respectively. The endosperm contains 13% of the total oil in the kernel; the germ, 76%; and the bran, 11%. The petroleum ether extract from sorghum bran consists mostly of wax rather than oil (Wall and Blessin, 1970).

Free lipids make up 2-4% of the grain and bound lipids 0.1-0.5%. The oil's properties are similar to those of maize oil. In other words, the fatty acids are highly unsaturated. Oleic and linoleic acids account for 76% of the total (NRC, 1996).

2.1.6.4 Vitamin and minerals

Compared to maize, sorghum contains higher levels of the B vitamin pantothenic acid, niacin, folate, and biotin; similar levels of riboflavin and pyridoxine; and lower levels of vitamin A (carotene). Most B-vitamin are located in the germ. Pellagra—a disease caused by too little niacin in the diet is endemic among certain sorghum eaters (as it is among some maize eaters) (NRC, 1996).

The grain's ash content ranges from about 1-2%. As in most cereals, potassium and phosphorus are the major minerals. The calcium and zinc levels tend to be low. Sorghum has been reported to be a good source of more than 20 micronutrients (NRC, 1996).

2.1.6.5 Polyphenols

The testa and pericarp of sorghum, and especially the birdproof varieties, contain high concentrations of polyphenols. The so called condensed tannins, which are flavonoid oligomers, also known as proanthocyanidins, form the most important group present, with respect to the amount as well as their functional properties, such as chemical defense agents against moulds, insects, and birds. Within birdproof varieties, the contents can vary between 0.36 and 3.48 catechin equivalents (CE; 1 CE = l mg catechine/l00 mg sample). In most white colored varieties and obviously the varieties which do not have a testa, the tannin level has diminished to 0 CE. The condensed tannins show the ability to strongly interact with proteins. This has implications for applications in human or animal nutrition, because digestive or endogenous sorghum enzymes can be inhibited. The polyphenols can be successfully removed by polishing and alkali extraction (Verbruggen, 1996).

The Chemical composition of sorghum whole grain and its fraction is presented in Table 2.2.

Table 2.2 Chemical composition of sorghum whole grain and its fractions

Kernel fraction	% of kernel weight	Protein (%)	Ash (%)	Oil (%)	Starch (%)
Whole kernel	100	12.3	1.67	3.6	73.8
Endosperm	82.3	12.3	0.37	0.6	82.5
Germ	9.8	18.9	10.4	28.1	13.4
Bran	7 9	6.7	2	4.9	34.6

Source: FAO (1995)

2.1.7 Health benefits of sorghum

2.1.7.1 Good source of vitamin, minerals and fibers

Sorghum, like other cereals, is an excellent source of the fat-soluble and B-complex vitamin. Amongst the B vitamin, concentrations of thiamine, riboflavin and niacin in sorghum were comparable to those in maize. The detectable fat-soluble vitamin are vitamin B, E and K. It is also an important source of mineral and amongst them, phosphorus is the most abundant. Minerals and vitamin are located at the pericarp and germ; therefore, refined sorghum products lose part of these important nutrients. Sorghum is also one of the best sources of dietary fiber. Sorghum does not have an inedible hull so that the whole grain could be eaten. This means it supplies even more fiber, in addition to many other crucial nutrients. One serving of sorghum grain contains 12 g of dietary fiber which is 48% of your daily recommended intake. High-fiber content of sorghum is important for digestion, hormone production and cardiovascular health (Anon., 2017b).

2.1.7.2 Sorghum may inhibit cancer tumor growth

Compounds in sorghum called 3-Deoxyanthoxyanins (3-DXA) are present in darker-colored sorgums, and to a lesser extent in white sorghum. Scientists at the University of Missouri tested extracts of black, red, and white sorghums and found that all three extracts had strong antiproliferative activity against human colon cancer cells (Yang *et al.*, 2009).

2.1.7.3 Rich in antioxidant

Sorghum contains polyphenol compounds in its pericarp which have the health-protective effect that is superior to many of the more popular consumed grains, fruits and vegetables. The antioxidant activity of sorghum was even 3-4 time higher than some of other whole grains. Black sorghum is especially rich in antioxidants because of its high content of anthocyanins. The antioxidants found in sorghum has anti-inflammatory, anti-cancer, anti-diabetic effects (Anon., 2017b).

2.1.7.4 Protect against diabetes and insulin resistance

Advanced glycation end products (AGEs) are increasingly implicated in the complications of diabetes. A study from the University of Georgia Neutraceutical Research Libraries showed that sorghum brans with a high phenolic content and high anti-oxidant properties inhibit protein glycation, whereas wheat, rice or oat bran, and low-phenolic sorghum bran did not. These results suggest that "certain varieties of sorghum bran may affect critical biological processes that are important in diabetes and insulin resistance" (Farrar *et al.*, 2008).

2.1.7.5 Sorghum is safe for people with celiac disease

Up to one % of the U.S. population (and about 0.5% worldwide) is believed to have Celiac Disease, an autoimmune reaction to gluten proteins found in wheat, barley and rye. While sorghum has long been thought safe for celiacs, no clinical testing had been done until researchers in Italy made a study. First, they conducted laboratory tests; after those tests established the likely safety, they fed celiac patients sorghum-derived food products for five days. The patients experienced no symptoms and the level of disease markers (anti-transglutaminase antibodies) was unchanged at the end of the five day period (Ciacci *et al.*, 2007).

2.1.7.6 Sorghum may help to manage cholesterol

Scientists at the University of Nebraska observed that sorghum is a rich source of phytochemicals, and decided to study sorghum's potential for managing cholesterol. They fed different levels of sorghum lipids to hamsters for four weeks, and found that the healthy fats in sorghum significantly reduced "bad" (non-HDL) cholesterol. Reductions ranged from 18% in hamsters fed a diet including 0.5% sorghum lipids, to 69% in hamsters fed a diet

including 5% sorghum lipids. "Good" (HDL) cholesterol was not affected. Researchers concluded that "grain sorghum contains beneficial components that could be used as food ingredients or dietary supplements to manage cholesterol levels in humans" (Carr *et al.*, 2005).

2.1.7.7 Sorghum may help to treat human melanoma

Scientists in Madrid studied the effect of three different components from wine and one from sorghum, to gauge their effects on the growth of human melanoma cells. While results were mixed, they concluded that all four components (phenolic fractions) "have potential as therapeutic agents in the treatments of human melanoma" although the way in which each slowed cancer growth may differ (Gomez-Cordoves *et al.*, 2001).

2.2 Malting

Malted barley, or 'Malt' as it is most commonly known, is a wonderful package of starch, enzymes, protein, vitamin, and minerals plus many other minor constituents that provide the brewer and distiller with their main raw material. 60-65% of the weight of malt is un-degraded starch and malt contains all the key enzymes for starch degradation during the mashing stage of both the brewing and distilling process. These enzymes produce fermentable sugars to supplement the other key nutrients for yeast growth that malt provides. These include amino acids, vitamin, and minerals (Anon., 2017a).

The Malting Process consists of 4 stages which are steeping, germination, kilning and roasting.

a) Steeping

The purpose of steeping is to increase the moisture in the grain from around 12% to approximately 45%. This is achieved through successive immersions and air rests over a period of 2-3 days. During this process, the grain begins to germinate and therefore produces heat and carbon dioxide. In the immersion cycles, the grain is immersed in water and air is blown through the wet grain to keep the level of dissolved oxygen in the water high enough so as to not stifle the developing embryos. In the air rests, the carbon dioxide is removed (Anon., 2017a).

Due to the varying degree of moisture tolerance of the different grains, steeping is a crucial step in the malting process. When the steeping process is complete, all of the grain should be evenly hydrated and show signs of germination (Anon., 2017a).

b) Germination

The Germination phase is the 'control' phase of malting. Germination continues for a further 4-5 days depending on the product type being made. The germinating grain bed is kept at temperature and oxygenated by providing a constant flow of humidified air through the bed at specific temperatures. The grain is turned regularly to prevent rootlets matting and to maintain a loosely packed grain bed. The maltster manipulates the germination conditions to vary the type of malt being manufactured (Anon., 2017a).

c) Kilning

Kilning, the third phase of malting, dries the grain down to 3-5% moisture and arrests germination. Large volumes of hot air are blown through the grain bed. By varying air flows and kiln temperatures, malts of different colors can be produced with varying flavor profiles. At the end of kilning the malt is cooled and the tiny rootlets removed before analysis and storage. The final malt is analyzed extensively according to malt type and customer profile. The malt may be dispatched in bags, in containers or in bulk (Anon., 2017a).

d) Roasting

Roasting is done in 4 distinct stages: steeping, germinating, roasting and cooling. At GWM Malt, grain spends 34-46 h in steep tanks where we aim for a target moisture of 42-44%. The grain is transferred to germination which lasts for around 4 days in Wanderhaufen style streets. This is a semi continuous moving batch germination process. Once germination is complete, the green malt is then transferred to the roasting drum (Anon., 2017a).

The roasting takes place in two roasting drums. The average roasting time is 2.5-3 h with an air on temperatures of up to 460°C. Our roasters take a batch size of 2.4–3.5 tonnes. The roasted malt is then transferred to the cooler and spends 35-60 min there in order to drop the temperature to <15°C and fix the color and flavor compounds. The malt is analyzed before storage and thereafter awaits dispatch to our customers (Anon., 2017a).

The general flow chart for malting is shown in Fig. 2.2.

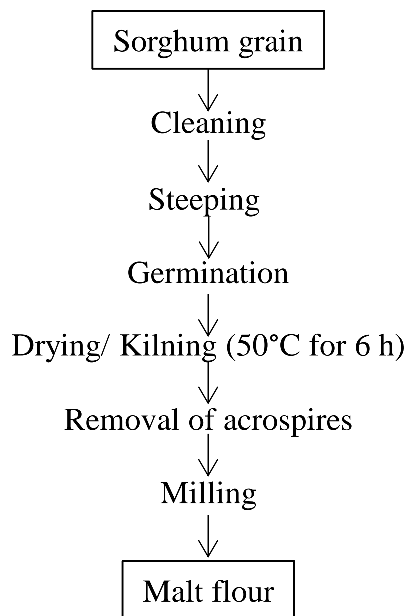

Fig. 2.2 General flow chart of malting

Source: Ratnavati and Chavan (2016)

2.2.1 History of malting

Malt, in substantially the same form as we know it today, was an important product long before the days of recorded history. Although its actual origin is buried in antiquity, there is a legend that early Egyptians manufactured malt by placing it in a wicker basket, which was then lowered into the open wells of that time. It was first lowered into the water for steeping, after which it was raised above the water level for germination (Anon., 2018).

The rate of germination was controlled by adjusting the height of the basket within the well. As germination progressed and heat developed, the basket would be lowered to a lower temperature level thus retarding growth and dissipating heat. To accelerate germination, the basket was simply raised to a higher level (Anon., 2018).

The malt was kept from matting by raising it to the top of the well and agitating the basket. Drying was by natural means, probably a simple process of spreading on the ground, and subjecting it to the direct rays of the sun. The use of malt at this time was thought to be exclusively for beverage purposes (Anon., 2018).

The making and selling of malts was often controlled, in Nurnberg in 1290 only barley was allowed to be malted, while in Augsberg between 1433 and 1550 beer was only to be made from malted oats. In England malt carried a tax for many years until 1880. By 1588, European settlers in North America were trying to make beer from malted maize. Beer can be brewed from a range of cereals, but by the 17th century beers brewed from barley malt predominated in Europe. By the 17th century floor malting was the method being used to malt larger quantities. Floor malting was the only method of malting in use until the 1850's. In floor malting, steeped barley is laid in piles on tiled or concrete floors and allowed to build up some heat and begin growth. The malt is turned manually with wooden shovels to reduce heat buildup and aerate the grain. This method is very labor intensive and time consuming (Briggs, 1998).

By the 19th century the development of large breweries led to the industrialization of malting and an increase in the size of production units. Pneumatic malting was developed and reached commercial success in the late 1800s. Two Belgian malting engineers; Galland and Saladin are considered to be the fathers of the modern malting equipment. Galland introduced the first aerated rectangular boxes in 1873 and Saladin introduced turning machines in 1880s. Saladin boxes are in common use today (Briggs, 1998).

With the expansion of trade and the discovery of the New World, making beer from barley malt spread across the globe. Currently, approximately 1,400 million hectolitres of beer are brewed annually around the world (Mallett, 2014).

2.2.2 Enzymes involved during malting

2.2.2.1 α-amylase

Carbohydrates consist of simple sugars (monosaccharides and disaccharides), as well as more complex compounds composed of multiple sugar units (polysaccharides), such as starch and cellulose. The starch is generally a mixture of amylose, which is composed of α-1,4-linked glucose polymers (link between C1 and C4 atoms), and amylopectin, which is composed of α-1,4-linked glucose polymers branched by α-1,6-linkages (McDowall, 2006).

Amylase is an enzyme that acts as a catalyst to hydrolyze carbohydrates. The role of amylase in plants is for breaking down starches. Starches are usually processed in this way during seed germination, and turned into sugars. These sugars then provide the main source

of energy for the plant during its early development. Plants are able to store energy from the sun by creating sugars. Amylase assists in the initial development of the plant, before it is able to use energy from photosynthesis. The amylase enzymes begin their role in plant development as the plant's seed begins to germinate, root, and sprout. In a study of the germination of cereal seeds, alpha amylase was found in the aleurone layer. The amylase works to hydrolyze the endosperm starch into usable sugars. These sugars provide the necessary energy for root growth and act as reserve food storage. Amylase in plants is important for the production of healthy shoots, so they form properly (Anon., 2018b).

Alpha-amylase is an essential enzyme in alpha-glucan metabolism, acting to catalyse the hydrolysis of alpha-1,4-glucosidic bonds of glycogen, starch and related polysaccharides. Although all alpha-amylases possess the same catalytic function, they can vary with respect to sequence. In general, they are composed of three domains: a TIM barrel containing the active site residues and chloride ion-binding site (domain A), a long loop region inserted between the third beta strand and the alpha-helix of domain A that contains calcium-binding site(s) (domain B), and a C-terminal beta-sheet domain that appears to show some variability in sequence and length between amylases (domain C). Amylases have at least one conserved calcium-binding site, as calcium is essential for the stability of the enzyme. The chloride-binding functions to activate the enzyme, which acts by a two-step mechanism involving a catalytic nucleophile base (usually an Asp) and a catalytic proton donor (usually a Glu) that are responsible for the formation of the beta-linked glycosyl-enzyme intermediate (Anon., 2018a).

2.2.2.2 β-amylase

Beta-amylase is an exoamylase that hydrolyses α-1,4-glycosidic linkages of polyglucan chains at the nonreducing end to produce maltose (4-O-α-D-Glucopyranosyl-β-D-glucose) during starch degradation (Neuhaus and Schulte, 1996). β-amylase stops hydrolysis 2–4 glucose residues ahead of a branch point on the glucan branches generated by the branch points, or on those that carry other branch points. It is the major enzyme for transient starch granule breakdown in leaves. Lack of β-amylase leads to a starch-excess phenotype in the leaves of potato and Arabidopsis which is caused by a slow rate of degradation of starch over the dark period (Fulton *et al.*, 2008; Scheidig *et al.*, 2002).

Most β-amylases are monomeric enzymes (Thoma *et al.*, 1971). However, that of sweet potato is tetrameric consisting of four identical subunits. Each subunit consists of a large (α/β) 8-barrel region also seen in a-amylase. A smaller, globular region is formed by long loops extending from β-strands. Between these two regions is a cleft believed to open and contain the conserved Glu187. Cys96 is located at the entrance of the (α/β)8-barrel region and is important to inactivation by sulfhydryl reagents (Thoma *et al.*, 1963).

2.2.2.3 β-D-glucanase

Cellulose or (1,4)-β-D-glucan is the major component of plant cell walls (Minic, 2008) while the mixed (1,3)(1,4)-β-D-glucan is present mainly in the cell walls of starchy endosperm (Havrlentova and Kraic, 2006). The (1,3)-β-D-glucans are commonly referred to as callose. During plant growth development it can be found in the cell plate during cell division. Callose deposition is important during pollen development, microsporogenesis or seed germination (Pirselova and Matusikova, 2013). Besides, callose is synthesized between the cell wall and the plasma membrane after exposure of plants to various (a)biotic stresses (Zavaliev *et al.*, 2011).

Callose degradation and thus various plant physiological processes are regulated by the β-glucan endohydrolases namely β-1,3-glucanases (EC 3.2.1.39) (Pirselova and Matusikova, 2013). The β-1,3-glucanases (GH 17) catalyse the hydrolysis of (1,3)-β-D-glucosidic linkages in (1,3)-β-D-glucans but not in (1,3)(1,4)-β-D-glucans (Hoj and Fincher, 1995). The β-1,3-glucanases have widely been studied for their potential to inhibit fungal pathogen (Moravcikova *et al.*, 2004), while recently their importance in plant defence against abiotic stresses has also been proven e.g. low temperature, drought or heavy metals (Pirselova *et al.*, 2011).

Plant β-1,3-glucanases are referred to as “Pathogenesis-related proteins” (PR2). They are grouped into the classes I-IV. The class I are vacuolar basic proteins that are accumulated in mature leaves and roots upon pathogen infection. The classes II and III are acidic extracellular proteins. The class IV is similar to the class II; however, β-1,3-glucanases of the class IV are not inducible upon pathogen attack (Leubnermetzger, 2003; Minic, 2008).

2.2.2.4 Limit dextrinase

Limit dextrinase is one of many enzymes involved in the degradation of starch and starch-derived oligosaccharides. This enzyme specifically attacks the α-1,6-glucosidic linkage, which occurs in α-glucans such as amylopectin, pullulan, and amylopectin /3-limit dextrin, as well as in starch-derived a-limit dextrins (Lee and Pyler, 1983). The hydrolytic action of this enzyme results in the formation of linear α-(1,4)-linked chains that can be extensively depolymerized to glucose and maltose by the combined actions of α- and β-amylases. The limit dextrinase in germinating barley hydrolyzes the α-(1,6) linkages of branched dextrins faster than those in large amylopectin molecules. Mature barley contains low levels of the enzyme but during germination, the activity of the enzyme increases due to de novo synthesis in the aleurone. However, barley contains a heat-stable protein that inhibits the enzyme. This inhibitor decreases in amount during malting, but there is a sufficient amount left in malt to inhibit most of the limit dextrinase. Although purified limit dextrinase has no action on starch granules, it enhances the rate of granule dissolution when combined with the actions of α- or β-amylases (Izydorczyk and Edney, 2003).

2.2.2.5 Pentosanases

The cell wall has a high content (85%) of a linear arabino-(1,4)-β-xylan polysaccharide, there is a little cellulose (8%). Three pentosanases capable of degrading this polymer, viz (1,4)-β-endoxylanase, β-xylanopyranosidase and α-arabinofuranosidase, increase in activity in germinatedgrains and are stimulated by gibberellins in isolated aleurone layers. These enzymes may also play a role in degrading the walls of the endosperm, thus rendering the starch and other reserves more accessible for enzyme attack (Loewus and Tanner, 2012).

2.2.2.6 Proteolytic enzymes

More than 40 endopeptidases have been identified in malt, broadly classified into cysteine-, metallo-, aspartic-, and serine-proteinases (Jones and Budde, 2005). There are also exo-peptidases classifiable into carboxypeptidases and amino peptidases (Sopanen and Mikola, 1975). A substantive reason for the limited action of the endo-peptidases in mashing is the presence of inhibitor proteins. Principal among such inhibitors are lipid transfer proteins that block the cysteine-proteinases (Jones, 2005). Jones and Budde (2005) suggest that 32% of the soluble protein in malt is already in the ungerminated barley form, 46% is released in malting and the rest solubilized in mashing. It was shown that over the pH range 5 to 6.6,

the proteolytic activity of malt can vary more than 7-fold (Jones and Budde, 2005). Various factors may come together in causing the release of the enzyme in an active form during malting (Buttimer and Briggs, 2000). It was recently suggested that serine-proteinases have a key role to play here (Schmitt and Marinac, 2008).

The proteolytic enzyme showed a significantly greater improvement than the cytolytic enzymes, yielding a 57% increase in β-amylase, a 173% increase in limit dextrinase, rapid starch solubilization during mashing and a higher percentage of fermentable sugars in resultant wort. The increases in limit dextrinase and β-amylase promoted by the protease suggest that sufficient proteinaceous inhibitor existed in the wort during mashing to inhibit their activities, leading to the unavailability of β-amylase and especially limiting dextrinase (Hu *et al.*, 2014).

2.2.2.7 Lipase

Lipid metabolism of germinating barley differs from that of other macromolecules. Lipase activity is already present in barley" and the activity is known to increase by over fourfold during malting. Lipoxygenase activity has also been reported to increase during germination of barley. Lipases and phospholipases act in concert with lipoxygenase: the polyunsaturated fatty acids released from triglycerides and phospholipids are preferentially oxidized by lipoxygenase. However, only minor differences exists between barley and malt lipids (Kaukovirta-Norm and S. Laakso, 1993). During the germination period, the triacylglycerols stored in "oil bodies" or "oleosomes" are quickly used up in the production of energy for the synthesis of the sugars, amino acids (mainly asparagine, aspartate, glutamine and glutamate) and carbon chains required for embryonic growth (BarrosI *et al.*, 2010).

2.2.3 Enzyme activation during sprouting

In germination seed, enzymes may arise from two sources:

1. They may be released or activated from existing proteins or

2. They may be synthesized through nucleic acid-directed protein synthesis.

Enzymes of the particular interest are starch degrading enzymes, α- amylase and β-amylase. Germination leads to the production of both enzymes, with α – amylase is formed and the quantity of β-amylase increases; the β-amylases predominating in the final malt.

Generally, the temperate climate cereals, sorghum, wheat and rye produce on germination high diastatic power. The hot climate cereals, sorghum, maize, millets produce much lower diastatic powers. The diastatic power developed during malting depends on (McDonald, 2014)

1. Temperature of malting;
2. The moisture level;
3. The % of germination, germination vigour;
4. Duration of malting; and
5. The cultivar employed.

β-amylase, which normally represents the minor portion of the total amylolytic activity, is resent in the unfermented seed in an inactive form probably attacked in wheat at least by disulphide linkages to gluten in. Its activation is due to its relies from the gluten in with the formation of active SH groups. The mediator of this reaction is unknown. Low temperature kilning schedule produce malts with greater α- amylase activity. According to Dewar *et al.* (1997), germination with the high watering regime gave the highest level of total β-amylase activity. They also reported that α – amylase enzyme is inactivated at the high water regime.

2.2.4 Change on physical structure

During germination enzymes migrate from the germ and partially break down the endosperm starch granules and protein bodies and matrix (Hough, 1985).

During steeping, the grain swells and increases its volume by about a quarter. Space is allowed in the steep tanks to accommodate the swollen grain. The first microscopic indication of germination after casting is the appearance of the 'chit'. The white coleorhizae or root – sheath that breaks through the pericarp and testa and produces from the base of the corn. In time seminal roots also called rootlets, culms, cooms, or malt sprouts bursts, through root sheath and form a tough at end of the grain, at the same time the first 'leaf- seat' or coleoptiles. Variously called by maltsters the 'acrospires', 'spire', 'blade', penetrates the apex between pericarp and the husk. In conventional malting practice, the malt is kilned and growth terminated before the acrospires grows beyond the end of the grain (Hough, 1985).

Starch appears in small amounts in the embryonic structures after the onset of germination. Coincident with the appearance of this starch the first sign of the breakdown of the starchy endosperm are seen as an enzymes partial dissolution of some cell walls. This process, cytolysis, begins in the compressed layer, adjacent to the scutellum and progressively spreads through the starchy endosperm towards the apex of the grains (Hough, 1985).

As these hydrolytic breakdown processes precede alterations may be detected in protoplasm of cells of the aleuronic layer of columnar cells between the compressed cells endosperm and the scutellum. As germination proceeds, the cells of epithelium tend to separate and elongate so forming a 'pile' which projects into the solubilized part of the endosperm. This alteration in similar form greatly increases the surface area of the cells and makes the epithelium a more efficient absorptive organ (Hough, 1985).

Partial dissolution of the cell walls and reduction of starch grains are both characteristics of physical modification of sorghum. 'Modification' progresses form the embryo parallel to the scutellum towards the apex of the grain advancing fastest on the dorsal side of the grain beneath the aleuronic layer (Hough, 1985).

The softening of endosperm that occurs during malting is easily and conveniently detected by 'rubbing out' the green malt by hand. Chewing grains to see that they are 'crunchy' and devoid of hard tips may check the degree of modification of finished malt (Hough, 1985).

Kernel density decreased during malting. Density of malted sorghum correlated with diastatic power and reduction in pasting viscosity (Beta *et al.*, 1995). Physical and biochemical properties such as kernel weight, diastatic properties, malt yield and fermentable sugars are critical in the preliminary evaluation and selection of grain for malting. However, further studies on the optimum modification conditions for the cultivars, friability index of the malts, component fermentable sugars and free amino nitrogen (FAN) need to be undertaken before final certification of the cultivars for malting purposes (Makeri *et al.*, 2013).

2.2.5 Chemical changes during malting

The chemical changes occurring during malting are complex. They can only be understood by appreciating the range of, sometimes conflicting, processes that occur during steeping, germination and kilning, and the effects of deculming and dressing the malt. Polymeric reserve substances, such as starch and proteins, are partly hydrolysed in the endosperm; the low molecular weight degradation products diffuse through the grain. Those reaching the living tissues may be metabolized, together with the reserves of these tissues (e.g. sugars, lipids). The aleurone may release some of its metabolic products. There is a net movement of materials to the embryo where they may be respired, converted into new substances and/or be incorporated into the growing tissues of the acrospire and the rootlets. Thus the synthesis of new, complex molecules (proteins, polysaccharides) in the embryo partly offsets the degradative changes that occur elsewhere in the grain chiefly in the starchy endosperm (Briggs, 1998).

2.2.5.1 Proximate composition

The moisture content increased significantly by 37.13% which is a normal indication of rapid water uptake by a viable grain expected during steeping. This hydration process activated a wide array of enzyme systems which hydrolysed and solubilised food reserves during germination. The moisture content of Sorghum malt was found to be 10.7% for tabat and 10.1% for faterita by (Abbas, 2000). Elshewaya (2003) reported lower moisture content values for tabat malt (3.72%) and faterita malt (4.17%). Bolarinwa *et al.* (2015) also reported the very low moisture content of sorghum malt as 6.76%. While Wall and Blessin (1970) obtained the moisture content and crude fiber content of sorghum grain as 12-14% and 2.7-2.9% respectively.

The crude protein showed an initial significant decrease of 28.53% before a later increase of 0.1%. This may be due to the fact that storage nitrogen reserves may have been mobilized during sprouting after hydrolysis by proteolytic enzymes (which digest the macromolecular proteins into the more easily assimilable peptides and amino acids) to play a role in the synthesis of its cellular materials for the rapidly growing roots and shoots during germination. The carbohydrate content of the malted samples decreased significantly by 24.33%. This significant decrease could be attributed to metabolism (Ogbonna *et al.*, 2012). The carbohydrates may have been digested into simple sugars by amylolytic enzymes which

are rapidly taken up by the growing embryo to serve as its energy source during germination (Elkhier and Hamid, 2008). The reducing sugar increase with increase in time and relative humidity. It may be due to favorable condition established for the breakdown of starch at high relative humidity. As the time increase more starch will breakdown due to exposure of starch to amylase for long time (Tejinder, 2007). The total ash content of the malted samples decreased significantly by 34.38% from that of the control. This may be due to the incorporation of mineral elements into cell constituents during the germination process (Ogbonna *et al.*, 2012).

Mineral ions play vital roles in metabolism as enzyme stabilizers and transport cofactors. The crude fibre content of the malted samples increased significantly by 72.5% during the malting period compared to the control (Ogbonna *et al.*, 2012). Crude fibre consists mainly of cellulose, lignin and hemicellulose (Eggum *et al.*, 1981). This increase could be attributed to increased bran matter and the building of dry matter during the growth and development (germination) of the plant. A highly significant increase of 111.82% in the crude fat levels of the malted samples was observed at first and later decreased significantly by 22.75%. This suggests that there was a change in the crude fat content during the malting stage which may be due to its proportional increase as a result of decrease in the other food reserves like carbohydrates (Ogbonna *et al.*, 2012). Lipase activity increased during germination and the proportion of lipid bodies during germination will decrease due to the synthesis of lipase (Narsih *et al.*, 2012).

The structural change in starch after malting is shown in Fig. 2.3.

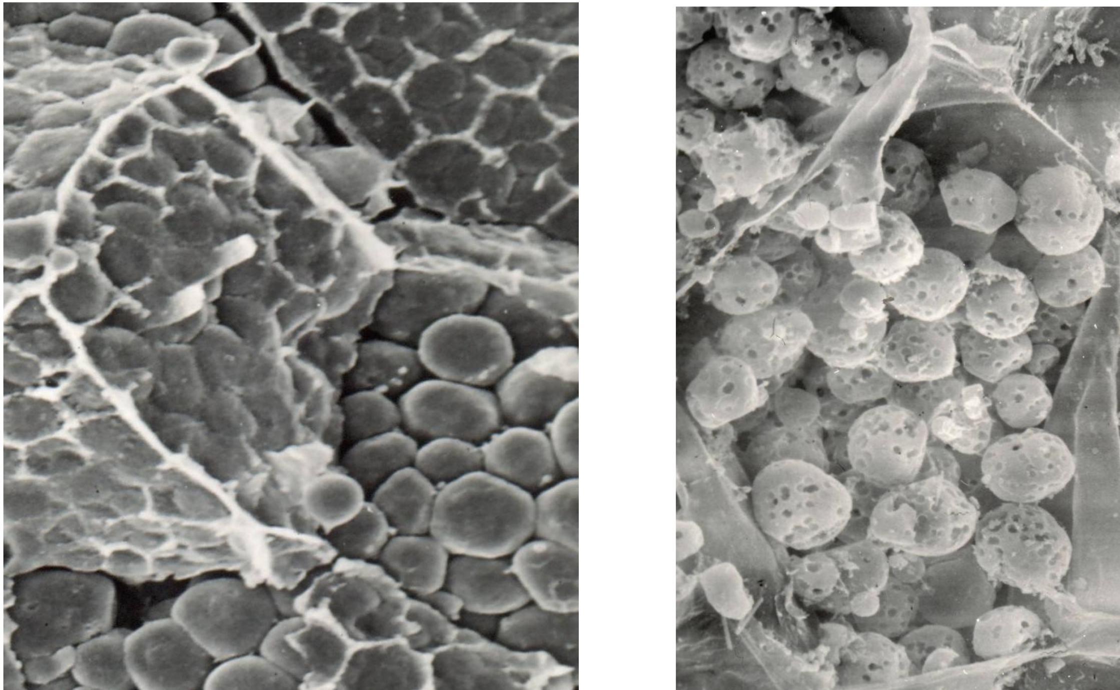

Fig. 2.3 Structure of starch before and after germination

Source: Taylor (2010)

The decrease in percentage of moisture content and crude fibre content after hydrolysis are good indication of increased nutritional value of the sorghum (Adebiyi *et al.*, 2005).

2.2.5.2 Effect on protein quality

A major objective of malting is to promote the development of hydrolytic enzymes, which are not present in the non-germinated grain. Sorghum malt quality (at least for sorghum brewing) is assessed primarily in terms of DP and FAN. DP is a measure of the joint activity of α- and ß-amylase. In general, the DP of the malts increased with increasing germination time to about 6 days. FAN is produced during malting by the action of endogenous proteinase and peptidase enzymes on the protein reserves of the grain and the breakdown products are collectively referred to as FAN. For all the sorghum varieties tested malting improved malt FAN, which increased with increasing germination time over the 8-days malting (Dewar *et al.*, 1997).

Malting also significantly improved the in-vitro digestibility and the quality of the sorghum protein, which increased with increasing malting time. For the Kenyan local white variety malting improved the digestibility of the protein by a staggering 110% and increased

the percentage protein, the nitrogen solubility index and lysine content by as much as approximately 8.5, 251 and 32%, respectively. It should be noted that the improvement in lysine content is not simply a consequence of protein concentration but rather a true increase in lysine. The significant increase in the NSI supports the idea that the increase in in-vitro protein digestibility is probably due to structural changes and the enzymic hydrolysis of proteins into more digestible forms such as amino acids and small peptides. The evidence would seem to support the suggestion that the simple technology of malting offers a means by which to improve the quality and digestibility of sorghum protein (Dewar *et al.*, 1997; Elmaki *et al.*, 1999).

The real nutritive value of sorghum protein after sprouting will have to await human or rat feeding studies. The sprouting of high-lysine sorghum is even more favorable than normal grain, because larger increases in lysine content (expressed as a % basis or absolute amount) were observed. The high percentage of sprouting grain may make the removal of unsprouted sorghum unnecessary while still realizing a large increase in lysine content of the sorghum. The sprouted sorghum can be used as vegetable, salad, or dried and ground to flour in more traditional food uses (Wu and Wall, 1980).

The structural change in protein is shown in Fig. 2.4.

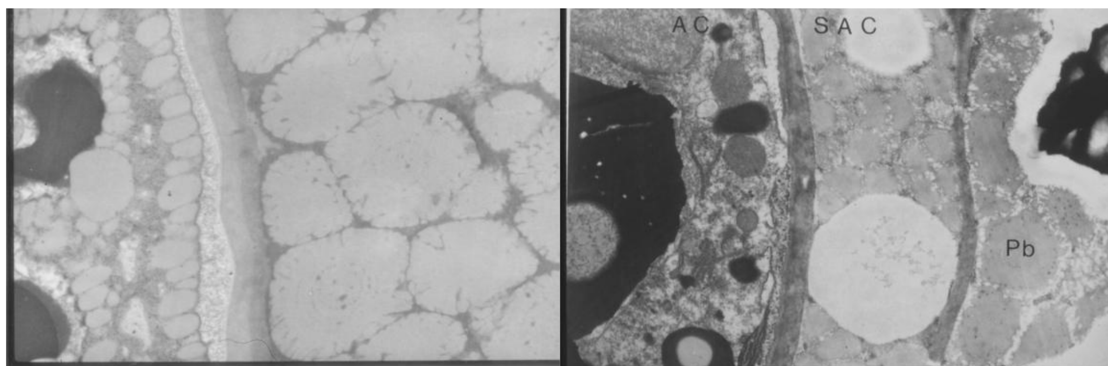

Fig. 2.4 Structure of protein before and after germination

Source: Taylor (2010)

2.2.5.3 Change in anti-nutritional factors

Following table reflects the levels of the anti-nutritional factors in both the control and malted samples. The levels of the anti-nutritional factors in the malted samples decreased significantly with increasing steeping and germination periods indicating the occurrence of

some form of modification during the malting process. Oxalate decreased by 34.13%, tannin by 8.45%, trypsin inhibitor activity by 36.5% while phytate dropped by 66%. Leaching during steeping was suspected to have contributed in the reduction of some of the anti-nutritional factors considering a change in colour of the steep water. Others such as phytate may have been significantly affected by the endogenous enzymes such as phytases activated during germination (Ogbonna *et al.*, 2012). Phytases degrade phytate into inorganic phosphorus and inositol and its intermediate forms (Idris *et al.*, 2007). This was confirmed by Vidal-Valverde *et al.* (1998) who reported that the germination of lentils greatly reduced phytate content compared to soaking and cooking.

Calcium is released from oxalate complexes and iron from protein-tannin complexes (Emmanbux and Taylor, 2003; Melaku *et al.*, 2005). The rate of reduction depended on the age of the malt. However, the rate of reduction in tannin was less than that of phytate possibly because while phytate is degraded by malt enzymes, tannins are only leached out (Idris *et al.*, 2005; Reichert *et al.*, 1980). Hence, the combined effects of the physical and enzymatic actions on the sorghum grain during malting dramatically decreased the concentration of anti-nutritional factors in the malted sorghum grist (Ugwu and Oranye, 2006).

2.2.5.4 Change in minerals

The mineral composition of the malted sorghum increased above the values of the unmalted sorghum with increasing steeping and germination periods. As result of malting, sodium can be increased up to 123%, potassium 29.55%, phosphorus 45.71%, calcium 157% and magnesium 93.33% (Ogbonna, 2011).

2.2.5.5 Amylase activity

The amylase activity ranged from 2 to 13.5 units in dry seeds and increased significantly during malting. Among the cultivars studied SPV86, CSH6, M35-1, SPV351, and SPV504 exhibited higher amylase activity than other cultivars after malting. The high amylase activity is desirable for rapid starch hydrolysis during malting. The cultivars with increased rate of release of amylase activity may be suitable for brewing purposes (Ratnavati and Chavan, 2016). The activity of enzymes depends on temperature, moisture content, and environmental conditions of germination (Moongngarm, 2010).

2.2.6 Functional properties of malt

Functional properties of sorghum flour are determined by its components such as protein, starch, etc., that affect the processing procedures and features of foods. Functional properties of proteins are desirable for the utilization of any food containing protein. Protein functions depend mainly on its size and structure as well as the interactions with other components such as carbohydrates and oils. It can be modified by various treatments. Modified proteins possess different functions. They can be added in small amounts to food products for a specific purpose (Ghavidel and Prakash, 2006).

Functional properties of sorghum proteins make its flour suitable to be used as supplement to replace toxic protein sources (e.g. wheat). Hence, sorghum flour can substitute gluten-rich cereals in the diet of people suffering from celiac disease (Elkhalifa and Bernhardt, 2010).

2.2.6.1 Water absorption capacity

The water absorption capacity of the flour obtained after germination is higher than the non-germinated grain flour. The increase may be due to the production of compounds having good water holding capacity such as soluble sugars (Ocheme *et al.*, 2015). Water holding capacity depends on the water bounding capacities of food components (Okaka and Potter, 1977). Gernah *et al.* (2011) observed an increase in the water absorption capacity of maize as a result of malting. Flours with good water absorption capacities are useful in baking (Ocheme *et al.*, 2015).

2.2.6.2 Swelling power

The swelling power of the flours increased significantly germination time increased (Ocheme *et al.*, 2015). Ocheme and Chinma (2008) reported an increase in the swelling power of millet flour as a result of germination. The increase in swelling power was probably due to an increase in soluble solids brought about by the breakdown of lipid, fiber and larger amount of amylose–lipid complex in flour that could inhibit the swelling of starch granules. Fats may complex with starch and limit swelling (Ocheme *et al.*, 2015).

2.2.6.3 Foaming stability

Foaming capacity varied from 14.30% to 16.20%, increasing with germination time. The non-germinated sorghum flour had the lowest foaming properties while germinated sorghum flour had the highest. Foam is a colloid of gas bubbles trapped in a solid or liquid. Foam formation and stability are dependent on protein type, pH, surface tension, viscosity and processing method. Eltayeb *et al.* (2011) reported that proteins in flours are surface active and that is why flours are able to produce foam. Adedeji *et al.* (2014) reported that the foaming capacity of germinated maize flour decreased with germination time. Food materials with good foaming capacity and stability are useful in the formulation of aerated foods. Phattanakulkaewmorie *et al.* (2011) reported that swelling power is positively related to the amount of soluble solids.

2.2.6.4 Gelatinization concentration

Gelation capacity enhanced with an increase in germination time. Non-germinating sorghum flour had the lowest gelation capacity, while that of germinating got the highest gelation capacity (Elbaloula *et al.*, 2014). Improvement in gelation capacity following an increase in flour concentration mainly resulted from a decrease in thermo-dynamic affinity of proteins for the solution, which increased the interactions of different proteins (Eltayeb *et al.*, 2011).

A variation in LGC could come from the aggregation of denatured protein molecules, which increased the protein concentration or solubility and caused intermolecular contacts during heating (Obatolu and Cole, 2000). The amylase released during germination would have interacted with the starch component of the flour, and led to an increase in its gelation property (Elbaloula *et al.*, 2014).

2.2.7 Factors affecting malting

2.2.7.1 Moisture content

The moisture content is needed to control the germination process. Water sensitive grain will usually germinate only if steeped to about 35% moisture. Following and air rest and the onset of germination, the moisture of water sensitive grain may safely be increased further to allow satisfactory modification. In the past in floor malting, low nitrogen-grain was steeped to 43% moisture, steeply grain to about 45% moisture. However, in pneumatic malting where evaporation from the grain is inevitable, the grain has to be sprinkled with water during

germination to maintain its moisture content. At higher moisture content, modification proceeds more rapidly than at lower moisture content. Without the methods of control, however, embryo growth and hence rootlet production and total malting loss may be proportionately greater (Hough, 1985).

2.2.7.2 Temperature

In traditional floor malting, a long cool germination period of up to 13 days at 12-14°C was preferred. As the temperature of malting increases, the respiration rate of the grain, the rate of growth of roots and the rates at which enzymes formed also increases. However, these changes are not all accelerated in the same proportion by increases temperature so that carrying out germination at progressively higher temperature do not merely produce the same malt in shorter time. This is illustrated by the fact that at lower germination temperature enzymes such as a-amylase and protease increases in amount slowly but ultimately reach levels higher than those attained at higher germination temperature (Hough, 1985).

2.2.7.3 Gases

Air is composed of about 20% oxygen, 0.03% carbon dioxide, and about 80% nitrogen gas. If one provides different proportions of each of these gases under experimental conditions, it soon becomes clear that oxygen is required for germination of most species. Carbon dioxide concentrations higher than 0.03% retard germination, while nitrogen gas has no influence (McDonald, 2014).

Part III

Materials and methods

3.1 Materials

The materials collected for the malting of sorghum were as follows:

3.1.1 Sorghum

The sorghum (*Sorghum bicolor* var. L. Moench) was collected from the Dakshinkali market, Kathmandu.

3.1.2 Containers

Polyethylene terephthalate (PET) containers were bought from the market of Dharan. The size of containers was 500 ml and plain in design. They were used to soak the sorghum grain before germination.

3.1.3 Plastic bags

Low density polyethylene (LDPE) plastic bags were bought from the market of Dharan. The size of plastic bags was 250 gm. They were used to keep the sorghum malt and sorghum malt flour.

3.1.4 Equipment and chemicals

The following equipment and chemicals used were available in campus. The list of chemical used for the analysis is shown in Table 3.1 and the list of equipment is shown in Table 3.2.

Table 3.1 List of chemicals used

Chemical	Supplier/Manufacturer	Other specifications
Sodium hydroxide (NaOH)	Thermo Fisher Scientific India Pvt. Ltd.	Pellets, AR grade, 98%
Hydrochloric acid (HCl)	Thermo Electron LLS India Pvt. Ltd.	36%, LR grade

Sulphuric acid (H_2SO_4)	Thermo Fisher Scientific India Pvt. Ltd.	97%, LR grade
Boric acid	Merck (India) Limited	Amorphous
Oxalic acid	Merck (India) Limited	Crystal
DNS	Himedia Laboratories Pvt. Ltd.	98%, LR grade
Maltose	Merck life Pvt. Ltd.	95% assay
Petroleum benzene	Merck life Pvt. Ltd.	B.P. 60°C-80°C
Dextrose	Merck (India) Limited	Amorphous
Sodium Carbonate (Na_2CO_3)	Qualigens fine chemicals	99.5%, LR grade

Table 3.2 List of equipment used

Physical apparatus	Specifications
Electric balance (620 g)	Phoneix instruments, India
Spectrophotometer	Labtronics, India
Soxhlet apparatus	Y.P. scientific laboratories, India
Hot air oven	Victolab, India
Incubator	Victolab, India
Muffle furnace	Accumax, India
Cabinet dryer	AIset YDL-2000

3.2 Methods

3.2.1 Work plan

The different temperature, RH and time for germination were obtained from design expert central composite rotatable design (CCRD) which were given in Table 3.3.

Table 3.3 Experimental combinations both in coded and uncoded levels for germination.

Treatments	Coded variables			Uncoded variables		
	A	B	C	Temperature (°C)	Time (Days)	RH (%)
A	0.4	-0.33	-1	27	4	70
B	0.4	-1	1	27	3	95
C	2	-0.33	1	35	4	95
D	2	-1	-1	35	3	70
E	0.4	1	0.6	27	6	88.5
F	2	1	0.48	35	6	90
G	-1	1	-1	20	6	70
H	-1	-1	-1	20	3	70
I	-1	-0.33	1	20	4	95
J	2	1	-1	35	6	70
K	2	1	1	35	6	95
L	2	-1	0.08	35	3	83.5
M	-0.2	-1	1	24	3	95
N	-0.2	-0.33	-0.2	24	4	80
O	-0.2	1	1	24	6	95

3.2.2 Procedure for malting

The malting procedure was adopted from Ratnavati and Chavan (2016) with slight modification. The steeping period and drying period were increased to obtain the similar condition that was described by Ratnavati and Chavan (2016). The germination condition were also varied according to the result obtained from design expert. The modified process that was adopted is shown in Fig. 3.1.

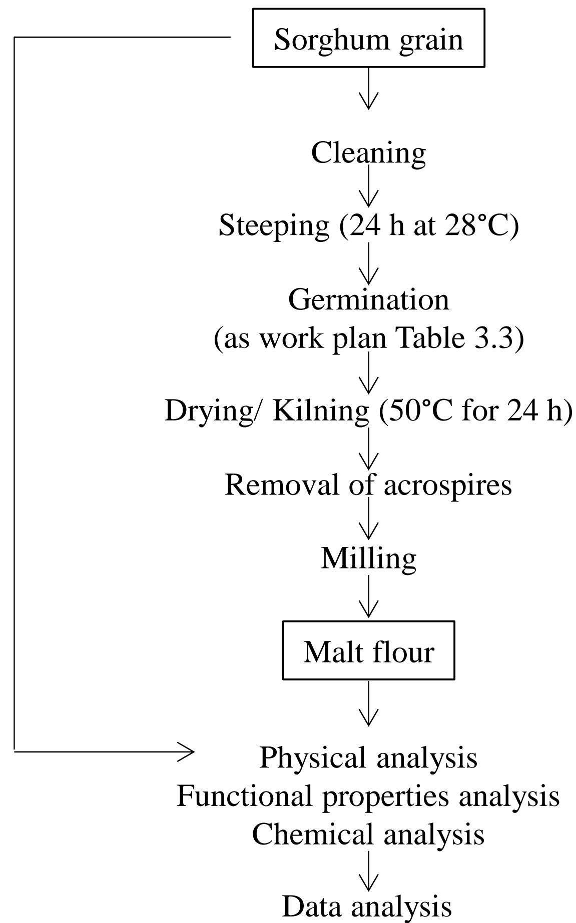

Fig. 3.1 Flow diagram for malting of sorghum

3.2.2.1 Cleaning

Sorghum grain was first winnowed with woven bamboo trays (nanglo). In this step; husk, immature grains and light particles was winnowed away and heavier particles such as specks and stones was separated by gravity during winnowing.

3.2.2.2 Steeping

Cleaned seeds were transferred to the plastic containers and water was added 3 times that of sorghum. Light materials present in the sample were skimmed off. Agitation was done to clean the seed. The grain was steeped for 24 h at room temperature (28±3℃) and drained to remove the excess water. Then it was dipped in KMS solution for 10 min to prevent the mold growth.

3.2.2.3 Germination

The steeped grain was first collected in a muslin cloth and swirled in order to drain excess water. The grains were spread over the another muslin cloth and kept at desiccator which were maintained at different relative humidity (RH) using H_2SO_4 solution and these desiccators were kept in incubator for the temperature control and the grains were left to germinate for the estimated interval of days as shown in Table 3.3.

3.2.2.3.1 Maintaining relative humidity

For maintaining humidity, the different solutions of sulphuric acid is used which is given in Table 3.4.

Table 3.4 Relative humidity control using H_2SO_4 solution

Relative humidity (%)	H_2SO_4 (%)
70	29.66
80	22.96
83.5	20.62
88.5	17.27
90	16.26
95	12.92

Source: Wilson (1921)

3.2.2.4 Drying/ Malt kilning

Different samples of germinating sorghum were taken and were dried to stop further germination. Drying was carried out in a cabinet drier at 50°C until the constant weight was obtained.

After drying, the rootlets were removed and the prepared malt was packed in airtight containers.

3.3 Experimental procedure

3.3.1 Physical analysis of raw materials and final product

3.3.1.1 1000 kernel weight

The 1000 kernel weight of raw materials and final products were determined by measuring the weight of 1000 kernels of sorghum grains after selecting the appropriate sample size by quartering method as stated in Buffo *et al.* (1998).

3.3.1.2 Bulk density

The bulk density was measured as mentioned in Clementson *et al.* (2010) by pouring the grains into the funnel- shaped hopper, the hopper was centered over the measuring bushel, the hopper valve was opened quickly, and the grains were allowed to flow freely into the measuring bushel. After the bushel was filled, the excess material was leveled off with gentle zigzag strokes using the standard Seedburo striking stick. The filled measuring bushel was then weighed, and the mass of grains in the bushel was determined by subtracting the mass of the measuring bushel itself. The bulk density (ρ) of grain was then calculated using the following expression:

$$\text{Bulk density} = \frac{\text{Mass of grain}}{\text{Volume of bushel}}$$

3.3.1.3 Particle density

True density of grain was measured by turpentine displacement method as mentioned in Simonyan *et al.* (2007).

3.3.1.4 Porosity

Porisity of grain was measured as mentioned in Ndirika and Mohammed (2005).

$$\text{Porosity} = [1\text{- Bulk density/ Particle density}] \times 100$$

3.3.1.5 Sphericity

Sphericity of grain was determined as mentioned in Simonyan *et al.* (2007).

$$\text{Sphericity} = (lbt)^{1/3}/\, l$$

Where, l = length of grain

b = breadth of grain

t = thickness of grain

3.3.2 Chemical analysis

3.3.2.1 Proximate analysis

3.3.2.1.1 Moisture content

The moisture content was determined by using hot air oven method. 5 g of sample was weighted and heated in an insulated oven at 110°C to constant weight. The difference in weight was the water that has evaporated as Ranganna (1986).

3.3.2.1.2 Crude fat

The fat content was determined by Soxhlet method. Solvent extraction of 10 g sample was done by recycling hot solvent for number of times until complete extraction and fat was recovered by evaporating away the solvent as standard method of Ranganna (1986).

3.3.2.1.3 Crude protein

The crude protein was determined by using Kjeldahl’s method. 2 g fatless sample was digested, steam distillated after decomposing the former NaOH. Titration of entrapped NH_3 boric acid was done with standard acid as standard method of Ranganna (1986).

3.3.2.1.4 Crude fiber

Crude fiber was determined by using chemical process, the sample was treated with boiling dilute Sulphuric acid, boiling sodium hydroxide and then with alcohol as standard method of Ranganna (1986).

3.3.2.1.5 Total ash

Ash content was determined using muffle furnaces according to Ranganna (1986). 5 g of weighed sample in silica crucible was charred in hot plate till no smoke raise from it and finally, ashing was done in muffle furnace at 550°C to the constant weight. The difference in weight was the total ash content remaining in crucible, under standardized condition (Ranganna, 1986).

3.3.2.2 Enzyme analysis (α-amylase)

Enzyme activity was determined by reacting with dinitrosalicylic acid solution which reduces the enzyme solution into a brown colored product. 1ml of sample solution was mixed with 1 ml of starch solution which was incubated at 27°C for 15 min and the reaction was stopped by adding 2 ml of dinitrosalicylic acid reagent which is then boiled for 5 min. 1 ml of potassium sodium tartarate is added to the warm solution, the solution is then cooled in tap water. Now the volume is make up to 10 ml by adding 6 ml distill water and absorbance was read at 560 nm as in Sadasivam and Manickam (1997).

For calculation the standard graph was prepared with 0-100 µg maltose and the unit of amylase is expressed as mg of maltose produced during 15 min incubation with 1% starch (Sadasivam and Manickam, 1997).

3.3.2.3 Reducing sugar

Reducing sugar is determined by Lane and Eynon’s method as mentioned in K.C. and Rai (2007).

3.3.2.4 Starch

Starch content can be determined by Lane and Eynon’s hydrolysis method as K.C. and Rai (2007).

3.3.3 Functional properties

3.3.3.1 Swelling power

One gram of the sorghum flour was mixed with 10 ml distilled water in a centrifuge tube and heated in a hot water bath at 80°C for 30 min while continuously shaking the tube. After heating, the suspension was centrifuged at 1000 rpm for 15 min. The supernatant was decanted and the weight of the paste taken. The swelling power was calculated as in Appiah *et al.* (2011):

Swelling power = weight of the paste / weight of dry flour

3.3.3.2 Water absorption capacities

One gram of sorghum flour is mixed with 10 ml distilled water in a pre-weighed 20 ml centrifuge tube. The slurry was agitated for 2 min, allowed to stand at 28°C for 30 min and then centrifuged at 500 rpm for 20 min. The clear supernatant was decanted and discarded. The adhering drops of water in the centrifuge tube were removed with cotton wool and the tube was weighed, the weight of water absorbed by 1 g of flour or protein was calculated and expressed as water absorption capacity (Appiah *et al.*, 2011).

3.3.3.3 Foam stability

The 1.0 g flour sample was added to 50 ml distilled water at 30 ± 2°C in a graduated cylinder. The suspension was mixed and shaken for 5 min to foam. The volume of foam at 30sec after whipping was expressed as foam capacity using the formula:

$$\text{Foam Stability} = \frac{\text{Volume of foam AW- Volume of foam BW}}{\text{Volume of foam BW}} \times 100\%$$

Where, AW = after whipping, BW = before whipping (Chandra and Samsher, 2013).

3.3.3.4 Gelatinization concentration

Gelatinization concentration was determined according to Coffman and Garcia (1977) as modified by Sathe and Salunkhe (1981). Appropriate sample suspension of 2, 4, 6, 8, 10, 12, 14, 16, 18 and 20% (w/v) were prepared in 10 ml distilled water. The test tubes containing these suspensions were heated for 1 h in a boiling water bath, followed by rapid cooling under running cool water. The test tubes then further cooled for 2 h at 20ºC. The least

gelation concentration was determined at that concentration when the sample from the inverted test tube did not fall down or slip out.

3.3.4 Statistical analysis

Design expert®10 central composite rotatable design was used to design the experiment having 3 factors (temperature, RH and time). Response surface methodology (RSM) was used for the optimization of the malting condition.

Part IV

Results and discussion

The malting of sorghum was performed by varying relative humidity, temperature and time. The physical properties, chemical composition and functional properties changes after malting was determined and optimization of malting procedure was done on the basis of chemical changes during malting.

4.1 Physical properties of sorghum grain and malt

Response surface methodology was used to analyze physical properties of sorghum malt. Linear model was suggested for bulk density and sphericity. Quadratic model was suggested for 1000 kernel weight. 2FI model was suggested for particle density and porosity. The equations for the 1000 kernel wt., bulk density, particle density, sphericity and porosity in coded forms were given in equations 4.1, 4.2, 4.3, 4.4 and 4.5 respectively.

$$\text{1000 kernel weight} = +21.466 - 2.490A + 0.0104B - 0.579C + 0.077AB - 0.413AC + 0.439BC + 1.820A^2 - 2.053B^2 + 0.751C \quad (4.1)$$

$$\text{Bulk density} = +73.039 - 0.663A - 0.195B - 1.851C \quad (4.2)$$

$$\text{Particle density} = +1.046 + 2.263^{-003}A - 0.028B - 0.026C + 0.027AB + 1.189^{-003}AC - 8.634^{-003}BC \quad (4.3)$$

$$\text{Sphericity} = +0.697 + 0.0197A - 0.0259B + 0.0113C \quad (4.4)$$

$$\text{Porosity} = +0.301 + 9.053^{-003}A - 0.013B - 2.086^{-004}C + 0.0129AB - 5.042^{-004}AC - 0.011BC \quad (4.5)$$

Where A represents temperature, B represents time and C represents RH

The positive coefficient represents the positive correlation while negative coefficient represents negative correlation. During malting of sorghum, 1000 kernel wt. is positive correlated with time and negative correlated with temperature and RH. Bulk density is negatively correlated with time, temperature and RH. Particle density and porosity are positively correlated with temperature and negatively correlated with time and RH.

Sphericity is positively correlated with temperature and RH and negatively correlated with time.

The mean value of triplicates of unmalted and malted samples for sphericity, 1000 kernel wt., bulk density, particle density and porosity are presented in Table 4.1.

Table 4.1 Physical properties of unmalted and malted sorghum grains.

Sample	Sphericity	1000 kernel wt. (g)	Bulk density (kg/HL)	Particle density (g/ml)	Porosity
Unmalted	0.62 (0.011)	29.91 (0.51)	82.33 (3.05)	1.23 (0.020)	0.33 (0.017)
A	0.75 (0.052)	25.02 (1.65)	73.33 (1.53)	1.05 (0.054)	0.30 (0.020)
B	0.77 (0.075)	19.87 (0.64)	70.33 (1.52)	1.00 (0.051)	0.29 (0.025)
C	0.75 (0.020)	19.30 (0.39)	66.00 (1.73)	0.98 (0.040)	0.32 (0.017)
D	0.73 (0.070)	18.52 (1.08)	73.66 (0.57)	1.12 (0.050)	0.34 (0.02)
E	0.65 (0.005)	26.03 (0.80)	78.66 (1.15)	1.10 (0.030)	0.28 (0.013)
F	0.71 (0.030)	21.46 (1.21)	75.66 (3.51)	1.07 (0.020)	0.30 (0.039)
G	0.72 (0.040)	24.16 (0.51)	72.67 (1.15)	1.05 (0.030)	0.31 (0.013)
H	0.73 (0.060)	22.85 (2.31)	75.66 (0.57)	1.09 (0.031)	0.31 (0.021)
I	0.71 (0.021)	26.68 (0.56)	73.33 (2.31)	1.01 (0.050)	0.27 (0.044)
J	0.72 (0.065)	20.51 (1.51)	71.33 (0.57)	1.10 (0.015)	0.35 (0.018)
K	0.66 (0.03)	21.87 (0.31)	74.67 (0.58)	1.07 (0.080)	0.30 (0.057)
L	0.65 (0.025)	23.94 (0.86)	73.67 (0.58)	1.01 (0.035)	0.27 (0.020)
M	0.70 (0.030)	23.38 (0.58)	71.67 (1.15)	1.09 (0.036)	0.34 (0.022)
N	0.72 (0.025)	19.38 (0.99)	66.67 (1.15)	0.99 (0.028)	0.33 (0.022)
O	0.67 (0.055)	19.07 (0.73)	68.33 (1.52)	0.94 (0.12)	0.26 (0.111)

* Values are the mean of triplicates and the values in bracket indicates standard deviation

Where, A, B, C, D, E, F, G, H, I, J, K, L, M, N and O were the malted samples at different treatments.

Ndirika and Mohammed (2005) reported the value of sphericity, 1000 kernel wt., bulk density, particle density (specific gravity), and porosity as 0.67, 32.41 g, 69.9 kg/HL, 1.18 g/cm^3 and 40.80% of sorghum grain (Farafara variety) which is similar to the mean values of unmalted sorghum grain of our study. Similar values were obtained by Simonyan *et al.* (2007) in their study.

The 1000 kernel wt., bulk density, and particle density decreased on malting. Similar result was observed by Beta *et al.* (1995) during malting of different varieties of sorghum grains. This decrease may due to hydrolysis of heavier starch molecules in lighter disaccharides like maltose by high amylase activity. Also decrease in weight may results due to the dry matter loss during malting and utilization of nutrients by growing shoots. This decrease may also be due to respiration of growing shoots during germination Beta *et al.* (1995). But Makeri *et al.* (2013) reported that there were not significant changes in most of the physical properties of barley grain after malting.

4.2 Chemical composition of grain and malt

Response surface methodology was used to analyze chemical composition of sorghum grain and malt. Linear model was suggested for moisture and crude fat content. Quadratic model was suggested for crude protein and crude fiber content and no any model was suggested only mean was found for crude fat content. The equations for the moisture content, protein content, total ash content, crude fat content and crude fiber content in coded forms were given in equations 4.6, 4.7, 4.8, 4.9 and 4.10 respectively.

$$\text{Moisture} = +8.49 - 0.057A - 0.474\,B - 0.211C \quad (4.6)$$

$$\text{Crude protein} = +9.816 + 0.312A - 0.454B - 0.119C + 0.0584AB + 0.082AC + 0.141BC + 0.049A^2 - 0.223\,B^2 + 1.329C^2 \quad (4.7)$$

$$\text{Total ash} = +1.87 \quad (4.8)$$

$$\text{Crude fat} = +2.930 + 0.205A - 0.322B + 0.124C \quad (4.9)$$

$$\text{Crude fiber} = +1.223 - 0.241A - 9.620^{-003}B + 0.175C + 2.523^{-003}AB - 1.679^{-003}AC - 0.0812BC + 0.156A^2 - 0.077B^2 - 0.068C^2 \quad (4.10)$$

Where A represents temperature, B represents time and C represents RH

The positive coefficient represents the positive correlation while negative coefficient represents negative correlation. During malting of sorghum, moisture content is negatively correlated with time, temperature and RH. Protein content is positively correlated with temperature and negatively correlated with time and RH. Fat content is positively correlated with temperature and Rh and negatively correlated with time. Fiber content is negatively correlated with temperature and time and positively correlated with RH.

The chemical analysis of grain and all 15 malt samples were performed and their mean values were obtained which is tabulated in Table 4.2

Table 4.2 Chemical composition of sorghum grain and malt

Sample	Moisture	Oil	Ash	Crude Fibre	Protein
Unmalted	10.37 (0.88)	4.71 (0.50)	1.45 (0.15)	1.13 (0.18)	12.8 (1.60)
A	7.98 (1.24)	3.25 (0.17)	1.59 (0.42)	1.04 (0.16)	11.39 (0.25)
B	9.05 (0.67)	3.65 (0.54)	1.73 (0.21)	1.09 (0.13)	11.89 (0.19)
C	7.64 (1.31)	3.67 (0.21)	2.24 (0.40)	1.35 (0.41)	11.72 (0.23)
D	10.21 (1.00)	4.03 (0.11)	1.64 (0.26)	0.95 (0.06)	12.60 (0.32)
E	6.93 (0.25)	3.25 (0.66)	2.96 (0.20)	1.17 (0.08)	10.26 (0.21)
F	9.03 (0.15)	3.21 (0.39)	1.75 (0.20)	1.24 (0.08)	10.43 (0.42)
G	9.11 (0.76)	2.58 (0.13)	1.81 (0.18)	1.42 (0.29)	10.40 (0.78)
H	9.53 (0.67)	2.83 (0.27)	1.67 (0.07)	1.12 (0.16)	11.30 (1.08)
I	9.31 (0.73)	2.70 (0.34)	1.8 (0.19)	1.83 (0.12)	11.17 (0.94)
J	7.14 (0.40)	2.25 (0.09)	1.84 (0.16)	1.04 (1.48)	10.96 (0.54)
K	8.76 (1.03)	3.18 (0.05)	1.85 (0.09)	1.50 (0.15)	12 (0.39)
L	8.11 (0.15)	3.27 (0.51)	1.93 (0.06)	1.42 (0.21)	10.41 (0.21)
M	8.47 (0.59)	3.37 (0.22)	1.69 (0.06)	1.58 (0.31)	10.53 (0.52)
N	8.26 (0.57)	2.50 (0.33)	1.84 (0.53)	1.12 (0.28)	10.05 (0.21)
O	6.96 (0.28)	2.48 (0.32)	1.69 (0.33)	1.04 (0.16)	9.62 (0.51)

* Values are the mean of triplicates and the values in bracket indicates standard deviation

The chemical composition of sorghum grain was observed as 12.3% protein, 3.6% fat, 1.67% ash and 73.8% starch by FAO (1995). Wall and Blessin (1970) obtained the moisture content and crude fiber content of sorghum grain as 12-14% and 2.7-2.9% respectively. This composition is similar to the composition that was obtained in our study. The result of chemical composition obtained is analogous to that obtained by Adebiyi *et al.* (2005).

Elmaki *et al.* (1999) reported decrease in proximate composition i.e. protein, fat, crude fiber and ash content during different soaking period and germination period of sorghum cultivars. Similar results were obtained in our study except ash content which is increased during germination period. According to Wu and Wall (1980) the percentage protein in germinated sorghum is greater than in the initial grain as a result of dry matter loss in the grain during germination, but the absolute amount of protein per kernel is not increased. Bolarinwa *et al.* (2015) reported the very low moisture content of sorghum malt as 6.76% and Narsih *et al.* (2012) reported the increase in ash content of sorghum malt which is similar to the results of our study. Narsih *et al.* (2012) also observed increase in protein content and decreased crude fat and crude fiber content during germination period of sorghum.

The slight change in protein content may attributed to the fact that water soluble nitrogen was lost during soaking of seeds and also, during seed germination, part of the protein was utilized for the growth and development of the embryo. During germination, starch and protein were degraded to soluble sugars and amino acids, respectively. Their degradations indicated the metabolic system interference to reserve starch and protein by amylases and proteases (Elbaloula *et al.*, 2014).

Changes in fiber content may attributed to the fact that part of the seed fiber may be solubilized enzymatically during seed germination (Elmaki *et al.*, 1999). Soaking process could also have decreased fiber content as sorghum contained soluble and insoluble fiber in water. Therefore, the longer soaking process may reduce water-soluble fiber content of sorghum seed namely β-glucan (Narsih *et al.*, 2012).

The reduction in fat content may be due to the fact that biochemical and physiological changes occurred during germination; such changes require an energy to proceed, and therefore part of the seed fat was utilized for the production of this energy (Elmaki *et al.*, 1999). Lipase activity increased during germination and the proportion of lipid bodies during

germination will decrease due to the synthesis of lipase. The lipase activity increased during germination possibly by the synthesis of the aleurone and scutellum (Narsih *et al.*, 2012).

The moisture content of sorghum malt is lower than that of grain this may be due to intense drying of malt after germination. During drying the moisture content at final stage should be about 5%. Germination would increase the mineral content due to an increase in fitase enzyme activity during germination. The enzyme will hydrolyze the bond between the protein-enzyme minerals become free, therefore increasing the availability of minerals (Narsih *et al.*, 2012).

4.3 Analysis of functional properties of grain and malt

Response surface methodology was used to analyze functional properties of sorghum malt. Linear model was suggested for foam stability and swelling power. Quadratic model was suggested for water absorption capacity. No model was suggested for gelatinization concentration. The equations for the water absorption capacity, foam stability, swelling power and gelatinization concentration in coded forms were given in equations 4.11, 4.12, 4.13 and 4.14 respectively.

$$\text{Water absorption capacity} = +1.771 + 0.013A + 0.119B + 0.0265C - 0.0127AB - 0.010AC + 0.080BC - 0.066A^2 + 6.901^{-003}B^2 - 0.287C^2 \quad (4.11)$$

$$\text{Foam stability} = +14.131 - 0.710A + 0.820B - 0.218C \quad (4.12)$$

$$\text{Swelling power} = +2.452 - 0.139A + 0.342B + 0.051C \quad (4.13)$$

$$\text{Gelatinization concentration} = +8.80 \quad (4.14)$$

Where A represents temperature, B represents time and C represents RH

The positive coefficient represents the positive correlation while negative coefficient represents negative correlation. During malting of sorghum, WAC is positively correlated with time, temperature and RH. Foam stability is positively correlated with time and negatively correlated with temperature and RH. Swelling power is positively correlated with time and RH and negatively correlated with temperature.

Functional properties of grain and all malt samples were analyzed according to standard procedures and the results obtained were presented in Table 4.3.

Table 4.3 Functional properties of sorghum grain and malt

Sample	WAC (g/g sample)	Foam Stability (%)	Swelling Power (g/g sample)	Gelatinization Concentration (%)
Unmalted	1.22 (0.006)	10.39 (0.35)	0.87 (0.021)	12
A	1.36 (0.030)	11.53 (1.68)	2.58 (0.123)	8
B	1.26 (0.011)	11.94 (1.73)	2.42 (0.269)	10
C	1.26 (0.006)	10.67 (1.15)	2.78 (0.111)	8
D	1.25 (0.005)	13.05 (0.48)	1.3 (0.233)	12
E	1.79 (0.036)	13.33 (0)	3.08 (0.105)	6
F	1.62 (0.012)	11.85 (1.28)	2.60 (0.037)	10
G	1.41 (0.012)	14.00 (0)	2.87 (0.045)	10
H	1.34 (0.015)	15.56 (1.93)	2.07 (0.101)	10
I	1.28 (0.012)	12.44 (0.77)	2.28 (0.199)	10
J	1.29 (0.020)	13.80 (0.83)	2.37 (0.202)	6
K	1.37 (0.047)	15.49 (1.736)	1.89 (0.058)	10
L	1.37 (0.072)	12.10 (1.068)	1.93 (0.04)	8
M	1.37 (0.045)	12.78 (1.95)	1.60 (0.058)	8
N	1.80 (0.058)	18 (0.656)	2.76 (0.02)	10
O	1.80 (0.078)	17.88 (0.95)	2.88 (0.025)	6

* Values are the mean of triplicates and the values in bracket indicates standard deviation

The above table shows that the WAC, foam stability and swelling power increases after germination but the gelatinization concentration decreases after germination. Ocheme *et al.* (2015) also reported the increase of WAC, swelling power and foam stability after germination. Elbaloula *et al.* (2014) observed the decrease in gelatinization concentration after germination of grain. This study also observed the increasing rate of foam formation and its stability as the time of germination increases during the germination of sorghum grain for 3 days. Phattanakulkaewmorie *et al.* (2011) also observed the increase in swelling power

and solubility of germinated sorghum flour during the preparation of bread from composite bread using germinated sorghum flour.

The increase in WAC may due to the production of compounds having good water holding capacity such as soluble sugars like glucose and maltose as a result of hydrolysis of complex starch molecules by amylase (Gernah *et al.*, 2011). An increase in foaming capacity might be initiated by a decrease in surface tension of the air and water interface, which consequently caused absorption of soluble protein molecules for hydrophobic interactions. Furthermore, germination might have caused surface denaturation of the proteins and reduced the surface tension of the molecules, which gave an improved foam stability (Elbaloula *et al.*, 2014). The increase in swelling power was probably due to an increase in soluble solids brought about by the breakdown of lipid, fiber and larger amount of amylose-lipid complex in flour that could inhibit the swelling of starch granules (Ocheme *et al.*, 2015). The decrease in least gelatinization concentration indicated that amylase released during germination would have interacted with the starch component of the flour, and led to an increase in its gelation property which results in gelation of starch at low concentration (Elbaloula *et al.*, 2014).

4.4 Effects of malting condition in starch content

The starch content for malted sorghum varied from 51.16% to 66.89%. Table B.15, B.16 and B.17 of appendix show the ANOVA of the model and other statistical attributes of starch content. Regression model fitted to experimental results of starch content showed that the model F-value of 11.20 was significant ($p<0.05$). The fit of model was also expressed by the coefficient of determination R^2, which was found to be 0.7534, indicating that 75.34 % of the variability of the response could be explained by the model. The predicted R^2 value of 0.5774 was in reasonable agreement with the Adjusted R^2 value. The Adjusted R^2 value 0.6861 and Adequate Precision value 8.678 showed an adequate signal. A ratio greater than 4 is desirable and hence this model may be used to investigate the design space (Myers *et al.*, 2009).

Considering all the above criteria the model (equation 4.15) was selected to represent the variation of starch content with the independent variables and further analysis. The linear model fitted for starch content obtained from regression analysis in terms of coded fator of the variables is represented by equation 4.15.

Starch = +58.941 - 0.548A - 4.269B - 0.881C……………………………………....(4.15)

Where A represents temperature, B represents time and C represents RH

The negative coefficient of all the factors temperature, time and RH indicates that there is negative correlation between all the 3 factors with starch content. Tester *et al.* (1991) obtained the result of decrease in starch content of barley grain with increase in temperature from 10-20^{0} C. Similar results was also obtained by Tejinder (2007) during the germination of barley at high temperature and relative humidity.

The linear model graph for starch content is presented in Fig 4.1

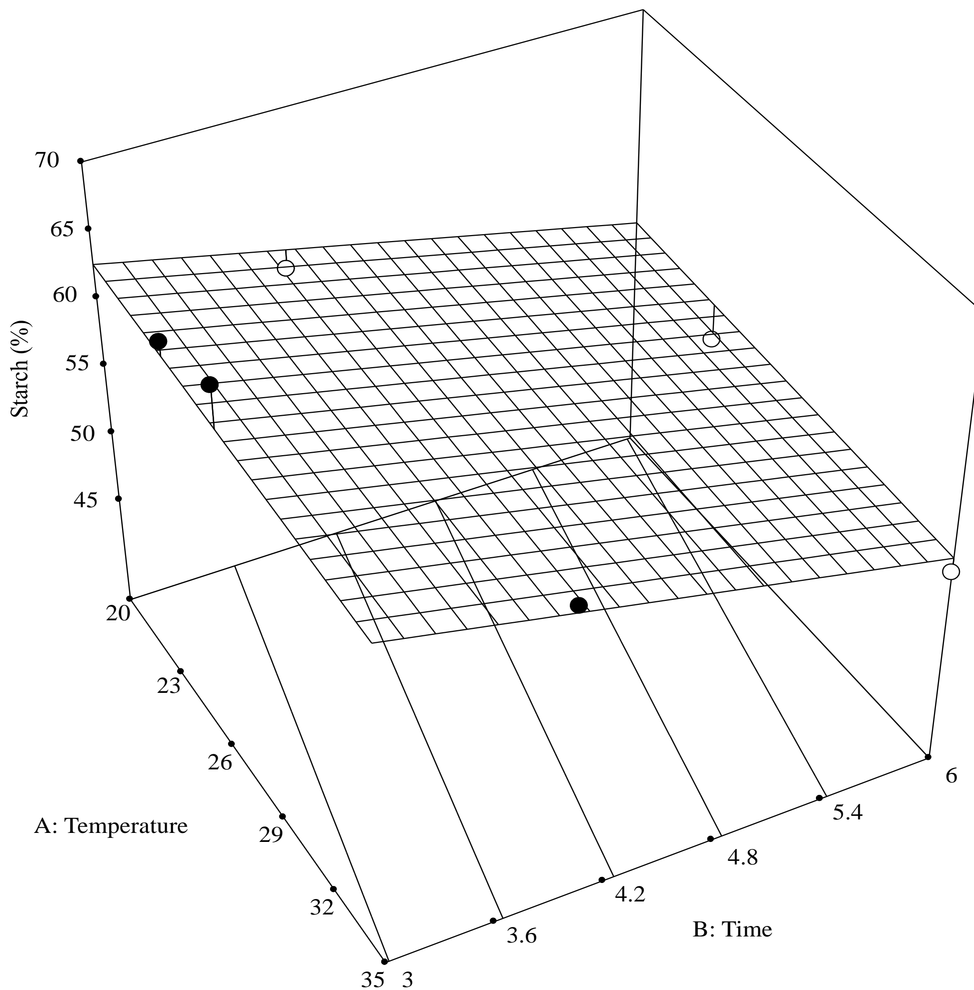

Fig 4.1 Central Composite plot for starch content for three factorial

This graph shows that the decrease in starch content as the temperature, time and relative humidity increases. This was also described in the result obtained by Moongngarm (2010).

The high temperature and high humidity for long time enhances the amylase activity which results in decrease in starch content of malted grain (Tejinder, 2007).

4.5 Effects of malting condition in reducing sugar content

The reducing sugar content for malted sorghum varied from 3.03% to 7.5%. Table B.18, B.19 and B.20 of appendix show the ANOVA of the model and other statistical attributes of reducing sugar content. Regression model fitted to experimental results of reducing sugar content showed that the model F-value of 20.62 was significant ($p<0.05$). The fit of model was also expressed by the coefficient of determination R^2, which was found to be 0.8490, indicating that 84.90% of the variability of the response could be explained by the model. The predicted R^2 value of 0.7369 was in reasonable agreement with the Adjusted R^2 value. The Adjusted R^2 value 0.8078 and Adequate Precision value 11.494 showed an adequate signal. A ratio greater than 4 is desirable and hence this model may be used to investigate the design space (Myers *et al.*, 2009).

Considering all the above criteria the model (equation 4.16) was selected to represent the variation of reducing sugar content with the independent variables and further analysis. The linear model fitted for reducing sugar content obtained from regression analysis in terms of actual factor of the variables is represented by equation 4.16.

$$\text{Reducing sugar} = +5.178 - 0.287A + 1.682B + 0.172C \quad (4.16)$$

Where A represents temperature, B represents time and C represents RH

The negative coefficient of the factors temperature indicates that there is negative correlation between temperature and reducing sugar. Similarly, the positive coefficient of time and RH indicates that there is positive correlation between reducing sugar with time and relative humidity. But the above equation shows that at overall the reducing sugar increases. Tejinder (2007) presented the similar results about reducing sugar content. This study shows that at high relative humidity for long time the reducing sugar content is higher. Abbas (2000) also mentioned the increase in reducing sugar during malting of sorghum.

The linear model graph for reducing sugar is presented in Fig 4.2

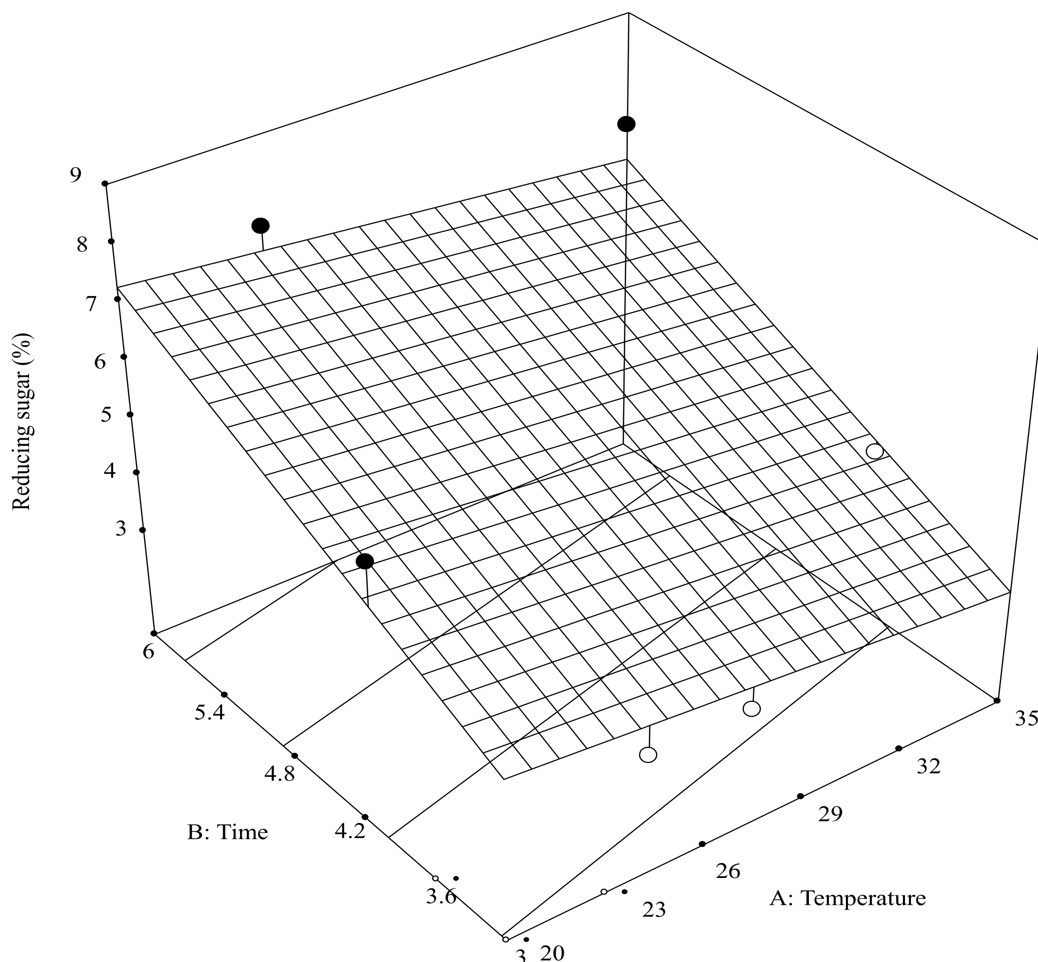

Fig 4.2 Central Composite plot for reducing sugar content for three factorial

The reducing sugar comparatively increases during germination due to the breakdown of starch by amylase (Abbas, 2000) but as the germination temperature increases the reducing sugar decrease relatively it may be due to less activity of amylase due to combined effect of malting conditions. Also the reducing sugar increase with increase in time and relative humidity. It may be due to favorable condition established for the breakdown of starch at high relative humidity. As the time increase more starch will breakdown due to exposure of starch to amylase for long time (Tejinder, 2007).

4.6 Effects of malting condition in amylase activity

The amylase activity for malted sorghum varied from 21.07% to 41.01%. Table B.21, B.22 and B.23 of appendix show the ANOVA of the model and other statistical attributes of amylase activity. Regression model fitted to experimental results of amylase activity showed

that the model F-value of 12.07 was significant ($p<0.05$). The fit of model was also expressed by the coefficient of determination R^2, which was found to be 0.7670, indicating that 76.70% of the variability of the response could be explained by the model. The predicted R^2 value of 0.6408 was in reasonable agreement with the Adjusted R^2 value. The Adjusted R^2 value 0.7035 and Adequate Precision value 7.618 showed an adequate signal. A ratio greater than 4 is desirable and hence this model may be used to investigate the design space (Myers *et al.*, 2009).

Considering all the above criteria the model (equation 4.17) was selected to represent the variation of amylase activity with the independent variables and further analysis. The linear model fitted for amylase activity obtained from regression analysis in terms of actual factor of the variables is represented by Eq. 4.17.

$$\text{Amylase activity} = +30.839 - 0.293A + 7.294B - 0.504C \quad (4.17)$$

Where A represents temperature, B represents time and C represents RH

The negative coefficient of temperature and relative humidity indicates that there is negative correlation of temperature and relative humidity with amylase activity. The positive coefficient of time indicates that there is positive correlation of time with amylase activity. Similar type of result was obtained by Tejinder (2007). In this study the amylase activity was increased during germination of barley. Moongngarm (2010) observed increase in amylase activity during germination of rice after 2 days of germination rapidly while after 6 days the amylase activity decreased. The increase in RH and temperature results in decrease in amylase activity (Moongngarm, 2010).

The linear model graph for amylase activity is presented in Fig 4.3

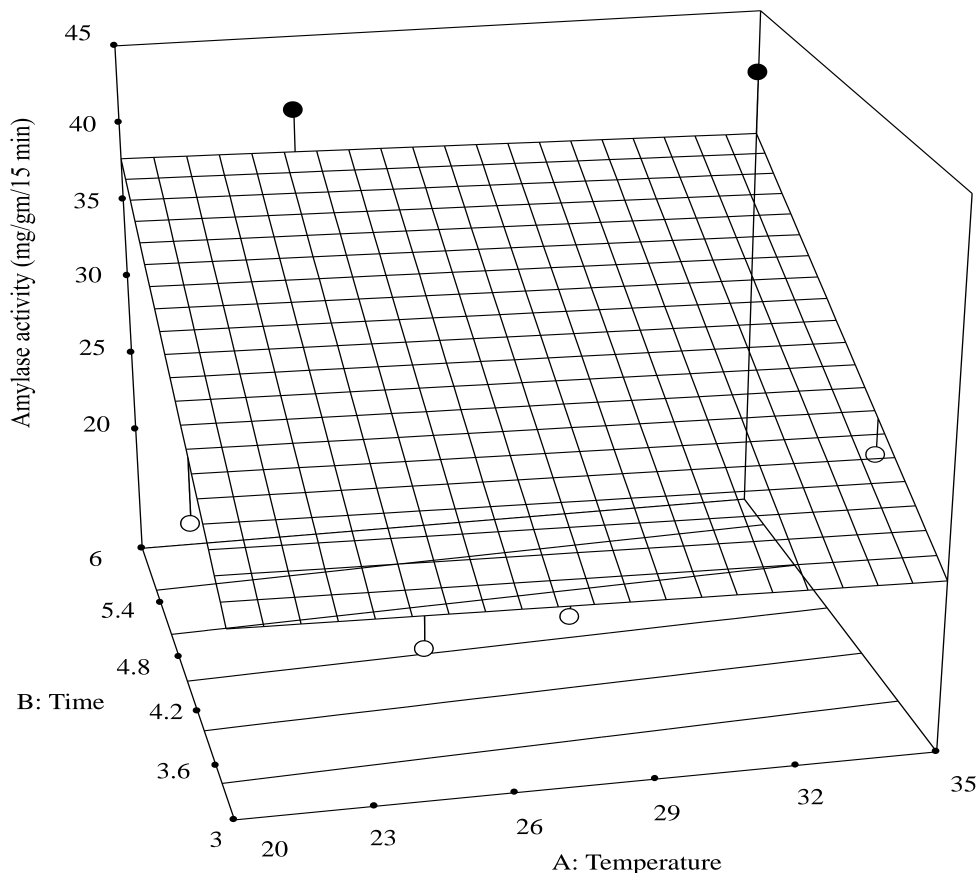

Fig 4.3 Central Composite plot for amylase activity for three factorial

The above graph shows that amylase activity increases with increase in time and decreases with increase in temperature linearly. As Moongngarm (2010) the activity of enzymes depends on temperature, moisture content, and environmental conditions of germination. So, the increase in amylase activity may due to the presence of favorable condition i.e. appropriate moisture, temperature and adequate presence of starch content during germination. Ratnavati and Chavan (2016) also observed the increased amylase activity during germination of sorghum grain as the time increases during the period of 3 days.

4.7 Multi response optimization criteria

As the reducing sugar and amylase activity should increase after malting and starch content should decrease. So, the optimized result was obtained by keeping maximum reducing sugar, maximum amylase activity and minimum starch content for minimum time and temperature and maximum RH as given in Appendix B.24. Total 23 solutions were found among 109 starting points. Among 23 solutions, solution no. 1 was selected having reducing sugar

content as 6.497% as dextrose, amylase activity of 34.352 mg/g sample/15 min and 56.429% of starch content with desirability of 61.2%. This solution was selected on the basis of desirability and high reducing sugar and amylase activity. The reducing sugar and amylase activity was taken as main criteria because they play important role during beer preparation from the sorghum malt. Hence, the optimized malting condition was found as 20°C at 95% RH for 5.266 days. Rounding off we can conclude that the optimized malting condition be 20°C at 95% RH for 5 days.

The optimized result was shown in Table 4.4 and Table 4.5

Table 4.4 Verification of malting variables

Response	Predicted Mean	Predicted Median	Observed	Std. Dev.	SE Mean
Reducing sugar	6.496683	6.496683	6.4967	0.731601	0.401369
Starch	56.43048	56.43048	56.429	2.666153	1.4627
Amylase activity	34.34885	34.34885	34.352	4.070372	2.23308

Table 4.5 Optimized malting conditions

Factor	Name	Obtained Level	Low Level	High Level
A	Temperature	20.00	20.00	35.00
B	Time	5.266	3.00	6.00
C	RH	95.00	70.00	95.00

Conclusion

5.1 Conclusion

On the basis of the analysis of sorghum grain and malt, the following conclusions were drawn.

- Malting of sorghum at different temperature, RH and time were performed successfully.
- The physical properties like 1000 kernel weight, bulk density and particle density decreased after malting.
- Proximate composition of sorghum (Moisture content, protein content, crude fiber and crude fat) decreased after malting but ash content increased after malting.
- Functional properties like water absorption capacity, swelling power, gelatinization concentration and foaming stability improved after malting. Water absorption capacity, swelling power and foaming stability increased while gelatinization concentration decreased after malting.
- Starch content of sorghum decreased after malting while reducing sugar and amylase activity increased.
- On the basis of maximum reducing sugar, the best malting condition was found to be 20^0 C at 95% RH for 5 days.

5.2 Recommendation

- Malting of sorghum at 20°C at 95% RH for 5 days was recommended to produce better malt.
- Soaking at different mold inhibitors solution can be done to inhibit the mold growth before germination of sorghum grain.
- Comparative study of malting of different varieties of sorghum (red and white) can be done at optimized condition.

Summary

Malting is the process comprising of steeping, germination and kilning. Steeping is one of the process that improve the nutritional value of sorghum by the breakdown of several complex components into simpler compounds which alter the texture and flavor. Germination is another process which improve the nutrition of sorghum seed as it helps in reducing starch component into simple sugars by the action of amylases, induces hydrolytic enzyme synthesis such as phytates and some flavonoids components. The last process is kilning which helps to stop germination of sorghum seed and reduces the grain moisture content to desirable limit.

For this study the sorghum was bought from the market of Kathmandu. The sorghum grains were soaked for 24 h at 28°C, at last steeped in KMS solution for 10 min to prevent mold growth and germinated at different temperature and relative humidity for various period of time which were obtained from design expert. After germination the sorghum grains were dried at 50°C for 24 h to obtain the desired final moisture content. The prepared sorghum malt samples were then taken for analysis. Analysis of physical, chemical and functional properties were carried out for all samples.

The 1000 kernel wt., bulk density and particle density were found to be decreased after malting from 29.91 g to 18.52 g, 82.33 kg/HL to 66 kg/HL and 1.23 g/ml to 0.94 g/ml respectively. While porosity of grain varies after malting from 0.26 to 0.35. Protein, starch, crude fiber, crude fat and moisture content were found to be decreased after malting from 12.8% to 10.05%, 69.77% to 51.16%, 1.13% to 0.95%, 4.71% to 2.25% and 10.37% to 6.93% respectively. While Ash content, reducing sugar and amylase activity were increased after malting from 1.45% to 2.96%, 1.04% to 5.14% and 0.00193 mg/g sample/15 min to 41.33 mg/g sample/ 15 min. The water absorption capacity, foam stability and swelling power increased after malting from 1.22 g/g sample to 1.80 g/g sample, 10.39% to 18% and 0.87 g/g sample to 3.08 g/g sample respectively while gelatinization concentration decreased after malting from 12% to 6%. Optimization of malting condition was done by using RSM on the basis of amylase activity, starch content and reducing sugar. The 18 solutions were found for optimized malting condition with maximized reducing sugar and amylase activity and minimized starch content. Out of 18 solutions solution no. 1 was found to be best by keeping minimum temperature and time and maximum relative humidity as criteria and

giving higher priority to reducing sugar. Hence, the optimum malting condition was found to be at 20°C, 95% RH for 5 days.

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Appendices

Appendix A

1. Calibration curve for amylase activity

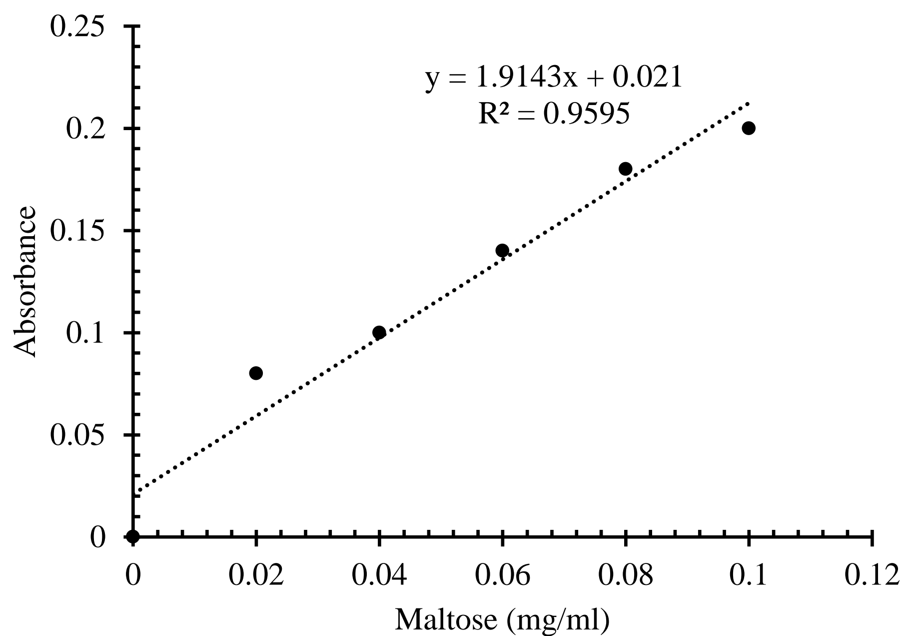

Fig. A.1 Standard curve of maltose for α-amylase activity

Table A.1 Relative humidity at different concentration of H_2SO_4

% RH	% H_2SO_4	% RH	% H_2SO_4
1	80	57	40
2.2	75	66.8	35
5.1	70	75.9	30
17	65	88.5	20
26.8	55	92.9	15
36.9	50	96.2	10
46.8	45	98.6	5

Source: Wilson (1921)

1. Graph for RH maintainance

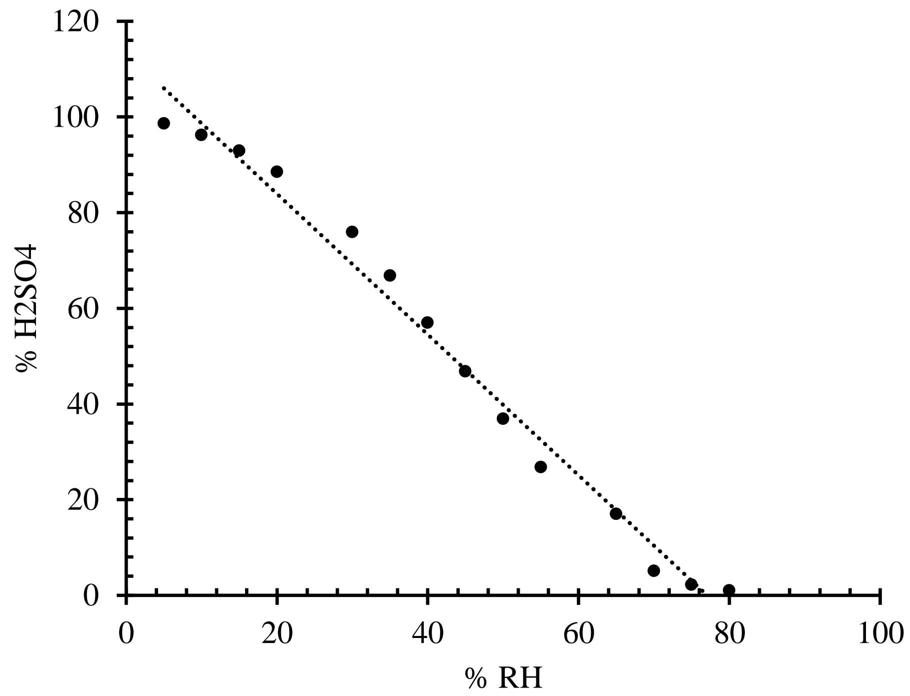

Fig. A.2 Graph showing % RH vs % H_2SO_4

Appendix B

Table B.1 ANOVA for sphericity

Source	Sum of Squares	df	Mean Square	F Value	p-value Prob > F	
Model	4.303E-003	3	1.434E-003	1.02	0.04228	significant
A-Temperature	1.450E-004	1	1.450E-004	0.10	0.54070	
B-Time	2.307E-003	1	2.307E-003	1.63	0.02275	
C-RH	1.375E-003	1	1.375E-003	0.97	0.03451	
Residual	0.016	11	1.413E-003			
Cor Total	0.020	14				

Table B.2 ANOVA for 1000 kernel wt.

Source	Sum of Squares	df	Mean Square	F Value	p-value Prob > F	
Model	23.50	9	2.61	0.17	0.0489	significant
A-Temperature	9.71	1	9.71	0.64	0.0415	
B-Time	0.39	1	0.39	0.026	0.1789	
C-RH	0.047	1	0.047	3.071E-003	0.0580	
AB	0.40	1	0.40	0.026	0.1776	
AC	0.058	1	0.058	3.826E-003	0.0531	
BC	0.76	1	0.76	0.050	0.0319	
A^2	1.42	1	1.42	0.093	0.0730	
B^2	0.22	1	0.22	0.014	0.9098	
C^2	3.77	1	3.77	0.25	0.6406	
Residual	76.44	5	15.29			
Cor Total	99.93	14				

Table B.3 ANOVA for bulk density

Source	Sum of Squares	df	Mean Square	F Value	p-value Prob > F	
Model	11.28	3	3.76	0.27	0.0489	significant
A-Temperature	8.144E-003	1	8.144E-003	5.750E-004	0.0813	
B-Time	5.37	1	5.37	0.38	0.0507	
C-RH	6.56	1	6.56	0.46	0.0103	
Residual	155.80	11	14.16			
Cor Total	167.08	14				

Table B.4 ANOVA for particle density

Source	Sum of Squares	df	Mean Square	F Value	p-value Prob > F	
Model	0.017	6	2.892E-003	1.00	0.04874	significant
A-Temperature	1.649E-003	1	1.649E-003	0.57	0.04727	
B-Time	8.240E-004	1	8.240E-004	0.28	0.06088	
C-RH	7.351E-003	1	7.351E-003	2.53	0.0503	
AB	6.110E-003	1	6.110E-003	2.10	0.0850	
AC	7.379E-004	1	7.379E-004	0.25	0.06278	
BC	4.842E-005	1	4.842E-005	0.017	0.9005	
Residual	0.023	8	2.905E-003			
Cor Total	0.041	14				

Table B.5 ANOVA for porosity

Source	Sum of Squares	df	Mean Square	F Value	p-value Prob > F	
Model	5.484E-003	6	9.139E-004	1.37	0.03303	significant
A-Temperature	1.103E-003	1	1.103E-003	1.66	0.02341	
B-Time	5.949E-004	1	5.949E-004	0.89	0.03722	
C-RH	1.888E-003	1	1.888E-003	2.84	0.01307	
AB	1.091E-003	1	1.091E-003	1.64	0.02363	
AC	8.929E-005	1	8.929E-005	0.13	0.07237	
BC	1.403E-003	1	1.403E-003	2.11	0.01847	
Residual	5.327E-003	8	6.659E-004			
Cor Total	0.011	14				

Table B.6 ANOVA for moisture content

Source	Sum of Squares	df	Mean Square	F Value	p-value Prob > F	
Model	3.42	3	1.14	1.25	0.0340	significant
A-Temperature	0.069	1	0.069	0.075	0.7888	
B-Time	2.53	1	2.53	2.76	0.0247	
C-RH	0.50	1	0.50	0.55	0.4738	
Residual	10.07	11	0.92			
Cor Total	13.49	14				

Table B.7 ANOVA for protein content

Source	Sum of Squares	df	Mean Square	F Value	p-value Prob > F	
Model	7.35	9	0.82	1.72	0.0286	significant
A-Temperature	0.47	1	0.47	0.98	0.3672	
B-Time	1.60	1	1.60	3.37	0.0260	
C-RH	0.12	1	0.12	0.25	0.6376	
AB	0.054	1	0.054	0.11	0.7502	
AC	0.10	1	0.10	0.22	0.6585	
BC	0.17	1	0.17	0.35	0.5813	
A^2	0.027	1	0.027	0.056	0.8224	
B^2	0.11	1	0.11	0.22	0.6562	
C^2	3.40	1	3.40	7.14	0.0442	
Residual	2.38	5	0.48			
Cor Total	9.73	14				

Table B.8 ANOVA for ash content

Source	Sum of Squares	df	Mean Square	F Value	p-value Prob > F
Model	0.000	0			
Residual	1.61	14	0.11		
Cor Total	1.61	14			

Table B.9 ANOVA for crude fat content

Source	Sum of Squares	Df	Mean Square	F Value	p-value Prob > F	
Model	2.01	3	0.67	4.59	0.0256	significant
A-Temperature	0.90	1	0.90	6.14	0.0307	
B-Time	1.17	1	1.17	8.01	0.0164	
C-RH	0.18	1	0.18	1.20	0.2963	
Residual	1.61	11	0.15			
Cor Total	3.62	14				

Table B.10 ANOVA for crude fiber content

Source	Sum of Squares	df	Mean Square	F Value	p-value Prob > F	
Model	0.61	9	0.068	1.40	0.03719	significant
A-Temperature	0.28	1	0.28	5.74	0.0619	
B-Time	7.172E-004	1	7.172E-004	0.015	0.9080	
C-RH	0.26	1	0.26	5.33	0.0691	
AB	1.005E-004	1	1.005E-004	2.068E-003	0.9655	
AC	4.346E-005	1	4.346E-005	8.944E-004	0.9773	
BC	0.055	1	0.055	1.12	0.3377	
A^2	0.27	1	0.27	5.61	0.0641	
B^2	0.013	1	0.013	0.26	0.6316	
C^2	9.142E-003	1	9.142E-003	0.19	0.6826	
Residual	0.24	5	0.049			
Cor Total	0.85	14				

Table B.11 ANOVA for WAC

Source	Sum of Squares	df	Mean Square	F Value	p-value Prob > F	
Model	0.53	9	0.059	5.74	0.0343	significant
A-Temperature	8.546E-004	1	8.546E-004	0.083	0.7854	
B-Time	0.11	1	0.11	10.70	0.0222	
C-RH	5.938E-003	1	5.938E-003	0.57	0.4829	
AB	2.559E-003	1	2.559E-003	0.25	0.6401	
AC	1.601E-003	1	1.601E-003	0.15	0.7103	
BC	0.053	1	0.053	5.16	0.0723	
A^2	0.050	1	0.050	4.78	0.0804	
B^2	1.014E-004	1	1.014E-004	9.800E-003	0.9250	
C^2	0.16	1	0.16	15.40	0.0111	
Residual	0.052	5	0.010			
Cor Total	0.59	14				

Table B.12 ANOVA for foam stability

Source	Sum of Squares	df	Mean Square	F Value	p-value Prob > F	
Model	16.92	3	5.64	1.20	0.0356	significant
A-Temperature	10.68	1	10.68	2.26	0.1605	
B-Time	7.57	1	7.57	1.61	0.0233	
C-RH	0.54	1	0.54	0.11	0.0413	
Residual	51.86	11	4.71			
Cor Total	68.79	14				

Table B.13 ANOVA for swelling power

Source	Sum of Squares	df	Mean Square	F Value	p-value Prob > F	
Model	1.63	3	0.54	2.90	0.0331	significant
A-Temperature	0.41	1	0.41	2.20	0.1660	
B-Time	1.32	1	1.32	7.01	0.0227	
C-RH	0.030	1	0.030	0.16	0.04985	
Residual	2.07	11	0.19			
Cor Total	3.70	14				

Table B.14 ANOVA for gelatinization concentration

Source	Sum of Squares	df	Mean Square	F Value	p-value Prob > F
Model	0.000	0			
Residual	46.40	14	3.31		
Cor Total	46.40	14			

Table B.15 Model summary statistics for starch content

Source	Std. Dev.	R-Squared	Adjusted R-Squared	Predicted R-Squared	PRESS	
Linear	2.67	0.7534	0.6861	0.5774	134.00	Suggested
2FI	2.60	0.8292	0.7011	0.6053	125.14	
Quadratic	2.83	0.8737	0.6463	-0.1216	355.64	
Cubic					+	Aliased

+ Case(s) with leverage of 1.0000: PRESS statistic not defined

Table B.16 ANOVA for starch content linear model

Std. Dev.	2.67	R-Squared	0.7534
Mean	58.57	Adj R-Squared	0.6861
C.V. %	4.55	Pred R-Squared	0.5774
PRESS	134.00	Adeq Precision	8.678
-2 Log Likelihood	67.33	BIC	78.17
		AICc	79.33

Table B.17 Summary of starch content linear model

Source	Sum of Squares	Df	Mean Square	F Value	p-value Prob > F	
Model	238.87	3	79.62	11.20	0.0011	Significant
A-Temperature	6.36	1	6.36	0.89	0.3646	
B-Time	204.90	1	204.90	28.82	0.0002	
C-RH	8.79	1	8.79	1.24	0.2898	
Residual	78.19	11	7.11			
Cor Total	317.07	14				

Table B.18 Model summary statistics for reducing sugar

Source	Std. Dev.	R-Squared	Adjusted R-Squared	Predicted R-Squared	PRESS	
Linear	0.731601	0.849005	0.807824	0.736859	10.26041	Suggested
2FI	0.787821	0.872659	0.777153	0.569994	16.76684	
Quadratic	0.875834	0.901636	0.724581	0.193872	31.43266	
Cubic					+	Aliased

+ Case(s) with leverage of 1.0000: PRESS statistic not defined

Table B.19 ANOVA for reducing sugar linear model

Source	Sum of Squares	df	Mean Square	F Value	p-value Prob > F	
Model	33.10448	3	11.03483	20.61662	8.04E-05	Significant
A-Temperature	1.747379	1	1.747379	3.264669	0.098188	
B-Time	31.80732	1	31.80732	59.42634	9.28E-06	
C-RH	0.33729	1	0.33729	0.630167	0.444082	
Residual	5.887633	11	0.535239			
Cor Total	38.99212	14				

Table B.20 Summary of reducing sugar linear model

Std. Dev.	0.73	R-Squared	0.8490
Mean	4.98	Adj R-Squared	0.8078
C.V. %	14.69	Pred R-Squared	0.7369
PRESS	10.26	Adeq Precision	11.494
-2 Log Likelihood	28.54	BIC	39.37
		AICc	40.54

Table B.21 Model summary statistics for amylase activity

Source	Std. Dev.	R-Squared	Adjusted R-Squared	Predicted R-Squared	PRESS	
Linear	4.07	0.7670	0.7035	0.6408	281.00	Suggested
2FI	4.43	0.7989	0.6482	0.4648	418.65	
Quadratic	4.82	0.8514	0.5840	-0.2008	939.31	
Cubic					+	Aliased

+ Case(s) with leverage of 1.0000: PRESS statistic not defined

Table B.22 ANOVA for amylase activity linear model

Source	Sum of Squares	Df	Mean Square	F Value	p-value Prob > F	
Model	599.96	3	199.99	12.07	0.0008	Significant
A-Temperature	1.82	1	1.82	0.11	0.7464	
B-Time	597.94	1	597.94	36.09	< 0.0001	
C-RH	2.87	1	2.87	0.17	0.6850	
Residual	182.25	11	16.57			
Cor Total	782.21	14				

Table B.23 Summary of amylase activity linear model

Std. Dev.	4.07	R-Squared	0.7670
Mean	30.42	Adj R-Squared	0.7035
C.V. %	13.38	Pred R-Squared	0.6408
PRESS	281.00	Adeq Precision	7.618
-2 Log Likelihood	80.03	BIC	90.86
		AICc	92.03

Table B.24 Solutions of optimization result

S.N.	Temperature	Time	RH	Reducing sugar	Starch	Amylase activity	Desirability	
1	20.000	5.266	95.000	6.497	56.429	34.352	0.612	Selected
2	20.000	5.255	95.000	6.486	56.458	34.302	0.612	
3	20.000	5.281	95.000	6.514	56.386	34.424	0.612	
4	20.000	5.243	95.000	6.472	56.493	34.243	0.612	
5	20.000	5.296	95.000	6.531	56.343	34.498	0.612	
6	20.000	5.269	94.908	6.500	56.425	34.373	0.612	
7	20.000	5.309	94.959	6.545	56.308	34.564	0.612	
8	20.052	5.263	95.000	6.492	56.430	34.337	0.612	
9	20.000	5.168	95.000	6.388	56.705	33.879	0.611	
10	20.000	5.358	95.000	6.601	56.166	34.801	0.611	
11	20.048	5.200	95.000	6.421	56.610	34.030	0.611	
12	20.000	5.147	95.000	6.364	56.766	33.775	0.611	
13	20.133	5.255	95.000	6.478	56.443	34.294	0.610	
14	20.000	5.071	95.000	6.279	56.982	33.406	0.608	
15	20.000	5.464	95.000	6.719	55.865	35.314	0.607	
16	21.051	5.217	95.000	6.382	56.452	34.054	0.596	
17	20.000	5.241	91.706	6.424	56.732	34.363	0.596	
18	22.795	5.408	95.000	6.497	55.716	34.882	0.566	

Color plates

P.1 Steeping of sorghum

P.2 Germination of sorghum

P.3 Germinated sorghum grain

P.4 Analysis of functional properties