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Studies on teff (*Eragrostis tef*) malt as alternative raw material for lactic acid fermented gluten-free beverages

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LIST OF PUBLICATIONS

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3. **Mekonnen M. Gebremariam**, M. Zarnkow and T. Becker: Effect of teff (E*ragrostis tef*) variety and storage on the malt quality attributes. Journal of the Institute of Brewing (2013), 119 (1): 64–70.
4. **Mekonnen M. Gebremariam**, M. Zarnkow and T. Becker: Thermal stability of starch degrading enzymes of teff (*Eragrostis tef*) malt during isothermal mashing. Process Biochemistry (2013), 48 (12): 1928–1932.
5. **Mekonnen M. Gebremariam**, Ahmed Hassani, M. Zarnkow and T. Becker: Investigation of fermentation conditions for teff (*Eragrostis tef*) malt-wort by *Lactobacillus amylolyticus.* LWT - Food Science and Technology (2014), http://dx.doi.org/10.1016/j.lwt.2014.11.008.
6. **Mekonnen M. Gebremariam**, Kebede Abegaz, M. Zarnkow and T. Becker. Studies on the mashing conditions of teff (*Eragrostis tef*) malt as a raw material for lactic acid fermented gluten-free beverage (2014), (in preparation).
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CONTENTS

Summary

Interest in teff has been increased noticeably due to its attractive nutritional profile and gluten-free characteristics. Fermentation further improves the health benefits of its final products. This project was aimed to develop kilning and mashing programs for teff malt, and investigate fermentation condition for teff malt wort in order to utilize it as a raw material for gluten-free lactic acid fermented beverages. The first phase of the project was aimed to study the influence of kilning on the enzyme activities and DMS level, and develop a kilning program that yields teff malt with low DMS (< 7 mg/kg) with no or little damage on its enzyme activities. The teff malt samples were dried using isothermal kilning at temperatures ranging between 30 and 70 °C for 40 h with sampling at certain time interval. The results showed that enzyme activities, DMS, and moisture contents were significantly affected (P < 0.05) by kilning time and temperature. To set a kilning program, two temperature regimens 18 h at 30 °C followed by 1 h at 60 °C and (3 or 5) h at 65 °C (R1), and 18 h at 30 °C followed by 1 h at 60 °C and (3 or 5) h at 80 °C (R2) were selected based on results from isothermal kilning and other trial experiments. The results from the two kilning programs showed that prolonged (5 h) curing in both kilning regimens caused an adverse effect on enzyme activities. The first kilning program (R1) with shorter curing time (3 h) was found to be the best condition in relation to preserving the enzyme activities, and it was considered as optimal kilning program for teff malt samples.

The second phase of the project was designed to study thermal stabilities of amylolytic enzymes, and develop mashing program for 100% teff malt. The target in developing the mashing program was producing wort with highest possible extract that yields at least 40 g/L fermentable sugar, FAN values of higher than 100 mg/L, and pH in the range of 5 to 6, preferably the lowest pH in this range. Before proceeding to the mashing program, different malt to water ratios (1:3, 1:3.5, 1:4, 1:4.5 and 1:5) were tested to identify suitable grist to liquor ratio that results in thicker wort with highest possible extract. Due to its high extract content and thicker mash, 1:4 was selected as the optimal ratio. The thermal stability study of the enzymes in teff malt showed that alpha-amylase was relatively stable at higher temperatures when compared to beta-amylase and limit dextrinase. Hence, the temperature at which most of the alpha-amylases are deactivated (78 °C) was taken as mashing-off temperature for the teff malt mashing program. Temperatures between 65 and 74 °C

were tested for investigation of the conversion rest temperature. The highest extract content (86%) was observed at 71 °C. Low pH (5.69) and high FAN (98 mg/L) values were also recorded at this temperature when compared to most other tested temperatures. Inclusion of lower mashing-in temperature in the mashing program substantially improved the concentrations of FAN (128 mg/L) and fermentable sugar (58 g/L) in the final wort. The highest FAN content was observed at the mashing-in temperatures between 40 and 50 °C. But the extract content at 50 °C was low when compared to the values at 40 and 45 °C. Taking the advantage of lower pH and viscosity at 40 °C, this temperature was selected as mashing-in temperature. Therefore, 30 min rest at 40 °C followed by 60 min rest at 71 °C and 10 min rest at 78 °C was selected as a suitable mashing program for teff malt.

The third phase of the project was aimed to study the influences of fermentation factors such as temperature, fermentation time, initial pH and initial bacterial cell concentration on the change in lactic acid concentration, pH of the fermentation medium, lactic acid to sugar ratio, and extract content. In addition to that it was also designed to investigate fermentation condition for the teff malt-wort. The results from fermentation of the teff malt-wort by *Lactobacillus amylolyticus* showed that temperature was the main factor influencing formation of lactic acid, change in lactic acid to sugar ration, and pH of the fermentation medium. The optimum conditions of the factors such as temperature, initial pH, initial cell concentration, and fermentation time for fermenting teff malt-wort were found to be 42 °C, 5.4, 1.86×10^5 cells/ml, and 52 h, respectively. The sensory quality assessment revealed that the beverage produced under the optimal fermentation condition had high acceptance of odour, taste and flavor, and its pH (3.5) was sufficiently low to suppress the growth of food spoilage microorganisms.

Zusammenfassung

Das Interesse an Teff wuchs in den letzten Jahren deutlich, aufgrund seines attraktiven Nährwertprofils und den glutenfreien Eigenschaften. Die gesundheitlichen Vorteile der Endprodukte können durch Fermentation verbessert werden. Das Vergären mit Milchsäurebakterien kann mehrere zusätzliche Vorteile, einschließlich ihrer möglichen Verwendung als Probiotikum und antimikrobielle Wirkungen, einbringen. Das Projekt zielte darauf ab, Mälzung, Maischen und Gärbedingungen von Teff Malz als potenzieller alternativer Rohstoff für glutenfreie Milchsäure vergorene Getränken zu untersuchen. Die Sorten eines Getreides haben erheblichen Einfluss auf die Braugerstenqualität. Aus diesem Grund wurden fünf Teff Sorten wie DZ-Cr-387 (*Kuncho*), Ivory, Brown, Dessie, und Sirgaynia analysiert, um eine Sorte mit niedriger Verkleisterungstemperatur auszuwählen. Die Verkleisterungstemperaturen der Sorten lagen in einem Bereich zwischen 69,7 bis 72,8 °C, wobei der niedrigste Wert für die Sorte Kuncho Teff ermittelt wurde.

Die erste Phase des Projekts zielte darauf ab, den Einfluss des Darrens auf die Enzymaktivitäten und den DMS-Gehalt zu studieren, und die Entwicklung eines Programmes für das Darren von Teff Malz mit geringem DMS-Gehalt (<7 mg / kg) und keinem oder wenig Schaden der Enzymaktivitäten. Die Teff Malz Proben wurden mit isothermem Darren bei Temperaturen zwischen 30 und 70 °C für 40 h, mit einer Probenahme in bestimmten Zeitintervallen, getrocknet. Die Ergebnisse zeigten, dass Enzymaktivitäten, DMS-Gehalt und Feuchtigkeitsgehalt deutlich ($P < 0,05$) von der Darrzeit und –temperatur betroffen waren. Als Darrprogramm basierend auf den Ergebnissen der isothermen Darrversuche und weiterer Experimente wurden zwei Temperatursequenzen ausgewählt: 18 h bei 30 °C, 1 h bei 60 °C und (3 oder 5) h bei 65° C (R1) und 18 h bei 30 °C nach 1 h bei 60 °C und (3 oder 5) h bei 80 °C (R2). Die Ergebnisse der beiden Darrprogramme zeigten, dass längeres (5 h) Heizen bei beiden Sequenzen sich nachteilig auf die Enzymaktivität auswirken kann. Als bestes Programm wurde R1 festgestellt, aufgrund seiner kürzeren Heizzeit (3 h) und der damit einhergehenden Erhaltung der Enzymaktivitäten. Für weitere Versuche wurde dieses Darrprogramm als optimal für Teff Malz Proben betrachtet.

Die zweite Phase des Projektes diente dazu, die thermische Stabilität der amylolytischen Enzyme zu studieren, und ein Maischprogramm für eine 100 % Teff Malz-Schüttung zu finden. Das Ziel bei der Entwicklung des Maischprogramm war die Erzeugung von Würze mit höchstmöglichem Extrakt, welche mindestens 40 g/l fermentierbaren Zucker, mehr als 100 mg/l freien Aminostickstoff (FAN) enthielten und

sich in einem pH-Bereich zwischen 5 bis 6 lagen, bevorzugt wurde der niedrigste pH-Wert. Vor der Erstellung des Maischprogrammes wurden verschiedene Malz-Wasser-Verhältnisse (1:3, 1:3,5, 1:4, 1:4,5 und 1:5) getestet, um das geeignete Schrot-Flüssigkeitsverhältnis für weitere Experimente zu identifizieren. Aufgrund seines hohen Extraktgehaltes und der dickbreiigen Maischen wurde das 1:4 Verhältnis als bestes ausgewählt. Die Untersuchung der thermischen Stabilität der Teff Malz Enzyme zeigte, dass α-Amylase, verglichen mit β-Amylase und Grenzedextrinase, relativ stabil bei höheren Temperaturen war. Daher wurde die Temperatur (78 ° C), bei der alle α-Amylasen deaktiviert wurden, als Abmaischtemperatur für die Teff Malz Maischprogramm ausgewählt. Temperaturen zwischen 65 und 74 °C wurden als Rasttemperaturen zur enzymatischen Umwandlung untersucht. Der höchste Gehalt an Extrakt (86%) wurde bei 71 °C beobachtet. Verglichen mit anderen Temperaturen, wurden bei dieser ebenfalls ein niedriger pH-Wert (5,69) und ein hoher FAN-Gehalt (98 mg/l) gemessen. Unter Einbeziehung einer niedrigen Einmaischtemperatur im Maischprogramm verbesserten sich die Konzentrationen an FAN (128 mg/l) und vergärbaren Zucker (58 g/l) in der finalen Würze. Die höchste FAN-Konzentration wurde bei Einmaischtemperaturen zwischen 40 und 50 °C detektiert. Wohingegen der Extraktgehalt bei 50 °C geringer war als zwischen 40 und 45 °C. So wurde eine Einmaischtemperatur von 40 °C gewählt, aufgrund der Vorteile niedriger pH-Werte und Viskositäten. Das gewählte Maischprogramm sah folgende Parameter vor: 30 min Rest bei 40 °C, gefolgt von 60 min Pause bei 71 °C und 10 min 78 ° C.

Die dritte Phase des Projekts zielte darauf ab, die Einflüsse der Fermentation, wie Temperatur, Fermentationszeit, pH-Wert und Bakterienzellkonzentration zu Beginn der Gärung auf die Änderungen in Milchsäurekonzentration, pH-Wert des Fermentationsmediums, Milchsäure-Zucker-Verhältnis und Extraktgehalt zu studieren. Zusätzlich dazu wurde auch darauf abgezielt, die optimalen Voraussetzungen für die Fermentation Teff Malz-Würze zu finden. Die Ergebnisse aus der Gärung der Malz-Teff Würze durch *Lactobacillus amylolyticus* zeigte, dass die Temperatur der dominierende Einflussfaktor für die meisten Zielgrößen war. Die optimalen Bedingungen für Temperatur, pH-Wert, Zellkonzentration und Fermentationszeit für die Vergärung von Teff Malz-Würze wurde gefunden als 42 ° C, 5,4, $1{,}86 \times 10^5$ Zellen/ml, und 52 h. Die sensorische Qualität hat ergeben, dass das unter optimalen Fermentationsbedingungen erzeugte Getränk eine hohe Intensität des harmonischen Geschmacks, eine hohe Akzeptanz in Geruch, Geschmack und Aroma, und einen ausreichend niedrigen pH-Wert (3,5) hatte, um das Wachstum von lebensmittelverderbenden Mikroorganismen zu unterdrücken.

1 INTRODUCTION

1.1 Background

Fermentation is one of the oldest methods of food preparation and preservation. Cereals are globally major food crops as well as substrates for fermentation, and a large proportion of the world cereals production is processed by fermentation prior to consumption (Chavan and Kadam, 1989; Minamiyama et al., 2003). The enhancement of attractive flavour and texture, the improved shelf-life and formation of healthy ingredients as a result of the fermentation process are among the main reasons for fermenting the cereals before consumption. The beneficial effects associated with fermented foods and beverages also include reduced loss of raw materials, reduced cooking time, improvement of protein quality especially the level of available lysine, and carbohydrate digestibility, improved bioavailability of micronutrients, and elimination of toxic and anti-nutritional factors (Addo et al., 1996; Amoa-Awua et al., 1997; Chavan and Kadam, 1989; Kreisz et al., 2008; Minamiyama et al., 2003; Steinkraus, 1994). Cereal based drinks contain natural sugar and are excellent sources of antioxidants, vitamins and other health-promoting substances (Kreisz et al., 2008; Minamiyama et al., 2003). In addition to the aforesaid health benefits, cereals contain biologically active ingredients like dietary and functional fibres contributing to about 50% of the fibre intake in western countries (Lambo et al., 2005).

Africa is the center of origin and still today the major producing area for several cereal crops, notably sorghum, pearl millet, finger millet, teff, fonio, and African rice. These traditional African cereals are sometimes called “Orphan Crops”, or even “Lost Crops” (National Research Council, 1996). This is despite the fact that they are staple foods for millions of people in the semi-arid regions of the world. African cereal grains are extremely versatile foodstuffs and are processed in to a very wide range of traditional food and beverage products. However, large-scale commercial

production of these cereal grain food products is still rather limited. Among these cereals, teff is commonly used in Ethiopia. The preparation of many indigenous or traditional fermented foods and beverages remain today as a house art (FAO, 1995; Ketema et al. 1998).

Teff (*Eragrostis tef*) is a low risk cereal that grows in a wider ecology and can tolerate harsh environmental conditions. It originated in Ethiopia where it is locally processed into different foods and beverages including porridge, *kitta* (unleavened bread), *atmit* (gruel), a pancake-like bread called *injera,* and local alcoholic beverages such as opaque beer called *tela,* a spirit called *arake* and *shamit* at household level (Selinus, 1971; Ketema, 1997). Processing of teff into different foods and beverages is usually done by traditional ways and is mostly limited to the household level. On the other hand, there is a high global demand of the cereal, and it is gaining popularity as a health food. Its flour is becoming the most preferred ingredient in gluten-free diets and sport foods (Dekking et al., 2005). Beverage industries are among the food industries in quest of alternative raw materials for gluten-free beverages. Although beverages are not major foods, they serve as source of energy and important nutrients for humans in several countries. It has been reported that nutrient absorption from cereal beverages is higher than that from the constituents, which are used to prepare the beverage (Ade-Omowaye et al., 2006; Derman et al., 1980). The fermentations of cereal based beverages are usually performed using starter cultures (Josephsen and Jespersen, 2004). Health-conscious consumers are in quest of natural foods without chemical preservatives that will fit in their healthy lifestyle. Hence, fermenting with lactic acid bacteria (LAB) could be a good solution to address the problems associated with use of chemical preservatives. Lactic acid fermentation is the simplest and often the safest way of preserving foods. LAB are used as ‘natural’ or ‘selected’ starters in food fermentations and exert antimicrobial effect as a result of different metabolic

processes (Corsetti et al., 2004; Zotta et al., 2009). Particularly, they have a key function in the development of the sensory and safety features of fermented food products (Franz and Holzapfel, 2011). Due to their potential use as probiotic and natural antimicrobial agents to enhance the safety of food products, lactic acid bacteria have got significant attention as novel approach to the control of pathogens in foods (Klaenhammer, 1993; Settani et al., 2005). They have strong inhibitory effects on the growth and toxin production of pathogenic bacteria (Kalantzopoulos, 1997). In general, they are of particular interest as biopreservatives. The preservative effect of LAB relates to the formation of antimicrobial compounds such as lactic acid, acetic acid, hydrogen peroxide, and bacteriocins (Lowe and Arendt, 2004). The formation of organic acids in the course of lactic acid fermentation reduces the pH of the product to a limit that prevents the growth of undesirable microorganisms. They also contribute to the development of the desired sensory qualities in the final product (Pederson, 1971; Steinkrause, 1986).

LAB are Gram-positive, non-spore forming rods (or cocci), and facultative anaerobes that can ferment in the presence or absence of air, but prefer reduced oxygen levels (Axelsson, 2004). These days, LAB starter cultures are applied in the brewing industry for their ability to improve mash and wort characteristics while ultimately resulting in a better beer. The use of lactic acid starter culture has also led to significant effects in the mashing and brewing processes (Lowe and Arendt, 2004). Beta-glucans have been linked to slow lautering, poor beer filtration, and formation of haze during storage of packaged beer (Home, 1993). However, lactic acid starters solve these problems as they have the ability to improve mash filterability, lower viscosity of wort, and improve clarity of wort (Pittner and Back, 1995; Lowe and Arendt, 2004; Lowe et al., 2005). Lowe et al. (2005) found that the addition of all strains of LAB tested in their experiments resulted in higher volumes of filtrate (264–300 ml) after 1 h and lower viscosities (1.53–1.54 mPa s) when compared with the

low filtrate volume (235 ml) and high viscosity (1.59 mPa s) of untreated malt. This could be attributed to the additional proteolytic and amylolytic activity of the strain, which may have aided in breaking down the larger starch and protein components of the mash. LAB may be classified into homofermentatives, which mainly produce lactic acid, and heterofermentatives, which, in addition to lactic acid, yield a large variety of fermentation products such as acetic acid, ethanol, carbon dioxide, and formic acid (Mayo et al., 2010). One of the homofermentative bacteria getting attention in brewing process is *Lactobacillus amylolyticus.* It is a Gram-positive, non-spore-forming rod with rounded ends, occurring singly, in pairs or as short chains producing solely lactic acid from a range of sugars (Bohak et al., 1998; Vriesekoop et al., 2012). The positive attributes of *L. amylolyticus* include production of L-lactic acids, its high amylolytic activity (uptake of dextrins and starch), it originates from malt, can be grown at high temperatures (52 °C), and it is not a beer contaminating microorganism. It can also be grown in a medium with wide range of pH (3.5–6) even though the optimum growth is in pH range of 5–5.5 (Bohak et al., 1998). In this project, *L.amylolyticus* was used as a starter culture for fermenting the teff malt wort, which was our main focus as alternative raw material for gluten-free beverage.

Gluten is a general term used to describe the protein fraction in wheat, and can be divided into two main fractions based on their solubility in aqueous alcohols: the soluble gliadins and the insoluble glutenins (Lewis, 2005; Phiarais and Arendt 2008). This type of protein, gliadins in wheat, hordein in barley, secalin in rye and possibly avenin in oats are toxic to people with celiac disease (Kasarda, 2001). Celiac disease (CD) is a syndrome characterised by damage to the mucosa of the small intestine caused by ingestion of gluten proteins (Fasano and Catassi, 2001). It is an immune-mediated response in the small intestine triggered by the ingestion of gluten in genetically susceptible individuals. The only successful treatment for CD is a strict gluten-free diet or avoidance of gluten-rich cereals in the diets (Fasano and Catassi,

2001; Fasano and Cureton, 2009; Gallagher et al., 2004; Vader et al., 2003). According to the European Union Regulations (EC, 2009), a food material to be labeled as gluten-free it must have no more than 20 mg gluten/kg, and foods containing less than 100 mg gluten/kg can be claimed to be very low gluten. The term gluten-free is used to indicate that the labeled amount is harmless level of gluten rather than a complete absence, and it is suitable for individuals with celiac disease. On the other hand, gluten sensitive people are not recommended to eat much amount of foods labeled with very low gluten on a daily basis (EC, 2009; FDA, 2013).

Research reports on the nutritional quality of gluten-free cereal based foods showed that many of these products are nutritionally inferior to wheat-based foods that they are intended to replace (Thompson, 2009). Studies on the nutritional adequacy of gluten-free diet suggest that adherence to a gluten-free diet may be a risk factor for inadequate intakes of certain nutrients, including fiber, folate and iron (Cotton et al., 2004; Thompson, 2009). But teff has comparable nutritional value with wheat and barley, and in some nutritional components it is even better than wheat and barley (Bultosa, 2007; Bultosa and Taylor, 2004; Jansen et al. 1962; Mengesha 1966; Seyfu 1997). Due to its very minute size, the embryo of the teff kernel, which contains the main portion of protein and lipid, occupies a relatively large proportion of the total weight (Parker et al., 1989); this resulted in nutritious flour. Although many research reports indicated that teff has the potential to substitute other commonly used cereals in their food and beverage applications (Bultosa, 2007; Bultosa and Taylor, 2004; Zarnkow *et al*., 2008), it has not been extensively studied for its use in malt and beverage production and for its industrial scale processing into other food items. To the best of our knowledge, no such detail experimental research has been carried out on utilizing teff for malting and brewing except that Zarnkow et al. (2008) studied its steeping and germination conditions. They used four different teff varieties

namely Ivory, Dessie, Sirgaynia, and Brown teff to optimize the steeping and germination conditions using response surface methodology (RSM). They reported that steeping of the teff samples at 24 °C to 48 % degree of steeping and germinating for 4 days at 24 °C in a temperature controlled chamber with relative humidity of 95 % yields malt with optimal quality for brewing. They concluded based on the results that teff malt can be a suitable alternative raw material for gluten-free beverages.

Investigations on the kilning, mashing, and fermentation conditions of a cereal are also vital to utilize it for production of fermented beverages. The kilning and mashing conditions of malts from different sources, and the fermentation conditions of the wort samples may vary from one cereal to another (Taylor, 1992; Wijngaard and Arendt, 2006; Zarnkow et al., 2010).

1.2 Malt kilning and its roles in beverage production

Numerous studies revealed that malting conditions of a cereal influence the enzyme activities, extract content, protein content and other malt quality attributes (Bathgate, 1973; Hough et al., 1977; Kiss et al., 2011; Zarnkow et al., 2008). Therefore, it is of great importance to identify suitable malting conditions that lead to have the required levels of malt quality attributes. The malting process comprises three unit operations namely steeping, germination and drying. During malting, germination is terminated by controlled drying of the seeds, which results in reduction of the water content from over 40% to less than 5% (Kunze, 2004). For this particular cereal grain, the moisture content at loading to the kiln is about 48% (Zarnkow et al., 2008). Having increased the moisture content of the grain from about 11% to 48%, it is then kiln dried to less than 5%. Kilning involves heat treatment which can have an adverse effect on enzyme activities. However, an increase in enzyme activity and modification of malt may continue during the first few hours of kilning when there is still free water in the kernels, and temperature is below the deactivation temperature

of the malt enzymes (Hämäläinen and Reinikainen, 2007; Bathgate, 1973; Uriyo and Eigel, 1999). Research findings revealed that kilned malt samples have lower enzyme activities when compared to their counterparts before kilning (Oyewole and Agboola, 2011). It is very important that maximum enzyme activity should be retained during malt preparation for industrial use. When heating the moist green malt during kilning, care must be taken not to destroy the enzymes by wet heat. To protect the enzymes, the malt must first be pre-dried before it is subjected to high temperatures (Kunze, 2004). Literatures on barley showed that at the end of kilning of barley-malt there could be about 15% more α-amylase activity than in the green malt, whereas the more temperature sensitive β-amylase activity is about 40% less than in the green malt (Kunze, 2004). The survival of enzymes in malt is greatly influenced by the temperature and time of the kilning regime (Briggs et al., 1981). Prolonged kilning may result in high consumption of energy, and possibly inactivation of high concentration of endogenous enzymes (Uriyo and Eigel, 1999). Hence, shorter heat treatment would be cost effective and may actually produce malt with high enzyme activity. Research reports showed that the levels of some enzymes including alpha-amylase, beta-amylase and endo-beta-glucanase increase in the early stages of drying at low initial kilning temperatures but showed substantial inactivation during the later stages of kilning (Lloyd, 1988). Other researchers (Bathgate, 1973; Uriyo and Eigel, 1999) also reported that low kilning temperature retains enzyme activities. According to these reports, germination may continue during kilning at temperatures lower than 60 °C, leading to an increase in the enzyme activities.

In addition to removal of the moisture content of the malt, kilning stops the biochemical processes in the kernels and contributes to the formation of malt colour and flavour compounds. In the kilning process, Maillard reaction occurs between amino acids and sugars generating melanoid that gives colour and flavour to the

beverage (Samaras et al., 2005). Research reports indicated that the formation of malt flavour and colour as well as maintaining other malt quality attributes during drying can be controlled by the heating conditions (Runkel, 1975). Low initial drying temperature for long initial drying time has a favorable effect on the flavor stability of the final beverage (Kunze, 2004). Malt kilning also plays an important role in controlling the dimethyl sulfide (DMS) level of the malt and the final beverage. The DMS precursor, S-methyl methionine (SMM), is formed during germination of the malt and 60 – 83% of the SMM breaks down to DMS and homoserine during kilning process. Part of the DMS may be lost in the kiln exhaust, and some other portions will be oxidized to dimethyl sulfoxide (DSMO) (Lloyd, 1988; Yang et al., 1998).

1.3 Thermal stability of amylolytic enzymes and factors affecting starch hydrolysis

Amylolytic enzymes such as alpha-amylase, beta-amylase, and limit dextrinase are among the key enzymes responsible for the hydrolysis of starch into fermentable sugars in beverage industries. The activity of starch degrading enzymes in malt is considered to be an important quality attribute for malting and brewing (Delcour and Verschaeve, 1987). They play significant role in the complete degradation of starch to fermentable sugars during the malting and mashing of cereal grains. Alpha-amylases are endoamylases which act randomly on the 1, 4-glycosidic linkages yielding dextrin, oligosaccharides, and monosaccharides. On the other hand, beta-amylases are exoamylases acting on 1, 4-glycosidic linkages from the non-reducing ends producing maltose (Bernfeld, 1951). Involvement of limit dextrinase in the degradation of starch makes the process more complete as it hydrolyses α-1, 6-linkages of amylopectin (Kunze, 2004; Stenholm and Home, 1999), which are resistant to attack by alpha- and beta-amylases. The hydrolysis of starch to fermentable sugars during mashing will be successful only if the malt has sufficient starch degrading enzyme activity (Delcour and Verschaeve, 1987; Hough et al.,

1977). In general, high level of amylolytic enzyme activities in the malt is vital for the production of fermentable sugars during mashing. However, enzymes have a finite working life due to inherent physical instability and the action of antagonists/inhibitors. In food processing, physical instability can be brought about by pH and temperature effects (Whitehurst and Law, 2002). Other researchers also reported that temperature is one of the most important parameter affecting the effectiveness of the enzymes in hydrolysing the starch molecules (Muralikrishna and Nirmala, 2005). They reported that most of cereal amylases deactivate beyond certain temperature. But the temperatures at which the enzymes start to be deactivated, and the overall thermal stability profiles of starch degrading enzymes vary from one cereal to another (Lee et al., 1984; Phiarais et al., 2005), and their stabilities in commonly used cereals such as barley, sorghum and others as well as the mashing conditions of these cereals are reported in many literatures (Kumar et al., 2005; Taylor, 1992; Wijngaard and Arendt, 2006; Zarnkow et al., 2010). However, the thermal stabilities of starch degrading enzymes as well as mashing conditions of teff malt have not been studied yet.

The enzymes taking part in starch hydrolysis differ in their thermal stability. Literatures indicate that beta-amylases are deactivated first on thermal applications such as kilning and mashing leaving the alpha-amylases. The alpha-amylases then act on the starch yielding dextrins, which are not hydrolysed further. However, limit dextrinase has the potential to convert the dextrins to fermentable sugars although it has less stability at higher temperatures when compared to alpha-amylases. Limit dextrinase has somehow more thermal stability when compared to beta-amylase. But it is readily inactivated at temperatures higher than 65°C. On the other hand, α-amylase has high stability when compared to the others, and retains the major part of its activity at relatively higher temperatures (Evans et al., 2005; Li et al., 2008). One of the main problems associated with production of fermented beverages from

amylaceous raw materials is the inefficient conversion of starch into fermentable sugars either due to insufficient enzyme activity of the raw material or deactivation of the enzymes during killing and mashing processes (Kumar et al., 2005; Kunze, 2004). Other research reports showed that the enzyme activities found in commercial malts are excess for degrading the starch (MacGregor et al., 1999). Hence, the incomplete conversion of the starch into fermentable sugars is mainly due to the deactivation of the enzymes before playing their roles in starch degradation. Incomplete starch gelatinization and inaccessibility of enzymes to the gelatinized starch may limit the formation of extracts and fermentable sugars during the mashing process, which in turn leads to limitation in the fermentation process. Temperature and time are among the most important mashing conditions that influence the amount of the extract yield during mashing, and fermentability of the wort (Delcour and Verschaeve, 1987).

1.4 Purpose of this study

Owing to the presence of gluten proteins, celiac disease patients and gluten sensitive people do not drink barley based beers. For the celiac disease patients, every meal is a matter of sickness or health. As more people are diagnosed with celiac disease and other types of gluten sensitivity, the demand for gluten-free products is certainly increasing (Ceppi and Brenna, 2010; Dekking et al., 2005; Hopman et al., 2008). Besides, research findings showed that gluten-free foods are much more expensive than their regular counterparts (Stevens and Rashid, 2008). Therefore, offering more alternative raw materials in the market may solve this problem. Utilization of teff as a pure component of the wort in brewing and/or beverage industries will address the group of gluten-intolerant people who are still excluded from the beer market. Beside the gluten-free property, teff provides high attractiveness for farmers because of its multifaceted usability and high tolerance to

many diseases and pests as well as high nutritional values (Bultosa, 2007; Ketema, 1997).

Unlike barley, sorghum, wheat, and other cereals, little is known about the technology of teff malting and its utilization for beverage production although some researchers reported that it has a promising brewing potential (Zarnkow et al., 2008). One of the targets of this project was also fermenting the teff malt into a non-alcoholic beverage aiming to address all groups of the society. Abstaining from alcoholic beverages in pregnancy is the overwhelming recommendation of medical authorities (Goh et al., 2010) as it may have risk for the baby's health. Besides, the Muslim population, children and some Christian groups who forbid the drinking of alcoholic beer may also be addressed by producing non-alcoholic beverages. Therefore, the general objective of this project was studying on teff malt as alternative raw material for gluten-free non-alcoholic beverage. In order to achieve this general objective, the project was structured in the following parts:

- review on teff and its applications for production of gluten-free foods and beverages
- studies on influence of kilning on enzyme activities and DMS level of teff malt
- developing kilning program that yields teff malt with low DMS (<7 mg/kg) with no or little damage on its enzyme activities
- investigation on thermal stabilities of its starch degrading enzymes
- developing mashing program to produce wort with highest possible extract yielding ≥40 g/L fermentable sugar, ≥100 mg/L FAN, and pH in the range of 5 to 6, preferably the lowest pH in this range
- investigation of the optimal fermentation conditions for teff malt-wort to produce a beverage with highest possible lactic acid concentration that leads to have a pH between 3 and 4, and lactic acid to sugar ratio of lower than 0.6

2 RESULTS (PUBLICATIONS)

2.1 Summary of papers

Full copies of the publications are presented hereunder following short summaries of the papers.

Part 1 Teff (*Eragrostis tef*) as a raw material for malting, brewing and manufacturing of gluten-free foods and beverages 24

The demand for gluten-free foods is certainly increasing. On the other hand, studies on the nutritional adequacy of the gluten-free diets suggest that adherence to a gluten-free diet may be a risk factor for inadequate intakes of certain nutrients, including fiber, folate and iron. However, the nutrients of teff grain are promising and the grain is an excellent gluten-free alternative for people with celiac disease and other gluten allergy. Teff has attractive nutritional profile, and in some nutritional components it has even better nutritional value than commonly used cereals such as wheat, barley, and others. Thus, it has the potential to replace wheat, barley and other commonly used cereals in their food applications. The research on alternative gluten-free brewing materials is still in its infancy, and researchers in this field are continuously researching on utilization of different cereals, pseudo-cereals, and neglected cereals such as teff. The nutrient composition of teff grain, in general, indicates that it has a good potential to be used in foods and beverages worldwide. This review gives direction for further research on its applications for health foods and on improvement and standardization of traditional teff processing methods.

Part 2 Effect of drying temperature and time on alpha-amylase, beta-amylase, and limit dextrinase activities, and dimethyl sulphide level of teff (*Eragrostis tef*) malt 39

This research was aimed to study the influence of kilning on the enzyme activities, DMS level and moisture content of DZ-Cr-387 teff variety, and suggest a kilning condition that yields teff malt with low DMS and no or little damage on its enzyme activities. The results indicated that enzyme activities, DMS and moisture contents were significantly affected by kilning time and temperature, and it was concluded

from the results that most part of the drying process for teff malt should be achieved at lower temperatures. Hence, a kilning program involving drying of the teff malt in a step wise fashion from 30 to 65 °C for a total of 22 h that best preserves the amylolytic enzyme activities was developed. Following this study, other four varieties of teff namely Brown, Ivory, Dessie, and Sirgynia were considered to assess the influence of variety and storage on the malt quality attributes. It was found that the different teff varieties yield malts with significantly different malt quality attributes, and storage of teff sample for over a year may have negative impact on its malting qualities.

Part 3 Thermal stability of starch degrading enzymes of teff (*Eragrostis tef*) malt during isothermal mashing 57

This part of the project was aimed to study thermal stability of amylolytic enzymes of teff malt. Knowledge of thermal stability of the enzymes would provide a basis for understanding the interaction between starch degradation and the working temperatures during mashing. The study showed that deactivation rate constants of alpha- and beta-amylases for temperatures ranging between 40 and 75 °C were in the range of 0.0003 to 0.0409 min^{-1}, and 0.002 to 0.032 min^{-1}, respectively. Rate of deactivation of limit dextrinase was not significant at temperatures lower than 60 °C but showed high deactivation at higher temperatures with rate constants ranging from 0.02 to 0.1 min^{-1}. The present findings have significant applications in commercial processes where determination of the upper temperature limits for these enzymes is required. The Arrhenius type equations developed in this study are useful for estimating the activities of the enzymes at any mashing temperature, which intern helps in developing mashing program for production of teff malt-wort.

Part 4 Investigation of fermentation conditions for teff (*Eragrostis tef*) malt-wort by *Lactobacillus amylolyticus* 62

This study was focusing on the influence of fermentation factors on the variation in lactic acid, pH, lactic acid to sugar ratio, and extract content during fermentation of teff malt-wort. Optimal fermentation conditions were also identified to produce a

beverage with highest possible lactic concentration that leads to a pH between 3 and 4, and lactic acid to sugar ratio of lower than 0.6. These targets were set based on the results of sensory analysis of fermented teff malt-wort beverage in a preliminary experiment. The results of the study showed that temperature was the dominant factor influencing the three response variables viz. lactic acid to sugar ratio, lactic acid, and pH. However, the main factor influencing extract content was time followed by cell concentration. The feasibility of developing acceptable gluten-free fermented beverage from teff malt-wort has also been demonstrated. The optimum conditions of the factors such as temperature, initial pH, initial cell concentration, and fermentation time determined with RSM were found to be 42 °C, 5.4, 1.86×10^5 cells/ml, and 52 h, respectively. With this set of condition, a beverage with pH of 3.5, lactic acid concentration of 9.5 g/L, and lactic acid to sugar ratio of 0.26 was predicted to be produced. The validation experimental runs demonstrated a good correlation between the predicted and experimental values. The teff malt beverage fermented under the optimum condition was further assessed for its sensory qualities, and the result revealed that the beverage was well liked by most of the panellists.

2.2 Teff (*Eragrostis tef*) as a raw material for malting, brewing and manufacturing of gluten-free foods and beverages

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REVIEW

Teff (*Eragrostis tef*) as a raw material for malting, brewing and manufacturing of gluten-free foods and beverages: a review

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Abstract The demand for gluten-free foods is certainly increasing. Interest in teff has increased noticeably due to its very attractive nutritional profile and gluten-free nature of the grain, making it a suitable substitute for wheat and other cereals in their food applications as well as foods for people with celiac disease. The main objective of this article is to review researches on teff, evaluate its suitability for different food applications, and give direction for further research on its applications for health food market. Teff is a tropical low risk cereal that grows in a wider ecology and can tolerate harsh environmental conditions where most other cereals are less viable. It has an excellent balance of amino acid composition (including all 8 essential amino acids for humans) making it an excellent material for malting and brewing. Because of its small size, teff is made into whole-grain flour (bran and germ included), resulting in a very high fiber content and high nutrient content in general. Teff is useful to improve the haemoglobin level in human body and helps to prevent malaria, incidence of anaemia and diabetes. The nutrient composition of teff grain indicates that it has a good potential to be used in foods and beverages worldwide. The high levels of simple sugars and α-amino acids as a result of breakdown of starch and protein, respectively, are essential for fermentation and beer making.

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Introduction

Africa is rich in a wide range of less explored grain species, including teff and other different types of millets. These grains represent an important source of dietary proteins, carbohydrates, fibre, vitamins, and minerals for the people. They are fermented into a large number of foods and beverages with improved texture, taste, aroma, keeping quality, nutritional values, digestibility, and microbial quality and with reduced antinutrient contents (National Research Council 1996; Yetneberk et al. 2004). Teff (*Eragrostis tef* (Zuccagni) Trotter) is a tropical cereal that belongs to the family of Poaceae, subfamily Eragrostoidae, tribe Eragrosteae, and genus Eragrostis. About 350 species are known in the genus Eragrostis (Demissie 2000), of which teff is the only cultivated species. Chloridoideae is used synonymously for Eragrostoidae of teff (Costanza et al. 1980). Teff cultivars have been recognized and described based on the colour of the grains and inflorescences, ramification of the inflorescences and the size of plants. For marketing purposes, teff is classified on the basis of seed colour: *netch* (white), *qey* (red/brown) and *sergegna* (mixed) (Tefera et al. 1995). Teff grain is hull-less (naked) and comes in a range of colors from milky-white to almost dark brown. The most common colors are white, creamy-white, light brown, and dark brown. The word teff is thought to have been derived from the Amharic word *teffa* which means "lost" due to small size of the grain and how easily it is lost if dropped. The grain is oval-shaped with size 0.9–1.7 mm (length) and 0.7–1.0 mm (diameter). The individual grain mass is generally in the range of 0.2–0.4 mg, perhaps the smallest among carbohydrate-rich kernels (Belay et al.

2009; Bultosa 2007). Teff can adapt to a wide range of environment (National Research Council 1996) and it is considered to be very resistant to insect pests. Teff seeds remain viable for several years provided that direct contact with moisture and sunshine is avoided (Gamboa and Ekris 2008). In comparison with other common cereals, teff grain is less prone to attacks by weevils and other storage pests (Tadesse 1969). Thus, it can be safely stored under traditional storage conditions with no chemical protection. This paper reviews the nutritional value and other aspects of teff and its potential for different applications including brewing and other gluten-free foods and beverages for the health food market.

Chemical composition of teff

The chemical composition of cereals varies widely and depends on the environmental conditions, soil, variety and fertilizer. The importance of teff is mainly due to the fact it has attractive nutritional profile and has no gluten found in other common cereals such as wheat, barley and rye. The demand for gluten-free foods is growing as more people are diagnosed with celiac disease and other types of gluten sensitivity (Bultosa and Taylor 2004; Dekking et al. 2005; Hopman et al. 2008).

Carbohydrates

According a research report by Bultosa (2007) for 13 teff grain varieties, the proximate compositions and flour-starch amylose contents were in some ranges: moisture 9.30–11.22 % (mean 10.53 %), grain protein 8.7–11.1 % (mean 10.4 %), ash 1.99–3.16 % (mean 2.45 %), crude fat 2.0–3.0 % (mean 2.3 %), crude fiber 2.6–3.8 % (mean 3.3 %), and amylose from flour 20–26 % (mean 23.0 %). These values are similar to previous findings by other researchers (Bultosa and Taylor 2004; National Research Council 1996). Studies showed that like other cereals teff is predominantly starchy (73 %) (Bultosa and Taylor 2004; National Research Council 1996) and the starch content of teff is higher than that of most other cereals (Table 1). This makes it to be a potential gluten free cereal that replaces wheat and other cereals in their applications as sources of food energy. Teff starch granules are conglomerates of many polygonal simple granules (Bultosa et al. 2002; Helbing 2009) (Figs. 1 and 2).

The individual starch granules are very small (2–6 μm in diameter) and similar in size to rice starch granules (2–10 μm) but larger than amaranthus (1–2 μm) and quinoa (0.5–3 μm) starch granules (Bultosa et al. 2002; Jane et al. 1992; Lindeboom et al. 2004). The shape is polygonal, smooth with no surface pores. A few granules are essentially

Table 1 The proximate (db[1]) and microelement compositions of teff grain compared with some gluten containing and gluten free cereals

Component	Gluten rich cereals			Gluten-free cereals				
	Barley	Wheat	Rye	Teff	Maize	Brown rice	Sorghum	Pearl millet
Starch (%)	60.6[¤]	71.0[§]	69[α]	73.0[†]	72[β]	64.3[β]	62.9[β]	67.0[σ]
Crude protein (%)	11.1[¤]	11.7*	7.98[α]	11.0[†]	8–11[β]	7.3[γ]	8.3[γ]	11.5[Ω]
Crude fat (%)	3.2[¤]	2.0*	1.98[α]	2.5[†]	4.9[γ]	2.2[γ]	3.9[γ]	4.8[σ]
Moisture (%)	10.6[φ]	12.6 [¥]	–	10.5[□]	14.0[γ]	14.0[γ]	14.0[γ]	9.5[Ω]
Ash (%)	2.4 [¤]	1.6[§]	1.72[α]	2.8[†]	1.4[γ]	1.4[γ]	1.6[Ω]	1.7[Ω]
Crude fiber (g/100 g)	3.7[¤]	2.0[§]	1.56[α]	3.0[§]	–	0.6–1.0[γ]	0.6[Ω]	0.5[Ω]
Food energy (kJ/100 g)	–	1105[§]		1406[†]				
Calcium (mg/100 g)	34[‡]	39.45[¥]	31.5[α]	165.2[†]	48.3[β]	6.85[λ]	50[θ]	46[σ]
Copper (mg/100 g)	0.52 [‡]	0.23[a] [¥]		2.6[†]	1.3[β]	0.16[λ]	0.41[μ]	1.06[σ]
Iron (mg/100 g)	2.43 [‡]	3.5 [δ]	2.7[α]	15.7 [†]	4.8[β]	0.57[λ]	6[θ]	
Magnesium (mg/100 g)	94.3[‡]	103.5[a] [¥]	92[α]	181.0[θ]	107.9[β]	16.88[λ]	180.0[θ]	137[σ]
Manganese (mg/100 g)	8.97[‡]	0.95 [¥]		3.8[†]	1.0[β]	0.36[λ]		
Phosphorus (mg/100 g)	563[φ]	–	359[α]	425.4[†]	299.6[β]	61.7[λ]	263.3[μ]	379[σ]
Potassium (mg/100 g)	507[φ]	–	412[α]	380.0[†]	324.8[β]	181.71[λ]	225.23[μ]	
Sodium (mg/100 g)	25.4[φ]	–		15.9[†]	59.2[β]	0.54[λ]	6.18[μ]	
Zinc (mg/100 g)	2.2[c] [‡]	1.94 [¥]	3.0[γ]	4.8[†]	4.6[β]	2.0[γ]	2.0[γ]	3.1[σ]

[Ω] Ahmed et al. 1996; [¤] Aman et al. 1985; [μ] Awadalkareem et al. 2008; [Φ] Bultosa 2007; [†] Bultosa and Taylor 2004; [β] FAO 1992; [γ] FAO 1993; [σ] FAO 1995; [λ] Heinemann et al. 2005; [¥] Kashlan et al. 1991; [‡] Köksel et al. 1999; [δ] Leder 2004; [α] McCance et al. 1945; [θ] Mengesha 1966; [§] Obilana 2003; [φ] Riahi and Ramaswamy 2003; *Saturni et al. 2010

[1] Dry basis

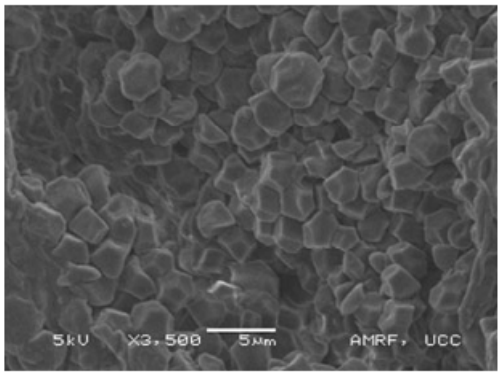

Fig. 1 Teff grain: Starch granules of the endosperm (Source: Helbing 2009)

cubic and at high magnification, and some appear as tortoise-shell shaped (Bultosa and Taylor 2004). According to Helbing (2009), Scanning Electron Microscopy (SEM) was better to identify these small structures accurately than Confocal Laser Scanning Microscopy (CLSM). The information contained in the endosperm starch granules look round from the CLSM images which contradicts the SEM images, there the starch granules were observed as rectangular or square. The reason for the difference in appearance is probably the treatment of CLSM teff grain with the fluorescent dyes that are dissolved in water. Through contact with water the grains are probably swollen and changed in structure.

The composition of teff starch granules is similar to other normal native cereal starches with 25–32 % amylose (Bultosa et al. 2002). However, Bultosa (2007) reported somehow smaller amylose contents for 13 teff varieties. According to Bultosa (2007), among teff flours the highest amylose contents were observed for DZ-01-354 (25.8 %), DZ-Cr-44 (25.6 %), DZ-01-1285 (24.2 %) and DZ-01-787 (23.8 %), and the lowest were for DZ-Cr-255 (20 %) and DZ-01-1681 (21.2 %). Bultosa et al. (2002) reported that the gelatinization temperature of raw teff is in the range of 68–80 °C, which is similar to that of other tropical cereal starches like sorghum (67–81 °C) (Dufour and Melotte 1992; Okolo et al. 1997), but narrower than that of maize (60–79 °C) (Narziss and Back 2009). Starch degradability is improved through the malting process due possibly to differences in the degree of modification of starch structure and composition (Glennie et al. 1983; MacGregor and Matsuo 1982; Okolo et al. 1997). The extent of gelatinization and the gelatinization temperature can also be affected by many other factors, including fat content, amylose content and granule size. As the gelatinization temperature of teff is too high for the amylolytic enzymes, an adjunct mashing regimen may be considered for wort production. The higher gelatinization temperature of raw teff may indicate that teff starches have high crystallinity and high perfect crystallites than barley and wheat starches which have lower gelatinization temperatures of 60–62 °C and 52–64 °C, respectively. This may be due to structural differences in their amylose and amylopectins (Briggs 1998; Sablani 2009; Tester

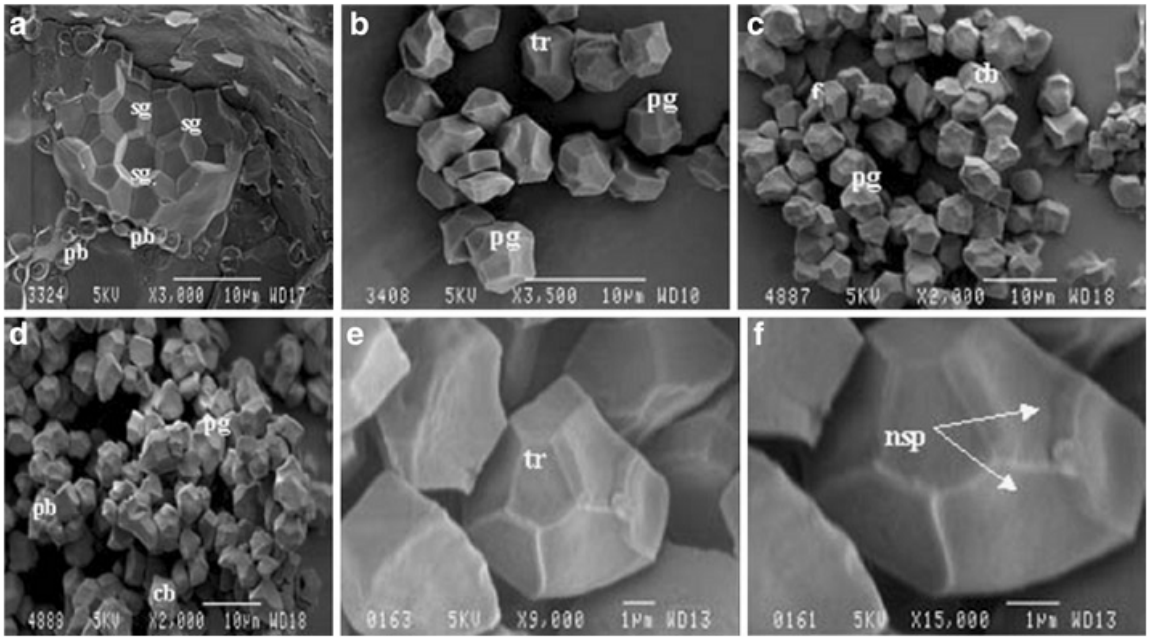

Fig. 2 Compound (**a**) and individual (**b–f**) starch granules from different teff varieties: (**a**, **b**) South African brown teff (**c**) DZ-01-1681, and (**d–f**) DZ-01-196; (sg = starch granules, pg = polygonal, cb = cubic, tr = tortoise-shell, pb = protein, f = fiber, and nsp = no surface pores). (*Source*: Bultosa and Taylor 2004)

and Morrison 1990). Gelatinization of starch is important in the processes such as baking of bread, gelling of pie fillings, formulation of pasta products, and thickening of sauces to produce a desirable texture or consistency. The proportion of raw and gelatinized starch in ready-to-serve starchy products may be critical in determining the acceptability. Texture of many foods such as breakfast cereals, beverages, rice, noodles, pasta, and dried soups depends on the fraction of gelatinized starch in the product (Sablani 2009). However, in most food systems the actual temperature at which starch gelatinizes is less important than those properties that depend on swelling, such as pasting behavior and rheological properties of the partially or fully swollen starch granules. The pasting temperatures for 13 teff varieties ranged from 67.7–75.9 °C with mean 72.7 °C (Bultosa 2007). The highest pasting temperatures were reported for teff varieties DZ-Cr-82 (75.7 °C) and DZ-01-1681 (75.9 °C) and the lowest was for DZ-Cr-44 (67.7 °C). The pasting temperatures reported by Bultosa (2007) were somehow similar to the reported Rapid Visco Analyzer (RVA) pasting temperatures (72.1–74.8 °C mean 74 °C) for five teff starches by Bultosa et al. (2002). According to Bultosa et al. (2002), the pasting temperature of teff (74 °C) is similar to that of maize starch (74.1 °C), but cooking time (4.19 min) to peak viscosity is longer than that of maize (2.90 min). The pasting character predicts the processing qualities of starch based raw material food ingredients. The pasting character is fundamentally determined by the starch granule composition and its nature (ultra-structures) and is also influenced by the non-starch flour components. Most of the gelling properties are due to amylose, which precipitates in aqueous solution to initiate gelatinization when heated. The paste clarity of teff starch is opaque, and the gel texture is short and smooth (Bultosa 2007). The branched molecules within the swollen granules undergo a slow association to rigidify the swollen granules. It is possible that the intra-granular amylose fraction enhances the rigidity of starch granules on bread staling. Specialty starches can provide a number of functional benefits in making snack products, including different expansion, crispness, oil pickup, and overall eating quality. The crisp texture of bakery products is due to recrystallization of sugar on removal of water during baking. Sugars also act to tenderize bakery products by slowing the rate at which starch molecules become interlinked and proteins break down (Hug-Iten et al. 1999).

The viscosity of teff starch is considerably lower than that of maize-starch. Peak, breakdown, and setback viscosities of teff starch 269 RVU, 79 RVU, and 101 RVU, respectively, are lower than those of maize starch 313 RVU, 129 RVU, and 161 RVU, respectively (Bultosa et al. 2002). Shimelis et al. (2006) reported that final viscosity is used to indicate the ability of starch to form various paste or gel after cooling and that less stability of starch paste is commonly accompanied with high value of breakdown. This implies that teff starch paste will have high stability after cooling when compared to that of maize starch paste. Sanni et al. (2001) reported that lower set back viscosity during cooling indicates higher resistance to retrogradation. This means that teff starch will exhibit higher resistance to retrogradation; thus, its products have high resistance to staling. Similar to other small-granule starches teff-starch granules offer good functionality as a fat substitute, flavor and aroma carrier since they are very small, smooth, and of uniform size. Teff starch has good resistance to shear breakdown, and thus it may find good application in high-shear processed foods. Its water absorption index is higher (108 %, db) than that of maize (86 %, db) whereas its water solubility index is lower (0.34 %, db) than that of maize (0.98 %, db) (Bultosa et al. 2002). Many carbohydrates are excellent scavengers of metal ions. Glucose, fructose and sugar alcohols have the ability to block the reactive sites of ions, such as copper, iron and to a lesser extent, cobalt. This is characteristic of monosaccharides and aids in food preservation by retarding catalytic oxidation reactions (Ferrier 1992).

Proteins

The average protein level of teff is comparable to that of barley, wheat, maize and pearl millet, and higher than that of rye, brown rice and sorghum (Table 1). The fractional composition of the protein in teff indicate that glutelins and albumins are the major protein storage components; and their order of fractional importance was: glutelins (44.55 %) > albumins (36.6 %) > prolamin (11.8 %) > globulins (6.7 %) (Seyfu 1997; Tatham et al. 1996). The distribution of these protein fractions varies among different cereals and there is considerable variation in the solubility classes among the cereals. Albumins range from 4 % in maize to 44 % in rye, globulins from 3 % in maize to 55 % in oats, prolamins from 2 % in rice to 55 % in maize, and glutelins from 23 % in oats to 78 % in rice. Among these fractions in cereals, the prolamin fraction has been the most studied (Haard et al. 1999). Prolamins are characterized by a particular amino acid composition with domains with a high content of proline and glutamine. It has been demonstrated that these protein domains are resistant to degradation by gastric, pancreatic and proteases in the human intestinal brush border membrane (Saturni et al. 2010). The prolamin fractions in teff were extracted from ball-milled teff samples from which Albumins and globulins were extracted by stirring with 1 M NaCl for 1 h and centrifuged (10000 g for 15 min). The prolamins were extracted with 70 % (v/v) aqueous ethanol for 1 h followed by 50 % (v/v) aqueous propan-1-ol, 2 % (v/v) acetic acid and 2 % (v/v) 2-mercaptoethanol for 1 h (Tatham et al. 1996). The amino acid composition of teff flour is favourable and its protein is easily digestible in comparison to

cereals such as maize and sorghum because the main protein fractions such as albumin, glutelin, and globulin are the most digestible types.

Bultosa (2007) reported that the grain protein contents of 13 teff varieties are ranged from 8.7–11.1 % with mean 10.4 %. The grain protein in DZ-01-354 (10.6 %), DZ-01-99 (10.8 %), DZ-Cr-44 (10.7 %), DZ-Cr-82 (10.6 %), DZ-Cr-37 (11 %), DZ-Cr-255 (11.1) and DZ-01-1281 (11.1 %) varieties were the highest; and in DZ-01-1285 (8.7 %) was the lowest. Belay et al. (2005) also reported the grain protein contents of these 13 released teff varieties in the range of 12.4–8.7 % with mean 11.0 % and the highest was for DZ-01-99 and the least was for DZ-01-1285 like Bultosa's (2007) findings.

Protein level is very important in malting and brewing processes. Grain protein content is a key "gateway" characteristic for malting quality. Excessively high protein level is commonly associated with lower soluble substance content and malt extract quality, resulting in unacceptable malt quality. However, if the protein content of malt is too low, brewing performance may be impaired through poor yeast amino acid nutrition. Protein levels in general must not be too low as proteins serve four basic functions in brewing. They are the origin of enzymes that catalyze the complex biochemical reactions involved in turning the cereal into malt, and malt into wort. They are required for yeast nutrition; they contribute to foam and are involved in the flavor development that malt contributes to beer and whiskey. The protein content of teff (11 %) is in the range (9.5 to 11.5 %) that a good quality malting material should possess to yield the above-mentioned quality-attributes (Asano and Hashimoto 1980; Bamforth 1985; Bishop 1930; Chen et al. 2006; Kunze 2004; Steiner et al. 2011). Thus, its protein content is an indicator for production of good quality gluten-free malt from teff grains. The proteins also play an important role in determining the texture of a food, and may be used as thickening, binding, or gelling agents and as emulsifiers or foaming agents (Vaclavik and Christian 2008).

Amino acid composition

Teff seeds appear to be similar to wheat in food value; however, the National Research Council of USA suggested that teff seeds are actually more nutritious (National Research Council 1996) and contain generally higher amounts of the essential amino acids (Table 2). Research findings (Jansen et al. 1962) indicated that teff has an excellent balance among the essential amino acids that makes it comparable to that of egg, except for its somewhat relatively low lysine and isoleucine contents. The other amino acids compositions of teff are also excellent and its lysine content (3.68 g/100 g) is also higher when compared to other cereals (Table 2). Isoleucine, leucine, valine, tyrosine, threonine, methionine, phenylalanine, arginine, alanine and histidine contents of teff are higher than in barley, wheat and most other cereals. Isoleucine, valine, tyrosine, serine, and glycine contents of teff are slightly lower than those of brown rice. The overall amino acid profile of teff can be regarded as well-balanced (Table 2).

Yigzaw et al. (2004) reported that spontaneous fermentation of teff caused decreases in threonine, valine, isoleucine, and lysine contents by 30, 8, 20 and 17 %, respectively. According to this research finding, amino acids such as leucine, phenylalanine, and histidine showed no significant change, whereas methionine was increased by 90 %. Fermentation with *Lactobacillus plantarum* (lactic acid bacteria) caused decreases in threonine, isoleucine, lysine, and valine by 28, 14, 17, and 6 %, respectively (Yigzaw et al. 2004). They found that fermentation with lactic acid bacteria increased the amounts of leucine (12 %) and methionine (110 %), whereas phenylalanine and histidine remained practically the same. In general, the bacterial fermentations did not improve the essential amino acid profile of the fermented food product. The fungal fermentation, on the other hand, improved essential amino acid profiles of teff. Almost all amino acids increased during fungal fermentation (or fermentation with *Aspergillus oryzae* and *Rhizopus oligosporus*) by 10 to 36 %. The effect was even higher for methionine, which was increased by 180 %.

The well balanced amino acid composition of teff makes it an excellent material for malting and brewing. Nutritional value and stability are the most important qualities of alcoholic beverages. In addition to some other food components, proteins and some amino acids are also responsible for nutritional value and stability of beer. Proline and lysine are the most important amino acids in beer. It is known that proline residues are responsible for the affinity towards proanthocyanidins and take part in the production of aromatic compounds and, in this way, influence beer quality. The difference in amino acid contents will affect beer flavors (Gorinstein et al. 1999; Jones and Pierce 1964; Outtrup 1989; Perpète et al. 2005). Amino acid contents also play a crucial role in yeast nutrition. As a result their concentration affects fermentability and flavour profile of the finished product (Clapperton 1971; Gibson et al. 2009; Procopio et al. 2011).

Fat

Bultosa (2007) found that the crude fat content of teff is in the range of 3.0–2.0 % with mean of 2.3 % which is similar to the review report (3.09–2.00 %) of previous findings (Bultosa and Taylor 2004). According to Bultosa (2007), the highest crude fat was for DZ-Cr-82 (3 %) and the lowest values were among DZ- 01-354 (2.1 %), DZ-01-99 (2.1 %),

Table 2 Amino acid content of teff (g/16 gN) compared with some gluten containing and gluten free cereals, and whole egg

Amino acid	Gluten rich cereals		Gluten free-cereals				
	Barley	Wheat	Teff	Pearl millet*	Rice[φ]	Sorghum[µ]	Whole egg*
Lysine	3.46†	2.08†	3.68†	2.89	3.7	0.34	6.6
Isoleucine	3.58†	3.68†	4.07*	3.09	4.5	0.65	7.5
Leucine	6.67†	7.04†	8.53†	7.29	8.2	2.13	9.4
Valine	5.04†	4.13†	5.46†	4.49	6.0	0.79	7.2
Phenylalanine	5.14†	4.86†	5.69†	3.46	5.5	0.87	5.8
Tyrosine	3.10†	2.32†	3.84†	1.41	5.2	0.70	4.4
Tryptophan	1.54†	1.07†	1.30*	1.62	1.2	0.22	1.4
Threonine	3.31†	2.69†	4.32†	2.50	3.7	0.53	4.2
Histidine	2.11†	2.08†	3.21†	2.08	2.3	0.36	2.1
Arginine	4.72†	3.54†	5.15†	3.48	8.5	0.62	6.9
Methionine	1.66†	1.46†	4.06†	1.35	2.7	0.28	3.8
Cystine	2.21[c §]	2.42 [d ¥]	2.50*	3.19	1.8	0.33	2.4
Asparagine + Aspartic Acid	4.62[a c §]	5.12 [d ¥]	6.4[¤]		9.0		–
Serine	3.51[c §]	4.98 [d ¥]	4.1[¤]		5.0	0.76	–
Glutamine + Glutamic Acid	18.86[b c §]	29.53[d ¥]	21.8[¤]		17		–
Proline	9.58[c §]	10.18[d ¥]	8.2[¤]		5.0	1.34	–
Glycine	3.29[c §]	4.04 [d ¥]	3.1[¤]		4.5	0.48	–
Alanine	4.51[c §]	3.56 [d ¥]	10.1[¤]		5.5	1.55	–

[a] Aspartic acid only; [b] Glutamic acid only; [c] Mean value of three varieties; [d] Mean value of 12 varieties

[¤] Bultosa and Taylor 2004; [§] Chatterjee et al. 1975; *Jansen et al. 1962; [φ] Khoi et al. 1987; [¥] Mosse et al. 1985; †Seyfu 1997; [µ] Shoup et al. 1969

DZ-01-974 (2.1 %), DZ-Cr-37 (2 %), and DZ-01-1681 (2 %). The crude fat content of teff grain, in general, is higher than that of wheat, rye, and brown rice but lower than that of barley, maize, sorghum and pearl millet (Table 1). The fat contents are mainly of fatty acids. Teff grains are rich in unsaturated fatty acids. The seeds contain 22 % w/w of fixed oil rich in unsaturated fatty acids (72.46 %), among which oleic acid is predominant (32.41 %), followed by linoleic acid (23.83 %) (El-Alfy et al. 2011). The unsaturated fatty acids are not only important for our nutrition, especially as some cannot be synthesized by humans (essential fatty acids), but also play important role in the production of beer. They are useful for the structure of the yeast cell wall. Their derivatives are also responsible for aging processes in beer taste after filling. Long-chain fatty acids (in particular linoleic and palmitic acid) play a key role in the improvement of fermentation performance in hot trub/break. Besides the contribution of long chain fatty acids for fermentation performance, their fast release from hot trub into wort improve yeast nutrition and therefore, favor its metabolism (Kunze 2004; Kühbeck et al. 2006).

Crude fiber

The crude fiber content in teff (3.0 g/100 g) is by far higher than in most other gluten containing and gluten-free cereals (Table 1). Inadequate fiber intake is likely to be related to the composition of many gluten-free foods made with starches and/or refined flours with low content in fiber (Thompson 2000). In fact during refining, the outer layer of grain containing most of the fibre is removed, leaving only the starchy inner layer. Exceptionally in the case of teff, due to the small size of its grains, it is almost always made into a whole-grain flour (bran and germ included), resulting in a very high fibre content compared with the other grains, and high nutrient content (Vinning and McMahon 2006). Teff has as much, or even more, food value (Tables 1 and 2) than the major grains: wheat, barley, and maize, for instance. This is probably because it is always eaten in the whole-grain form: the germ and bran are consumed along with the endosperm. Although germ in teff is known to occupy large proportion as in other small grains, its crude fat is known to be not as such high. The crude fiber ranged from 3.8–2.6 % with mean 3.3 % (Bultosa 2007) and apparently the crude fiber contents observed in these 13 varieties are almost similar with the earlier report of 3.5–2.0 % with typical value 3.0 % (Bultosa and Taylor 2004). According to Bultosa (2007), the crude fiber contents were high among teff varieties DZ-01-787 (3.5 %), DZ-Cr-82 (3.5 %), DZ-01-974 (3.5 %), DZ-Cr-358 (3.5 %) and DZ-01-1281 (3.4 %) and highest in brown teff varieties DZ-01-99 (3.8 %) and DZ-01-1681 (3.7 %). The

crude fiber for DZ-Cr-44 (2.7 %) and DZ-Cr-255 (2.6 %) were the lowest. Consumption of dietary fiber provides many health benefits. The dietary fiber content of teff (8.0 g/100 g) is high when compared to some fruits, nuts, pulses and cereals such as corn and rice (Saturni et al. 2010). Studies revealed that high fiber diets prevent many human diseases, colon cancer, coronary heart disease and diabetes (Anderson et al. 2009)

Minerals

Research data (Table 1) show that the proximate and mineral contents of whole teff grain are comparable with, in some cases higher than, wheat, barley and most other cereals. Bultosa (2007) reported that the ash content of 13 teff varieties ranged 3.16–1.99 % with mean of 2.45 %. The ash contents in the brown teff varieties DZ-01-99 (3.16 %) and DZ-01-1681 (2.99 %) and in DZ-Cr-255 (3.10 %) were comparatively high, and in teff varieties DZ-01-787 (2.06 %), DZ-Cr-358 (1.99 %) and DZ-01-1285 (2.02 %) appeared low. In general, compared to the other cereals, teff is rich in minerals such as calcium, zinc, magnesium, iron, phosphorous and copper (Abraham et al. 1980; Bultosa and Taylor 2004; Kashlan et al. 1991; Mengesha 1966; Seyfu 1997).

Calcium is the most common mineral in our body and is indispensable for the strength of the skeleton and hardness of teeth. It also plays numerous functions in the body. High calcium diets prevent gaining of weight and fat accumulation (Teegarden 2003; Zemel 2003). Epidemiologic evidence (Norat and Riboli 2003) and at least one intervention study (Holt et al. 2001) have shown that higher calcium intake lowers the risk of developing colon cancer. A disease caused by failure to build adequate bone mass or by progressive bone loss during aging called osteoporosis can be prevented by generous intake of calcium. Thus, a high consumption of calcium rich foods will help build optimum bone mass during childhood and adolescence and will also slow the rate of bone loss that naturally occurs with aging (Dickinson 2002). Teff contains an excellent concentration (0.165 %) of calcium and the level of this mineral in teff is by far higher than other cereals (Table 1). This leads us to conclude that teff is an excellent cereal to prevent the aforementioned health problems associated with less consumption of calcium. An invention by Roosjen (2007) revealed that flour should preferably contain at least 0.15 % calcium. None of the major cereals such as barley (0.034 %), wheat (0.039 %), rye (0.032 %), maize (0.048 %), sorghum (0.05 %), brown rice (0.0069 %), and pearl millet (0.046 %) fulfill this requirement. However, teff and some other cereals such as finger millet (0.182 %) contain naturally higher than 0.15 % calcium (Shukla and Srivastava 2011)

As magnesium deficiency alters calcium metabolism and the hormones that regulate calcium (Elisaf et al. 1997; Rude et al. 1999), magnesium deficiency could be a risk factor for osteoporosis. The concentration of magnesium in teff is much higher than in other cereals except sorghum which has magnesium concentration comparable to that of teff (Table 1). Malabsorption of iron, folate, and calcium is common, as these nutrients are absorbed in the proximal small bowel. In particular, it has been reported that the frequency of iron-deficiency in celiac disease varies from 12 to 69 % (Tikkakoski et al. 2007). Studies showed that teff consumers have higher level of haemoglobin in their blood than non-teff consumers, and they do not suffer from hookworm anaemia even when infested; however, hookworm anaemia develops in non-teff eaters if they are infested with hookworm (Molineaux and Biru 1965; Tadesse 1969). In Ethiopia, an absence of anaemia seems to correlate with the levels of teff consumption and is presumed to be due to the grain's high content of iron. In addition, according to the same studies, malaria is frequently found in the groups with lower haemoglobin levels. Teff has been implicated in the low incidence of anaemia in Ethiopia. The severity of these nutritional deficiencies is modulated by different factors: the length of time that people have lived with the active but undiagnosed disease, the extent of damage to the gut intestinal tract and the degree of mal-absorption. Previous studies have demonstrated that most of these nutritional deficiencies disappear after following strictly a gluten free diet (Annibale et al. 2001; Bardella et al. 2000).

Recent studies have examined athletes' endurance higher iron requirements. Runners, for example, might need 30 % to 70 % more iron due to losses from foot strike hemolysis and gastrointestinal blood loss (Food and Nutrition Board, 2002). It is well known in a worldwide level that the resistance and general good fitness of Ethiopian sport people is high. The general high nutritional value of teff and its iron content are believed to be the major contributors for this. The high iron content of teff increases the haemoglobin level of the blood that helps more oxygen to be transmitted (Andrews et al. 1999). Due to the aforementioned health benefits of teff, new scientists are interested to know all about the teff composition, nutritional properties, and the changes that happen at the moment of grain fermentation during the preparation of *injera*, a flat bread that is responsible for about 70 % of the Ethiopian population. More and more interest is being shown in teff research and in the future it might also be used for different food and beverages applications in the different parts of the world because of its high nutritional value and potential for health food market.

Metal cations are also well known to promote beer foam stability (Rudin 1957). More recently, Roza et al. (2006) confirmed that the inclusion of non-toxic metal cations in

beer has a significant foam stability benefit, such as the inclusion of Zn^{2+}. Kühbeck et al. (2006) reported that zinc plays a key role in the improvement of fermentation performance. They also reported that similar to the long chain fatty acids, fast release of zinc from hot trub into wort also improved yeast nutrition and favor its metabolism.

Vitamins

Vitamins are useful to prevent and treat various diseases including heart problems, high cholesterol levels, eye disorders, and skin disorders. Most of the vitamins also facilitate the body mechanism and perform functions which are not performed by any other nutrient (WHO 2003). Most of the vitamins in cereals and malt are solubilized into wort during the brewing process. Their importance to the brewing process depends on their content in wort, more than sufficient to ensure a regular yeast performance during fermentation. In particular the B-group vitamins are crucial as growth factor for yeast, especially biotin, inositol and panthotenic acid (Buiatti 2009). Teff contains good levels of certain vitamins such as vitamin C (88 mg), niacin (2.5 mg), vitamin A (8 retinol equivalent (RE)), riboflavin (0.2 mg) and thiamin (0.30 mg), all per 100 grams of grain (National Research Council 1996). Thiamin in teff is typically lower when compared to that of wheat (0.43 mg), and barley (0.37 mg) (Guerrant and Fardig 1947).

Enzymes

Enzymes, particularly amylases, are responsible for the degradation of starch molecules during mashing. Presence of too little enzyme activities in the mash may lead to several undesirable consequences such as low extract, longer time to separate the wort, slow fermentation process, too little alcohol in the final product, microbiological instability, reduced filtration rate of the beer, and inferior flavour and stability of the beer. Zarnkow et al. (2008) reported that teff malt has good level of enzyme activities though the recorded α- and β-amylase activities (75 and 213 U/g, respectively) are lower than those of barley malt 106 and 514 U/g, respectively (Phiaraise et al. 2005). The levels of the enzyme activities of four different teff varieties reported by Zarnkow et al. (2008) were enough to use them as suitable raw materials for malting. However, the gelatinization temperature of teff (68–80 °C) (Bultosa 2007) is too high for the amylolytic enzymes. Thus, it may be useful to consider an adjunct mashing regimen for wort production. Research findings showed that the highest limit dextrinase value of teff malt (894 U/Kg) is twice the average limit dextrinase value of barley malt (400 U/Kg) (Zarnkow et al. 2008).

Phenolic compounds

The other most important health-promoting aspect of teff as food is that like other millets it is generally assumed to contain substantial amounts of phenolics (Dykes and Rooney 2007). Research findings revealed that ferulic acid (285.9 μg/g) is the major phenolic compound in teff. Some other phenolic compounds such as protocatechuic (25.5 μg/g), gentisic (15 μg/g), vanillic (54.8 μg/g), syringic (14.9 μg/g), coumaric (36.9 μg/g), and cinnamic (46 μg/g) acids are also present in teff in considerable amounts (McDonough and Rooney 2000). Phenolics are notable for their antioxidant activity, which appears to be beneficial in terms of prevention of cardiovascular disease and cancer (Awika and Rooney 2004). They also act as natural antioxidants for the food industry. At the same time, they might inhibit digestive enzymes and reduce food digestibility (Maheshu et al. 2011; Qiang et al. 2006). The role of phenolic compounds in relation to the colour, taste, and stability of beer is well known. Beer rich in phenolic antioxidants shows higher quality, more stable sensory properties such as flavor and aroma, foam stability, and longer shelf life (Drost et al. 1990; Guido et al. 2007; McMurrough et al. 1996; Woffenden et al. 2001).

Teff utilization

Teff is a major food grain in Ethiopia but is a minor cereal crop worldwide. In Ethiopia, teff is traditionally grown as a cereal crop. The grain is ground to a flour mainly used for making a popular pancake-like local bread called *injera,* which has a honeycomb-like appearance. *Injera* is made from dough fermented for 2–3 days. Sometimes the flour is also used for making porridge, *kitta* (unleavened bread), and *atmit* or *muk* (gruel) (Selinus 1971). Various studies (Yetneberk et al. 2004; Zegeye 1997) showed that in its *injera* making and keeping quality features, teff grain appeared superior among other cereal grains because of its high resistance to staling. During the baking of *injera*, starch is completely gelatinized to form a steam-leavened, spongy matrix, in which fragments of bran, embryo, microorganisms and organelles are embedded (Bultosa et al. 2002; Parker et al. 1989). Yetneberk et al. (2004) studied the staling properties of *injera* prepared from teff (DZ-01-196) and 12 varieties of sorghum. They reported that *injera* prepared from teff grain is resistant to staling and the maximum force required for bending fresh teff-*injera* and *injera* stored for 24 and 48 h is lower than that of sorghum-*injera*. The maximum forces required for bending fresh teff-*injera* and *injera* stored for 24 and 48 h were 0.13, 0.12 and 0.15 N, respectively. However, the force required for

bending fresh sorghum-*injera* and sorghum-*injera* stored for 24 and 48 h were 0.23, 0.3, and 0.37 N, respectively. This indicates that sorghum-*injera* easily undergoes staling, and the keeping quality and palatability of teff-*injera* is much better than sorghum-*injera*. Various research findings also showed that teff flour can be mixed with barley or sorghum flour to make *injera*. But pure teff flour produces the best quality *injera*, pliable, soft with glossy appearance, which does not fall apart under handling or stick to the fingers, and has a slightly sour taste (Yetneberk et al. 2004; Zegeye 1997). *Injera* is traditionally consumed with *wot,* a sauce made of meat or ground pulses like lentil (*Lens culinaris*), faba bean (*Vicia faba*), field pea (*Pisum sativum*), and chickpea (*Cicer arietinum*). The traditional way of consuming teff-*injera* with *wot* provides a well balanced diet because of the high nutritional composition of the whole grain teff and the sauce made from nutritious raw materials; *wot* also supplements the lysine deficit in teff (Seyfu 1997). White-grained types of teff are preferred for food, but consumption of *injera* from red- or brown-grained types is also increasing, especially for health-conscious urban people.

An invention on processing of teff flour revealed that teff flour with falling number higher than 250 s is an excellent raw material for baking (Roosjen 2007). However, teff flour which is obtained by grinding the grain directly after harvesting causes instability and unattractive taste and structure to the final product. The main reason for this is that teff grain directly after harvesting has too low falling number. This can be solved by letting the grain to go through an after-ripening process (storing for some time) after harvesting in which the falling number of the grain increases (Hidetoshi 2001; Lukow et al. 1995). According to the invention (Roosjen 2007), a high quality product, which meets the market standard of 7.5 (on a scale of 1–10) by a test panel, can be obtained by using teff flour with falling number of at least 400 s. Teff flour having falling number between 400 and 550 s results in a dough or batter with high baking qualities. According to this invention, dough prepared from teff flour with falling number higher than 250 s can be used for the preparation of a wide range of baked food products such as bread, pastry, cookies, pizza, pasta, noodles, etc.

In wheat-bread-dough the gluten proteins naturally form a visco-elastic network required for the desired functional properties of bread products (Xu et al. 2007). Since these kinds of proteins are lacking in gluten free cereals, other techniques such as enzyme treatment (e.g. glucose oxidase) (Renzetti & Arendt 2009), and high pressure treatment (Vallons et al. 2011) can be used to improve the functional properties of gluten-free flours by promoting a protein network. According to these research findings, protein polymerisation can improve the bread making performance of gluten-free flours by enhancing elastic-like behaviour of batters. The teff grain, owing to its high mineral content, has started to be used in mixtures with soybean, chickpea, and other grains in the baby food industry (Seyfu 1997). The high fiber content of the grain makes it to be useful in preventing diabetes and other health problems as well as assisting with blood sugar control. Anderson et al. (2009) reported that individuals with high intakes of dietary fiber appear to be at significantly lower risk for developing coronary heart disease, stroke, hypertension, diabetes, obesity, and certain gastrointestinal diseases. Increased fiber intake lowers blood pressure and serum cholesterol levels, improves glycemia and insulin sensitivity in non-diabetic and diabetic individuals, and benefits a number of gastrointestinal disorders including the gastroesophageal reflux disease, duodenal ulcer, diverticulitis, constipation, and hemorrhoids. Fiber supplementation in obese individuals significantly enhances weight loss (Anderson et al. 2009). An additional advantage is that teff is almost always grown and stored under organic conditions. Several recipes that fit Western tastes have been developed from teff flour particularly in the United States, where it has found niches in the health food market and as a gourmet food. Teff flour is used as a thickening agent in a range of products, including soups, stews, gravies, and puddings (Seyfu 1997).

In Ethiopia, the grain is also used to make traditional local alcoholic beverages such as opaque beer called *tella,* a sprit called *katikala/arake,* and *shamit* at household level.

Tella has a smoky flavor due to the addition of bread darkened by baking and use of a fermentation vessel which has been smoked by inversion over smoldering *weyra*-wood. In addition to the cereal, the other most important ingredient in making *tella* is the leaves of *Gesho* (*Rhamnus prinoides*). It imparts characteristic bitterness of the beverage. Research findings indicated that *Gesho* regulates the microflora responsible for the fermentation process (Kleyn and Hough 1971). It is also revealed that the bitterness of the brew is directly related to the amount of *Gesho* added. *Tella* is not processed under government regulations and the alcohol content varies between 2 to 4%vol. Filtered *tella* has a higher alcohol content ranging from 5 to 6%vol (Selinus 1971).

Arake is a distilled beverage. Ground *Gesho*-leaves and water are kept for 3–4 days and after that unleavened bread called *kita* made of teff or other cereals, and germinated barley or wheat are added. The mixture is allowed to ferment for 5–6 days and then distilled. In the villages, distillation is carried out with primitive equipments made of gourds and wood. It is usually redistilled and its alcoholic content is in the range of 45–50%vol (Selinus 1971).

Shamit is a local beer made among the Gurage ethnic group. Unleavened teff bread, called *kita,* and germinated barley, called *bekel*, are milled and mixed with water, and the mixture is filtered after 3–4 days of fermentation. Dehusked barley is toasted on metal plate called *mitad*,

milled and added to the mixture, and the beverage is ready to serve the next day, when Ethiopian cardamom, *mitmitta*, black cummin and bishop's weed are added (Selinus 1971).

Teff as a potential gluten-free cereal for malting and brewing

The food alternatives for Celiac Disease (CD) patients are mostly based on maize, rice, and soy. Teff appears to be another interesting possibility. Beer is commonly produced from malted barley; therefore beer is excluded from the diet of celiac disease patients. No beers or other beverages of any kind made with any amount of malted barley will meet the standard of gluten-free, because the definition of "gluten-free" typically used by physicians and clinical nutritionists is "made from gluten-free raw materials only." That is the food must contain no particle of any material derived from wheat, barley and/or rye (FAO/WHO 1994). Celiac disease is a lifelong inflammatory condition of the gastrointestinal tract caused by permanent intolerance to gluten proteins present in cereals such as wheat, barley and rye (Green et al. 2005). The only effective treatment for celiac disease is the total lifelong avoidance of gluten ingestion; this is not easy because many staples of the Western diet are based on wheat flour. A cereal like teff lacking T-cell-stimulatory peptides would thus be of great value to patients with celiac disease. The present "raw-materials" definition of gluten-free foods will permanently exclude any malt-based beer, or any other malt-based beverages, regardless of how it might be made. This is not a good outcome for the brewing industry and their consumers, and the brewing industry should take a lively interest in this debate. The most obvious way to make a gluten-free beer is to start with gluten-free raw materials (Zarnkow et al. 2010). Gluten-free cereal products represent a growing market opportunity, within the global health and wellness market, for food manufacturers that develop consumer-led new products with high added-value levels, which ultimately gain consumer acceptance. Beer usually contains appreciable quantities of hordein (barley gluten prolamin) (Ellis et al. 1994); however, some low gluten beers have been presented in the market. Rice, corn and potatoes have been widely used as substitute of gluten containing grains. A number of nutrient dense grains, seeds, pulses offer increased variety, improved palatability and high nutritional quality for the gluten free diet (Gallagher et al. 2004; Lee et al. 2009).

There is a growing interest on teff grain utilizations because of nutritional merits (whole grain), and the protein is essentially free of gluten the type found in wheat (Dekking et al. 2005; Hopman et al. 2008; Taylor et al. 2006) making it a suitable substitute for wheat and other gluten containing cereals in foods for people with celiac disease. Thus, it can be an alternative food for consumers allergic to wheat glutens. However, other cereals have proteins that exert a toxic effect for celiac disease patients. Studies on structural composition of raw and malted grains indicate that like other cereals the main differences between the raw and malted teff grains are mainly in the endosperm (Helbing 2009). Enzymatic breakdown of the endosperm cell walls occur faster in germinated (malting) cereals (Figs. 3 and 4).

The endosperm cells have an angular, polygonal shape with a size of up to 70 microns (Helbing 2009). According to this research finding it was possible to see the endosperm structure somehow better in the SEM image of the whole grain in the top view of the belly. Teff produces nutritionally rich grain, comparable to or better than wheat, barley and maize (National Research Council 1996). Thus, it is a potential cereal for brewing process and can be used as a substitute for wheat flour in almost all its applications. Malting and brewing processes for teff have not been extensively investigated except for the study conducted by Zarnkow et al. (2008) on the optimization of the malting conditions of teff (Fig. 5).

They used four different varieties of teff (Ivory, Dessie, Sirgaynia and Brown) with average moisture of 10.0 %. Based on the result of this study, they concluded that the optimal malt is obtained after 4 days of germination with 48 % degree of steeping, and a set temperature of 24 °C for steeping and germination. Studies showed that like other cereals teff is predominantly starchy; the starch content and its malt quality are indicators of its suitability as raw material for brewing (Bultosa 2007; Zarnkow et al. 2008). Protein content of teff is comparable to those of wheat and barley, and it is nutritionally superior because of its high levels of amino acid profile (FAO 1970; National Research Council 1996), making it an excellent material for malting and brewing. Research findings (Zarnkow et al. 2008) indicate that teff malting causes an increase in the levels of simple sugars and free amino nitrogen (FAN) as a result of breakdown of starch and protein, respectively. These products are essential for beer making. Therefore, malt produced from teff is of high nutritional quality for brewing and food use. However, the extract values determined by using the

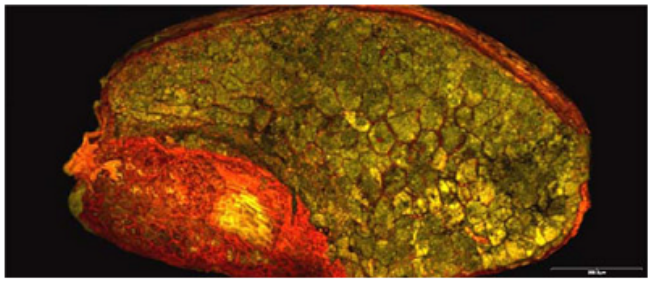

Fig. 3 Teff grain: Longitudinal section with germ and endosperm (SEM image) (*Source*: Helbing 2009)

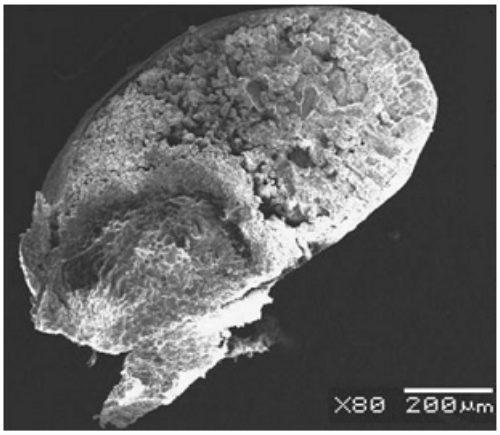

Fig. 4 Teff malt kernel: Scanning Electron Microscopy (SEM) image (*Source*: Helbing 2009)

congress mashing ranged at low level between 13.6 and 55.7 %, which is lower than that of barley (>80 %) (Briggs 1998). The low extract in teff may indicate that the congress mashing program is not suitable condition for mashing teff malt. The results reported by Zarnkow et al. (2008) could be used as a base for further studies on uses of various teff varieties as malt. Apart from teff's intrinsically good brewing quality, what is driving this brewing revolution is economics. In developing countries the commercial processing of these locally grown grains in to value-added food and beverage products is an important driver for economic development.

Research gaps and opportunities

Research gaps

Teff is used for many applications including products such as *injera* and *tella*. The production of *injera* and *tella* involve fermentation stages, and are dependent on temperature and other environmental factors (Magazoni et al. 2010) but there is no standard for the amounts of the starter culture and other ingredients, and little is known about the identity and relative importance of species of yeasts and bacteria involved in the preparation of *injera*. Standard processing conditions are also required for making these environment dependent products in the different parts of the world. Although *injera* and *tella* are parts of the main staple foods for Ethiopian community, their preparation techniques are still too traditional, labor intensive and time consuming. It is also a burden for women as it is considered as females work. Attention should be given mainly to its processing technology at small and/or large scale production. Teff-*injera* has high keeping quality than *injera* prepared from other cereals (Yetneberk et al. 2004). However, its shelf life is not more than 4 days if it is kept under room temperature with the local preserving techniques. Thus, there is a research need to develop packaging technique for preserving *injera* so as to supply it for local and export markets. The by-product during *tella* preparation is called *atela*. *Atela* (sediment) is a residue that settless at the bottom of a pot at the end of *tella* preparation. It is commonly used in Ethiopia across the country for cattle feeding. However, its nutritional value has not yet been studied.

Many research reports concluded the medicinal value including gluten free nature of teff (Dekking et al. 2005; Hopman et al. 2008). The growing demand of gluten free foods in the western countries needs an urgent research on teff products and application of teff in other food products as a substitute for gluten rich cereals.

Opportunities

- Teff is a highly nutritious (Bultosa and Taylor 2004; Guerrant and Fardig 1947; Jansen et al. 1962; Obilana 2003; Seyfu 1997) and gluten free cereal (Dekking et al. 2005; Hopman et al. 2008). It has an excellent amino acid profile that makes it comparable to egg, and the best cereal with respect to its nutritional value (Jansen et al. 1962; Seyfu 1997). Thus, it is an interesting alternative raw material for manufacturing nutritionally improved gluten free foods.
- Many gluten-free products may not meet the recommended daily intake for fiber, minerals, and vitamins.

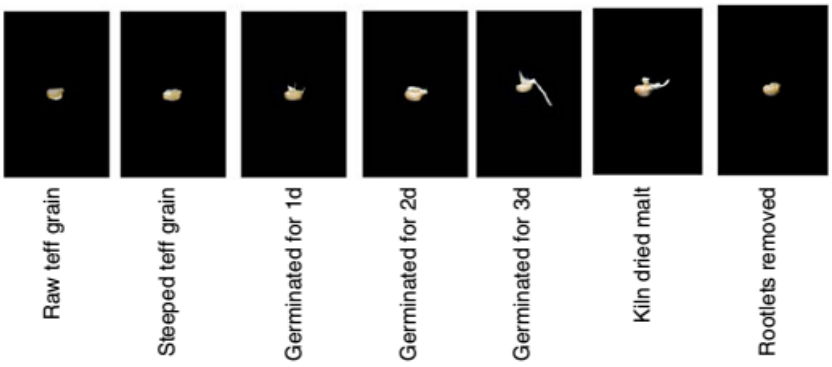

Fig. 5 Structures of teff grain under different stages of malting (*Source*: Zarnkow et al. 2008)

Thus, they need to be fortified to fulfill the requirements of the daily intake (Gallagher et al. 2004; Suliburska and Krejpcio 2011). Teff has naturally higher nutritional value when compared to many other grains, and doesn't need to be fortified.

- One of the major problems associated with gluten-free products is their inferior taste and/or structure (Gallagher et al. 2004). However, use of teff grain with falling number higher than 250 seconds at the moment of grinding solves these problems (Roosjen 2007). According to this report teff flour with the above-mentioned falling number has great advantage that it can be processed in to a stable gluten-free product with an attractive taste and structure.
- Due to its high fiber content (Bultosa 2007) and gluten free property, teff is getting acceptance as medicinal ingredient (Hopman et al. 2008). In connection to its medicinal values interests are growing in many countries to utilize teff for production of gluten free foods.
- Use of teff as food consumption is already well known in some parts of the world like Ethiopia, the Netherlands, North America and so on. It will not be difficult for the processors to introduce this cereal in other parts of the world.
- Teff-*injera* is one of the best staple foods and widely used in Ethiopia. In addition to the high consumption of teff in the country, there is a growing demand for *injera* and other teff products by Ethiopians living abroad and foreigners who are accustomed to Ethiopian dish.
- Production of teff is increasing by using improved varieties (Tefera et al. 1995; Tefera et al. 2001).
- It is a tropical low risk cereal that grows in a wider ecology and can tolerate harsh environmental conditions where most other cereals are less viable. Thus, it has the potential to be grown in every part of the world.

Conclusion

Teff is a reliable and low risk cereal that grows on a wider ecology under moisture stress and waterlogged areas with few plant diseases and grain storage pest problems. Processing of teff for different foods is usually done by traditional ways and is mostly limited to the household level. Processing of the grain for different commercial foods is needed to promote worldwide teff utilization. The nutrient composition of teff grain indicates that it has high potential to be used in foods and beverages worldwide. Teff has its own unique qualities and advantages, and thus its introduction to other parts of the world could benefit many nations. It has the potential to add variety to our diet and may have useful health promoting properties, particularly antioxidant activity. The very high concentration of calcium in teff makes it to be an excellent cereal to prevent health problems associated with less consumption of calcium such as gaining of weight, accumulation of fat, colon cancer, osteoporosis, weakening of skeleton and teeth, and so on. Teff grain nutrients are promising and it is also an excellent gluten free alternative for people with celiac disease and other gluten allergy. The high carbohydrate content, lack of gluten and its high germinative energy and malt quality make it to be a suitable raw material for brewing and producing other gluten free beverages. Though the α- and β-amylase activities of teff malt are lower than that of barley, it has sufficient level of enzyme activities to be used as a raw material for malting. Currently only sorghum, rice, maize, millet, and buckwheat appear to be successful gluten-free beer ingredients, while others have only shown adjunct possibilities. The search for new gluten-free brewing materials is still in its infancy and researchers in this field of study are continuously researching on the malting, mashing, fermentation conditions and other aspects of teff so as to use it as a raw material for gluten-free beer, functional beverages and other gluten-free foods.

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2.3 Effect of drying temperature and time on alpha-amylase, beta-amylase, limit dextrinase activities and dimethyl sulphide level of teff (*Eragrostis tef*) malt

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ORIGINAL PAPER

Effect of Drying Temperature and Time on Alpha-Amylase, Beta-Amylase, Limit Dextrinase Activities and Dimethyl Sulphide Level of Teff (*Eragrostis tef*) Malt

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Abstract Teff is a gluten-free cereal with attractive nutritional profile. This research was aimed to study the influence of kilning on the enzyme activities and dimethyl sulphide (DMS) level of DZ-Cr-387 teff variety and suggest a kilning condition that yields teff malt with low DMS with no or little damage on its enzyme activities. The malts were dried using isothermal conditions at 30, 40, 50, 60 and 70 °C for 40 h with sampling in certain time interval. To set up kilning program, two temperature regimens 18 h at 30 °C+1h at 60 °C+3 or 5 h at 65 °C (R1) and 18 h at 30 °C+1 h at 60 °C+3 or 5 h at 80 °C (R2) were selected. Results from isothermal kilning indicated that enzyme activities, DMS and moisture contents were affected ($P<0.05$) by time and temperature. The values of α-amylase, β-amylase, limit dextrinase activities and DMS content while using the first regimen (R1) with 3 h curing at 65 °C were 68 U/g, 440 U/g, 1,072 U/kg and 3.3 mg/kg, respectively. Whereas in the second regimen with 3 h curing at 80 °C, the values were 42 U/g, 406 U/g, 736 U/kg and 2.15 mg/kg, respectively. Prolonged curing in both kilning regimens caused an adverse effect on the amylolytic enzyme activities. R1 with shorter curing time is considered to be the best condition in preserving enzymes. The enzyme activities and DMS level show that teff can be an alternative raw material for production of gluten-free malt.

Keywords α-amylase · β-amylase · Kilning · Limit dextrinase · Teff

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Introduction

Malted barley traditionally remains the grain of choice in brewing industries although many other cereals are used mainly in conjunction with barley (Depraetere et al. 2004; Goldammer 2008; Narziss and Back 2009). However, owing to the presence of gluten proteins, celiac disease patients cannot drink barley-based beers. Since the number of victims of recognized celiac disease is increasing, the demand for gluten-free products is certainly increasing. Thus, researchers are examining alternative gluten-free starting materials in order to overcome this problem (Arendt and Bello 2008; De Meo et al. 2011; Zarnkow et al. 2008; Zarnkow et al. 2010). Teff is a tropical cereal that belongs to the family of Poaceae, subfamily Eragrostoideae, and genus *Eragrostis*. It is a gluten-free cereal with attractive nutritional profile (Bultosa 2007).

In the germination of cereals, most of the targeted changes, such as malt modification, and the synthesis and activation of enzymes take place (MacGregor and Ballance 1980; MacGregor and Matsuo 1982; MacLeod 1967; MacLeod et al. 1964). During the malting process, hydrolytic enzyme production and/or activation are maximized leading to cell wall degradation and protein solubilisation with minimal starch breakdown. When the

germination of kernels has reached a desired stage, germination is terminated by kilning the green malt. These results in reduction of the water content from over 40 % to less than 5 % (Kunze 2004). The kilning process is separated into three physical stages: removal of 'free water' (down to about 20 % moisture), intermediate stage (about 20–10 % moisture) and removal of bound water (about 10–2 % moisture) (Briggs et al. 1981; Kunze 2004; Schuster and Grünewald 1957). Sequential biochemical changes during kilning may also be classified into three phases (Bathgate 1973). The first is an extension of the germination stage of malting, during which enzyme syntheses continue and rates of enzyme reactions increase. In the second kilning phase, described as enzymic, enzyme-catalysed reactions accelerate as malt temperatures are increased and moisture levels are substantial (Bathgate 1973; Sebree 1997). As kilning proceeds and malt temperature increases, inactivation of some enzymes takes place. Finally, in the third or chemical phase, changes in colour, flavour and other minor constituents of the malt are accelerated. This process is often termed 'malt curing'; more enzymic activity is lost even though low moisture content affords some enzymic protection. The three phases just described are not completely discrete but are useful in the study of biochemical changes during kilning. For malts aiming at high enzyme activity, a curing temperature of only 65 °C can be used as it preserves the enzymes from being denatured. Prolonged thermal exposure results in increased energy consumption and possibly inactivation of endogenous enzymes (Palmer 1989; Uriyo and Eigel 1999). In a previous research (Kolbach and Schild 1935), it was reported that the effect of higher temperatures on the enzymatic activity level is largely dependent on the moisture content of the malt. Thus, shorter heat treatments would be more cost effective and may actually produce higher levels of enzyme activity. Enzyme systems in malt vary in their sensitivity to the moisture–temperature–time relationships of kiln environments, determined principally by air-on temperature, airflow rate and kiln bed depth.

Dimethyl sulphide precursor is also an important quality attribute of malt (Stewart 2004). Dimethyl sulphide (DMS) is often identified as the sulphur compound contributing to unpleasant odour and flavour in beverages and foods. Although sulphur compounds contribute in a positive way to the aroma and taste of many foodstuffs (Mussinan and Keelan 1993), their low sensory thresholds and powerful nature as well as often unpleasant characteristics make them to be the frequent cause of off-flavours and odours. When malt is kilned, a part of the inactive precursor S-methyl methionine, which is formed during germination, is converted to active precursor which probably involves only a slight structural change, and part is destroyed by the heat so that lightly kilned malts will contain more than those kilned at higher temperatures (Kunze 2004). For a good quality malt, the DMS content of the malt should not be higher than 5–7 ppm (Kreisz 2009). Since teff and barley have different physiological and chemical properties, it may not be worthy to apply the kilning regimens commonly used for barley directly to teff. Therefore, this study was aimed to examine the effect of kilning on the enzyme activities and DMS content of teff and suggest a suitable kilning condition that yields teff (*Eragrostis tef*) malt with low level of DMS and no or little damage on its enzyme activities.

Materials and Methods

DZ-Cr-387 (*kuncho*) teff variety with germination rate of 100 % in 24 h was used to assess the influence of kilning on the moisture content, DMS level and amylolytic enzyme activities of the malt. This teff variety was obtained from Debrezeit Agricultural Research Centre, Ethiopia. The samples were cleaned manually by siftings and winnowing to ensure that they are free from chaffs, dust and other impurities. After cleaning, exactly 500 g of the teff grain in duplicate was placed in nylon bags and steeped in tap water at 24 °C to a moisture content of 48 %. It was germinated at 24 °C in temperature-controlled chamber with 95 % relative humidity for 4 days (Zarnkow et al. 2008). The samples were dried using isothermal kilning conditions at 30, 40, 50, 60 and 70 °C for 40 h in order to study the influence of kilning time and temperature on the enzyme activities, moisture and DMS contents of teff, and the samples were withdrawn at certain time interval for analysis. Based on the results of the isothermal kilning and subsequent trial experiments, the green malts of another batch, which have been steeped and germinated in the same conditions as above, were dried by increasing the temperature of airflow in a stepwise fashion from 30 to 80 °C so as to find out the best kilning regimens for teff malt. Two different kilning regimens were applied. The first kilning regimen (R1) was kilning for 18 h at 30 °C, 1 h at 60 °C followed by 3 or 5 h at 65 °C, and the second kilning regimen (R2) was kilning for 18 h at 30 °C, 1 h at 60 °C followed by 3 or 5 h at 80 °C. All samples were analysed for α-amylase, β-amylase, limit dextrinase activities, DMS levels and moisture contents using standard methods. Congress mashing procedure was used for mashing the teff malt, and the worts were analysed for extract content, free amino nitrogen (FAN) and soluble nitrogen using standard procedures (Anger 2006; EBC 2007)

Analytical Procedures

Enzyme Activities

The enzyme activities were measured using the ICC standard methods (ICC 1998) using Megazyme kits (Megazyme International Ireland Ltd). α-amylase, β-amylase and limit dextrinase activities were assayed using CERALPHA method (kit K-CERA 08/05), BETAMYL-3 method (kit K-BETA3) and limit dextrizyme method, respectively.

In order to study the influence of kilning temperatures on the amylolytic enzyme activities of teff malt, the samples were dried at different temperatures in the range of 30–70 °C and withdrawn periodically for activity assay. An increase in temperature may increase the rate of an enzyme-catalysed reaction as the enzyme activity is a function of temperature or it may cause denaturation of the enzyme (Sadana 1991). The deactivation of enzymes is assumed to follow first-order kinetics (Kitz and Wilson 1962; Sadana 1991). The assumption is that the active enzyme (E) directly proceeds to inactive state (E_d) without providing significant amount of stable intermediate.

$$E \xrightarrow{k} E_d$$

Hence, the rate of enzyme deactivation is first-order in relation to the concentration of the active enzyme (E). The deactivation in enzyme activity, expressed as the ratio of the measured activity, E, at time ,t, of heat treatment to the initial activity. E_0 is described by the simple equation of a first-order process with k as the rate constant:

$$\frac{dE}{dt} = -k[E] \tag{1}$$

Integration of this equation with the initial condition $E = E_0$ for $t = 0$, gives

$$\ln\left(\frac{E}{E_o}\right) = -k\,t \tag{2}$$

Where E = the concentration of active enzymes at time t; E_o = the initial concentration of active enzymes; t = time elapsed during the kilning process; k = rate constant.

Thus, the first-order rate constants (k) were determined from the semi-logarithmic plot of residual activity as a function of time.

Activation energies for the enzymes thermal deactivations (E_a) were calculated from the Arrhenius plots of logarithms of the deactivation rate constants (k) versus reciprocals of the absolute temperatures.

$$k = k_0 \exp\left(-\frac{E_a}{RT}\right) \tag{3}$$

Where R is universal gas constant (8.3145 J/molK)

The values of the activation energy (E_a) and the pre-exponential factor (k_0) were estimated from the slope and intercept of the plot of the natural logarithm of the rate constant (ln k) as a function of the inverse of the temperature in Kelvin 1/T, respectively.

DMS

The DMS content of the malt was determined using gas chromatographic head space method following the MEBAK procedure 1.3.3 (Anger 2006)

Moisture Content

The moisture content was determined through the loss in mass during a standardized drying process using the MEBAK method 3.1.4.1 (Anger 2006).

Statistical Analysis

In order to assess the influence of the kilning conditions on the enzyme activities as well as DMS and moisture contents, the results were subjected to one-way analysis of variance (ANOVA) with Tukey's HSD statistical test at 0.05 significance level using SPSS for windows (SPSS 2007).

Results and Discussion

In this study, kilning conditions were studied in relation to their effects on the DMS level, moisture content and enzyme activities of teff malt.

Moisture Content

The effect of kilning time and temperature on moisture content of teff malt are outlined in Fig. 1 The temperatures during kilning were kept constant at 30, 40, 50, 60 and 70 °C in order to assess the influence of kilning time, and the different kilning temperatures on enzyme activities, DMS levels and moisture contents. In the free drying phase, moisture moves out of the grain relatively quickly, as it involves removal of water from the surface of the grain (EBC 2000). The boundaries indicating the three physical stages in the diagram (Fig. 1) are for the teff malt dried at 40 °C in order to compare it with a well-studied barley malt dried at similar condition. The free water drying stages during teff malting were accomplished after 12 and 9 h of kilning at 30 and 40 °C, respectively, and 3 h of kilning at higher temperatures. The next two phases involve the removal of moist held strongly within the grain (EBC 2000). The reduction of the drying speed at the intermediate stage showed that the major part of the free water contained in the

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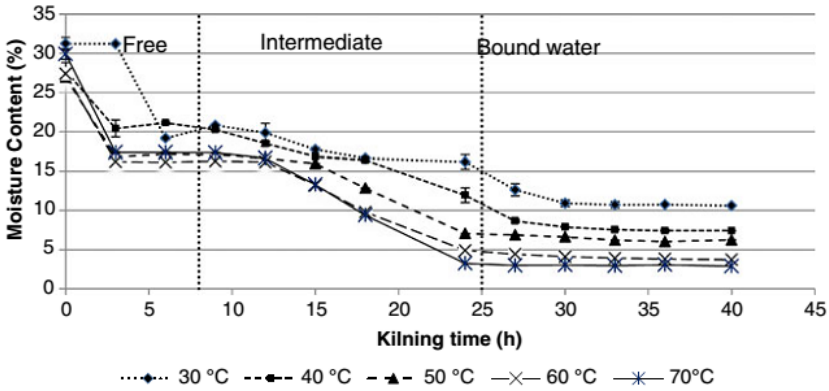

Fig. 1 Moisture contents of teff malts dried at different temperatures against kilning time

malt was evaporated in the free drying stage. The intermediate stages of the teff malt kilning were accomplished after 40, 24 and 18 h of kilning at 30, 40 and 50 °C, respectively, and after 15 h of kilning at both 60 and 70 °C. The rate of drying in the teff malt was much slower during the intermediate stage of kilning when compared to barley malt dried under similar kilning temperature (40 °C) (Schuster and Grünewald 1957), where the moisture content decreased from 19.3 to 10.5 % after 12 h of kilning. During teff drying at the same kilning temperature, this stage was achieved after 24 h of kilning. The final stage of kilning is characterized by the removal of firmly bound water in the grain (Briggs et al. 1981). The result of our study showed that the rate of drying in teff malt was highly slowed at this stage, and the moisture content was decreased from about 10 % to a final moisture content of 7.4 % after 40 h of kilning at 40 °C. In a previous research (Zarnkow et al. 2008), it was revealed that teff has a higher ability to absorb water than barley and barley takes up moisture more slowly than teff. This may be due to the fact that the covering layers present in the barley kernel limit water uptake during steeping. However, during the drying process, the rate of removal of moisture from barley kernel was faster than that of teff samples as most of the absorbed water in barley kernels remained as a free water in the husks. Research findings (Schuster and Grünewald 1957) showed that the moisture content in barley was reduced from approximately 43 to 11 % in the first 12 h of kilning at 40 °C. In contrast, the moisture content in teff malt was reduced only to approximately 19 % under the same kilning temperature and time frame. One reason for this may be due to the uptake of more free water because of the presence of extra outer layers or husks around the barley kernel (Pomeranz and Sachs 1972). This water is not bound to endosperm components; therefore, it is removed more easily by the kilning process.

One-way ANOVA (analysis of variance) showed that no significant change ($P > 0.05$) was observed in the moisture content during the first 3 h of kilning for the sample dried at 30 °C (Fig. 1). However, it was significantly decreased ($P< 0.05$) for the samples kilned at all other temperatures in the same time frame. In general, the moisture content was significantly decreased ($P< 0.05$) in the first 24 h of kilning at all kilning temperatures, but no significant change ($P> 0.05$) was observed after 24 h of kilning. After about 20 h of drying, the average malt moisture contents were reduced to lower than 10 % (Fig. 1), with the exception for the samples dried at 40 and 30 ° C. At 40 °C, this condition was attained after 27 h of kilning, whereas at 30 °C, it was not attained throughout the 40 h of kilning because the temperature was not too high to achieve this condition in the studied time frame.

Dimethyl Sulphide

The DMS content was not detected for raw teff grain but increased during the germination process. It was declined rapidly with high kilning temperature and longer time of kilning. The lowest results were obtained for the samples kilned at high temperatures and longer time (Fig. 2), and the highest values were recorded for the green malt (>7.0 mg/kg). The DMS level of teff malt was significantly affected ($P< 0.05$) by the kilning time and all kilning temperatures.

There was no significant change in the DMS level of the samples dried at 40 and 50 °C in the first 3 h of kilning. However, it showed a significant decrease in the first 3 h of kilning at 30, 60 and 70 °C. The results showed that an increase in the kilning time caused a significant decrease in the DMS content of teff malt at all kilning temperatures (Fig. 2). The DMS contents of the teff malt samples were also analysed during an experiment designed to obtain an optimum kilning conditions in which the samples were dried up to the temperature of 85 °C (data not shown). In general, lightly kilned malts contain more DMS than those kilned at higher temperatures; this may be due to the fact that during malt kilning, only part of the 'inactive precursor' of DMS is converted to 'active precursor' (Kunze 2004), which probably involves only a slight structural change, and the remaining portion of the precursor is destroyed by the heat. Some of the DMSs are driven off by heat in the kilning process.

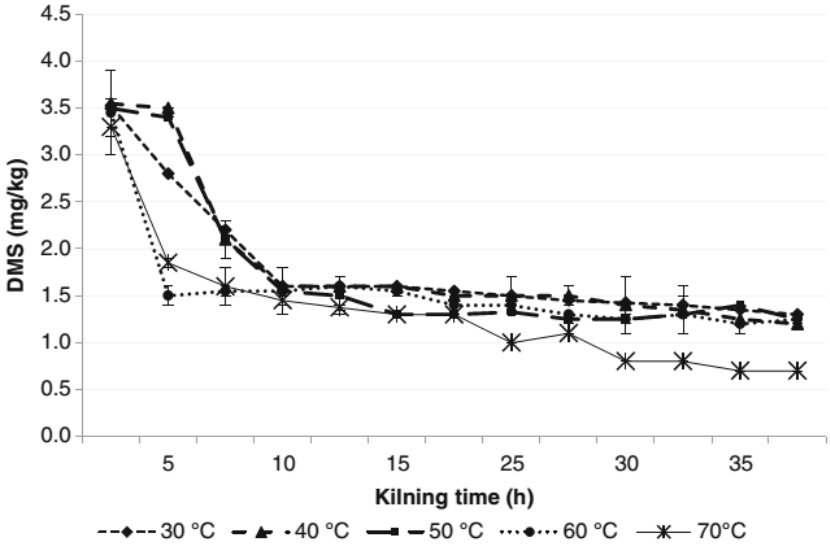

Fig. 2 Effect of kilning time and temperature on the DMS level of *kuncho* teff malt

Alpha-Amylase Activity

During kilning of the teff samples, both the activation and inactivation of alpha-amylase were observed. Thus, these two opposing processes were considered independently to study the kinetics of the enzymes during the kilning process. Alpha-amylase is a pacemaker enzyme for starch degradation, which randomly acts on α-1,4 links of starch releasing small dextrins and fermentable sugars.

The rate constants for the change in alpha-amylase activities of teff malt during kilning at different temperatures were determined from the plots of $\ln\left(\frac{E}{E_0}\right)$ versus time.

Temperature and duration of kilning were found to influence the α-amylase activities of teff. Enzyme activity measurements carried out at various temperatures indicate that alpha amylase activity increased in the first few hours at all studied temperatures. It was noticed that denaturation of the enzyme started after few hours of kilning, and the thermal unfolding profile of the enzyme had a single peak at every kilning temperature although it showed stability after the peak point at low temperatures for long time (Fig. 3). The increment of the alpha-amylase activities in the first few hours of kilning confirms the findings of other researchers for other cereals (Okungbowa et al. 2002) where it was noted that when kilning sorghum at lower temperatures, the enzyme denaturing phase was avoided and increased enzyme development was observed. Other research findings also indicate that low kilning temperature yields greater survival of enzymes. Elevated temperatures also allow greater enzyme survival when the malt is less moist (Briggs et al. 1981; Owuama and Asheno 1994).

The increase in α-amylase activity at the early stages and low kilning temperatures of teff kilning may be attributed to continuation of the germination process. The rate constants during this activation stage ranged from 0.0111 h^{-1} at 70 °C to 0.0319 h^{-1} at 30 °C. The rates of increase in the alpha-amylase activities at low temperatures were high when compared to those at higher kilning temperatures. At the later stages of the kilning process, there was a significant decrease in the alpha-amylase activities of teff malt. The deactivation rate constants at these stages ranged from 0.0035 h^{-1} at 30 °C to 0.0241 h^{-1} at 70 °C. The deactivation rate constant (k) increased with temperature during the deactivation stages, whereas the activation rate constant in the first stages of kilning decreased with temperature.

The Arrhenius energies for the first stages (enzyme activation stage) and the later stages (enzyme deactivation stage) of kilning the teff malt were calculated to be −18.2 and 41.8 kJ/mol, respectively. Thus, the Arrhenius equation for alpha-amylase activation rate constant in the first few hours of teff kilning process was

$$k_a = 2.212 \times 10^{-5} \exp\left(\frac{2187}{T}\right)$$

and for the later stages of teff kilning process, the deactivation rate constant was found to be

$$k_d = 7.23 \times 10^{4} \exp\left(\frac{-5024}{T}\right)$$

Beta-Amylase Activity

Beta-amylase is the key saccharifying enzyme of the brewers' malt. It is an exo-enzyme that releases maltose molecules from the non-reducing ends of amylose and amylopectin (Lewis and Young 1995). Figure 4 shows the deactivation of beta-amylase in the kilning process as a function of time.

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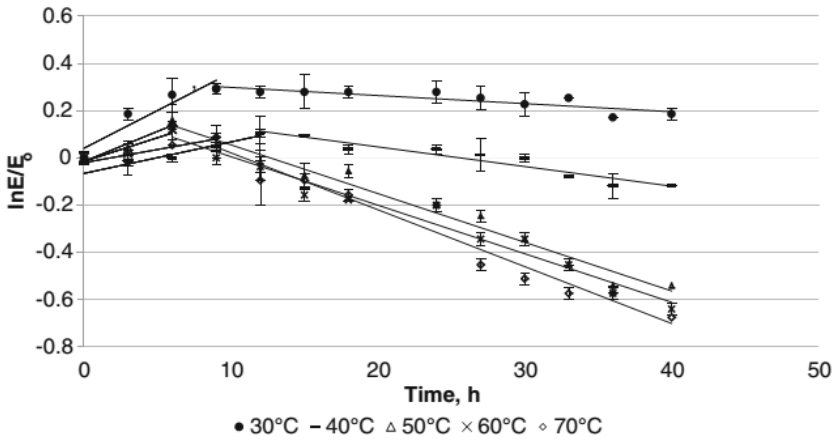

Fig. 3 Changes in the α-amylase activity of teff malt during kilning process

From the plots of $\ln\left(\frac{E}{E_0}\right)$ versus kilning time, the rate constants of the deactivations of beta-amylase at the different kilning temperatures were estimated. In contrast to the biphasic pattern observed in the alpha-amylase activities (Fig. 3), there was no activation of the beta-amylase activities during the early stage of the kilning process. The beta-amylase started to be deactivated immediately after the commencement of the drying process, which could be attributed to the thermolabile nature of this enzyme (Kunze 2004). The deactivation rate constants were in the range of 0.0034 h^{-1} at 30 °C to 0.0048 h^{-1} at 70 °C. The results showed that β-amylase activity of teff malt was highly affected by higher kilning temperatures and time. The β-amylase activity decreased as the thermal exposure during the kilning process is increased, which confirms the findings of other researchers (Nic Phiarais et al. 2006), where it has been reported that β-amylase activity of buckwheat decreased as the thermal exposure during the kilning regimen is increased. The rates of losses in beta-amylase activities of the teff malt were somewhat similar at higher temperatures and slower during the later stages of kilning at all these studied high temperatures. This could be attributed to the presence of only bound forms of the enzymes at high temperatures, which are bound to insoluble material in the endosperm (Sopanen and Lauriere 1989) and have high stability towards high temperature when compared to the free forms of the enzymes. All the free forms of the enzymes in teff malt might be denatured at the first few hours of the kilning process.

The Arrhenius energy for inactivation of the beta-amylase activities was found to be 6.93 kJ/mol. Thus, the Arrhenius rate constant equation for inactivation of beta-amylase in teff malt during kilning process was found to be

$$k_d = 5.05 \times 10^{-2} \exp\left(-\frac{833}{T}\right)$$

Limit Dextrinase Activity

Limit dextrinase is one of the many enzymes involved in the degradation of starch and starch-derived oligosaccharides. This enzyme is the only enzyme in germinating cereals

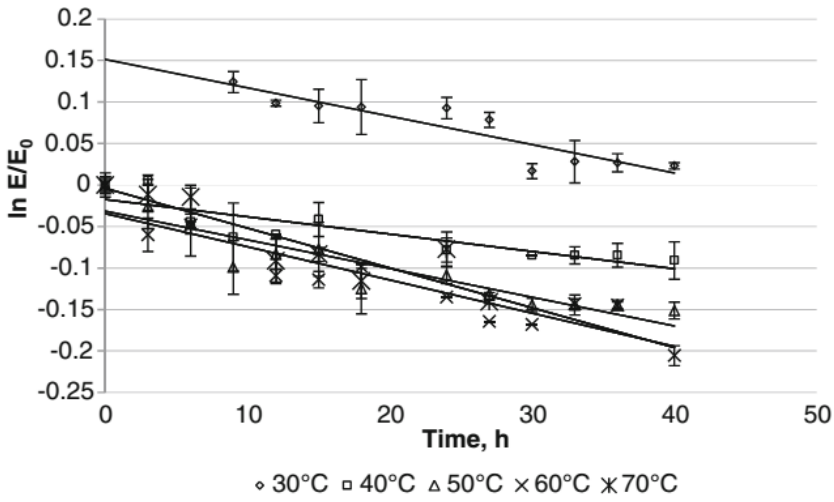

Fig. 4 Inactivation of β-amylase activities of teff malt during kilning process

capable of cleaving α-1, 6 linkages in branched dextrins formed by the action of α- and β-amylases on the amylopectin fraction of starch (Bryce 2003).

The results of this study showed that teff malt has higher limit dextrinase activity than barley and many other potential malting cereals. In combination with α- and β- amylases, limit dextrinase contributes to the degradation of starch. There are some evidences suggesting that malt limit dextrinase activity might be correlated to wort fermentability (Lee and Pyler 1984; Stenholm and Home 1999), an important malt quality attribute. Therefore, elevated limit dextrinase activity in malt may enhance hydrolysis of unfermentable branched dextrins into fermentable sugars, leading to increased total fermentability of wort.

The limit dextrinase activity continued to increase at all temperatures for some hours except for samples kilned at 70 °C (Fig. 5). The increase in the limit dextrinase activity may be attributed to the continuation of the germination process at low temperatures as well as the release of limit dextrinase from a bound form to a free form (Ross et al. 2003). The rate constants for activation of the limit dextrinase in the first stage of teff kilning ranged from 0.0241 h^{-1} at 30 °C to 0.0088 h^{-1} at 60 °C. In the later stages of kilning, deactivation of the limit dextrinase was observed at higher temperatures, and slight deactivation of the enzyme was also observed at 30 and 40 °C after 40 h of kilning. In the deactivation stage, the rate of deactivation of limit dextrinase during the kilning process was slow. The Arrhenius energies for the activation and deactivation of limit dextrinase were determined to be −28.2 and 5.72 kJ/mol, respectively. Thus, Arrhenius rate constant equation for the deactivation of limit dextrinase was found to be

$$k_d = 6.6 * 10^{-2} \exp\left(-\frac{688}{T}\right)$$

and the rate constant equation for the activation of the limit dextrinase at the first stage of kilning was found to be

$$k_a = 2.73 * 10^{-7} \exp\left(\frac{3395}{T}\right)$$

Unlike barley, where the limit dextrinase activity was stable at lower kilning temperatures and highly decreased at high temperatures (Kneen and Spoerl 1948; Lee and Pyler 1984), the limit dextrinase activity of teff malt significantly increased at lower kilning temperatures for longer time. This may be due to the retention of more water for longer kilning period at lower temperatures than barley, leading to continuation of the germination, which in turn causes development of the enzyme. Other research findings also showed that the limit dextrinase activity of barley malt was quite stable at 49 °C, showing almost no increase and loss in activity over 24 h. When the temperature was raised to 65 °C, only a slight decline in activity was observed, but a dramatic decline was observed at 82 °C (Lee and Pyler 1984); this could be attributed to the adverse effect of high-heat treatment on the enzyme activity.

Kilning Regimens

Enzymes that have been carefully developed in germination are easily destroyed by heat, especially when moisture content of the seed is high. To prevent this, the moisture content of the kernel needs to be lowered initially with warm (<50 °C), rather than hot (>60 °C), air at high airflows. As the seed moisture content is lowered, the enzyme system becomes more resistant to destruction by heat, and the drying temperature can be increased (Sebree 1997). The survival of enzymes in malt is also greatly influenced by the temperature and time of kilning regimen. Low initial drying temperature of below 50 °C for long initial drying time has a favourable effect on the

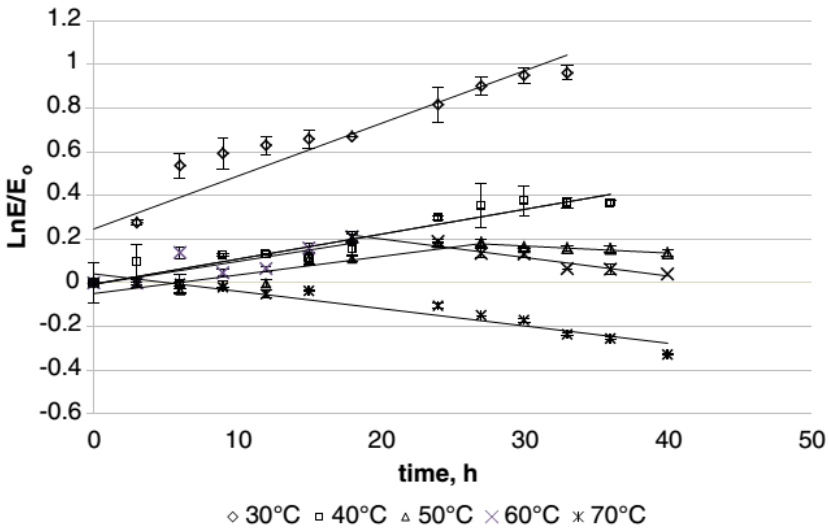

Fig. 5 Changes in the limit dextrinase activities of teff malt in the kilning process

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flavour stability of the beer (Kunze 2004). Developing an appropriate kilning regimen relies on different factors including the type of cereal to be used, type of required malt, and other factors which have impact on the final malt quality. Usually, the air temperature is raised from about 30 °C initially to a final temperature of about 80 °C over a period of 24 to 30 h. Under low moisture and high temperature (70 to 80 °C) conditions, complex chemical reactions take place between products of starch (sugars and dextrins) and protein (peptides and amino acids) degradations forming compounds that provide colour and aroma to the malt (CMBTC 2011). The non-germinated teff samples showed moderate amounts of β-amylase (<25 U/g) and limit dextrinase (<350 U/kg) activities but only traces of α-amylases (<3 U/g). After steeping, the activities increased to about 35, 355 and 6 U/g, respectively. The germination process increased the activities of all the three enzymes by several folds.

The results of kilning showed that over the final 22 h of kilning using the first kilning regimen (R1); there was an increase of the α-amylase activity from 61 U/g in the green malt to 68 U/g in the final dried malt. Whereas both β-amylase and limit dextrinase activities decreased from 502 to 440 U/g and from 1,297 to 1,072 U/kg, respectively. When the samples were cured for longer time (5 h) during the first kilning regimen, the α-amylase, β-amylase and limit dextrinase activities decreased to 60, 421 and 780 U/kg, respectively (Fig. 6).

All these enzyme activities during kilning with the second kilning regimen (R2) using both shorter (3 h) and longer (5 h) curing times decreased significantly (Fig. 7). Kilning the teff malt by using the second kilning regimen (R2) with shorter curing time reduced the α-amylase, β-amylase and limit dextrinase activities to 41, 406 and 736 U/kg, respectively. The same kilning regimen with longer curing time reduced these enzyme activities to 37, 395 and 594 U/kg, respectively. As the curing temperature increased, all the three amylolytic enzyme activities decreased. In both studied kilning conditions, the amylolytic enzyme activities of teff malt were significantly affected by the increase in temperature ($P < 0.05$) at the 95.0 % confidence level.

Like barley amylases, where α-amylase is more thermostable than β-amylase (Kunze 2004), α- and β-amylases in teff malt were also found to have different thermostability. It has been reported by other researchers that α- and β-amylases in some other malting materials such as buckwheat have almost similar thermostability (Nic Phiarais et al. 2005). Teff β-amylase showed an increase in inactivation after some hours of kilning at all kilning regimens due to heat denaturation. Our result for α- and β- amylases showed similar trends that occur in barley amylases. The α-amylase activity of teff malt, which was dried by using the first kilning regimen with shorter curing time, increased by about 11 %, but the decrease in β-amylase activity (12 %) of teff malt was much lower than that of barley malt (40 %) (Kunze 2004). The limit dextrinase activity of teff malt was also about 17 % less than in the green malt (Fig. 6).

DMS, a highly volatile sulphur compound, in malt and wort has a significant effect on the flavour of the finished product. Thus, the brewer aims at low levels in beers. The DMS level of teff reduced from 7.6 mg/kg in the green malt to 3.3 mg/kg in the final malt dried by using the first kilning regimen (R1) with shorter curing time and to 2.5 mg/kg while curing for longer time using the same kilning regimen. A reduction from 7.7 mg/kg in green malt to 2.15 and 1.7 mg/kg was recorded for the samples dried by using the second kilning regimen (R2) with shorter and longer curing times, respectively. The enzyme activity levels in teff malt kilned using R1 with shorter curing time were compared with barley malt and some other potential malting cereals and pseudo-cereals as highlighted in Table 1.

Teff malt was found to have a final α-amylase activity (68 U/g) lower than malted barley, which was found to have values in the range of 84–210 units per gram but higher than that of buckwheat malt (19.9 U/g) and comparable to some sorghum varieties (Alhassam and Adedayo 2010; Narziss and Back 2009; Nic Phiarais et al. 2005; Phiarais et al.

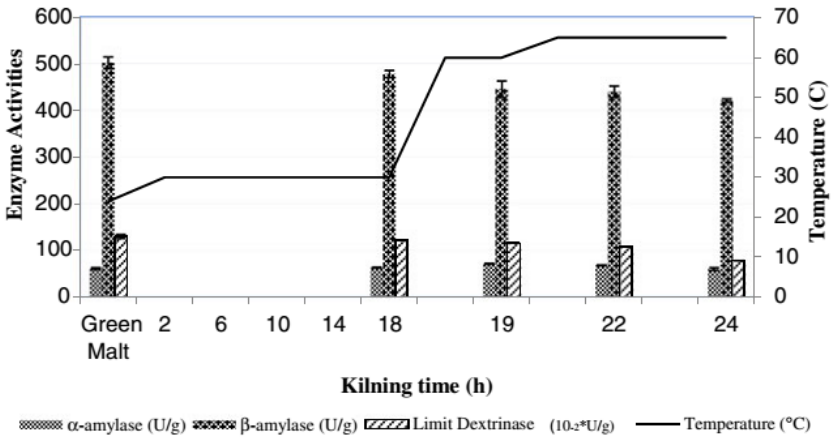

Fig. 6 Effect of kilning on amylolytic enzyme activities of teff malt dried by increasing the temperature of airflow in a stepwise fashion from 30 to 65 °C

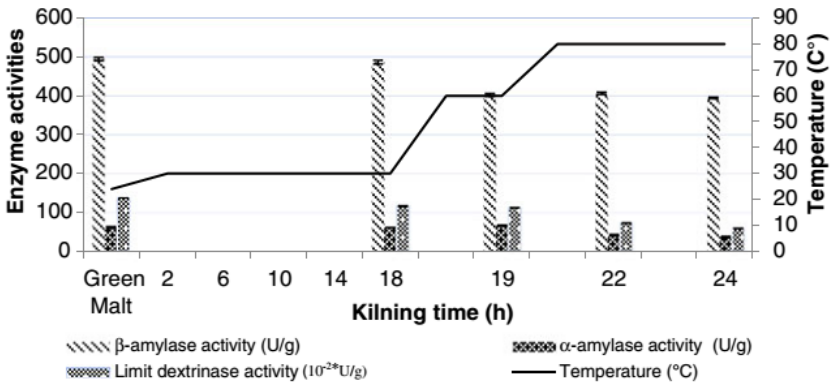

Fig. 7 Effect of kilning on amylolytic enzyme activities of teff malt dried by increasing the temperature of airflow in a stepwise fashion from 30 to 80 °C

2005). One of the possible problems responsible for low α-amylase activity in teff malt could be the high concentration of phenolics in teff (White and Xing 1997), which have the capacity to inhibit amylase activity in grains (Chukwura and Muller 1982; Daiber 1975). However, possibly, a teff seed does not require a higher level of alpha-amylase activity due to differences in the starch structure. Teff starch granules have a much smaller size (2–6 μm) than barley (10–25 μm); therefore, the enzymes in teff malt have a large total surface area to act upon (Bultosa et al. 2002; Lauro 2001). More surface area per volume of fine particles allows for a better solubilization of the grist ingredients. Thus, the enzyme reactions with the solubilized substrates will be high. The amylose–amylopectin ratio of teff starch (1:3) is lower (Bultosa 2007; Bultosa et al. 2002), which has also consequences for the enzymatic degradation. Evidences presented in literature lead to the conclusion that amylopectin is more readily degraded than amylose (Briggs et al. 1981).

The β-amylase activity of teff malt (440 U/g) is comparable to that of barley malt and is higher when compared to buckwheat, proso millet and sorghum malts (Agu and Palmer 1997; Alhassam and Adedayo 2010; Nic Phiarais et al. 2005; Phiarais et al. 2005; Zarnkow et al. 2010). Among the starch-debranching enzymes, limit dextrinase is an important enzyme found at high level in teff malt. The limit dextrinase activity found in teff malt was much higher than values reported in barley malt (mean value of 400 U/kg) (Narziss and Back 2009). This malt enzyme is important in the brewing industries because it has the potential to convert non-fermentable dextrins to fermentable sugars (Longstaff and Bryce 1993). The extract of teff malt (68 %) from a congress mashing procedure was lower than that of barley malt (76–85) (Narziss and Back 2009). This may be due to the higher gelatinization temperature of teff as well as different optimal temperature for the enzyme activities in teff malt than barley malt which makes the congress mashing procedure unsuitable for teff malt.

The total soluble nitrogen needs to be sufficiently high so that the 'body' and mouth-feel of the beer is adequate, and the beer foam (or 'head') will be stable. The soluble nitrogen

Table 1 Comparison of some quality attributes of teff malt with barley and some other gluten-free potential malting cereals and pseudo-cereals

Quality attribute	Teff	Barley[a]	Sorghum[b]	Buckwheat[c]	Proso millet[d]
α-amylase activity, U/g	68±1.4	84–210	52–170	19.9	124
β-amylase activity, U/g	440±17	225–627	38–131	24.7	107
Limit dextrinase activity, U/kg	1072±10	Nd to 800			2,073
Extract, % dm	68±3.27	76–85			64.1
Kolbach index, %	45±0.4	35–45			39.5
FAN, mg/L	182±2.1	85–237			219
DMS, mg/kg	3.3±0.1	4.1			
Gelatinization temperature, °C	69±0.71	58–66	69–75		67.1

Teff malt underwent congress mashing for wort production

Nd not detectable, *dm* dry matter

[a] Source: Narziss and Back (2009)

[b] Source: Alhassam and Adedayo (2010)

[c] Source: Nic Phiarais et al. (2005)

[d] Source: Zarnkow et al. (2010)

ratio (or Kolbach index) of the malt serves as measure of modification, and the value is often included in malt specification. FAN values (chiefly amino acids and small peptides) must be sufficiently high to ensure that lack of nitrogenous yeast nutrients does not limit fermentation (Briggs et al. 2004). Both Kolbach index and FAN values of teff malt were comparable with those of barley malt (Table 1). The soluble nitrogen in teff malt (710 mg/100 g) was also comparable with those of barley malt (600–900 mg/100 g) (Narziss and Back 2009) and proso millet malt (786 mg/100 g) (Zarnkow et al. 2010).

Conclusion

The results of this study indicated that the influences of kilning temperature and time were significant ($P<0.05$) for the amylolytic enzyme activities as well as DMS level of *kuncho* teff malt. Low kilning temperature yields greater survival of enzymes, particularly kilning at 30 and 40 °C resulted in the highest enzyme activities. This is a good indicator that most part of the drying process for teff malt should be achieved at low temperatures. Of the two selected kilning regimens, the first kilning regimen (R1) with shorter curing time was much better in preserving the survival of enzyme activities. It can be concluded from the results of this study that curing at 80 °C and prolonged curing at 65 °C can cause greater inactivation of the malt enzyme activities. Thus, the first kilning regimen with shorter curing time was considered as the best kilning regimen for good quality teff malt with respect to DMS level and survival of amylolytic enzyme activities. The β-amylase activity of teff malt is comparable to that of barley malt and much higher than some other potential brewing materials. The limit dextrinase activity of teff malt is more than twice of that of barley malt. However, its alpha-amylase activity is much lower than that of barley malt. The high limit dextrinase activity of teff malt will have high contribution to convert unfermentable dextrins to fermentable sugars. These simple sugars are highly required for both yeast and bacterial fermentations in food and brewing industries. In general, the amylolytic enzyme activities and DMS level of teff malt kilned in a step wise fashion from 30 to 65 °C for a total of 22 h show the potential of teff grain as a gluten-free brewing ingredient. Thus, it can be concluded from its malting qualities that teff can be an excellent alternative raw material for gluten-free foods and beverages. Wheat- and barley-based foods including beer are excluded from the diet of celiac disease patients, which is not a good outcome for food and brewing industries. The most obvious way to make a gluten-free food is to start with gluten-free raw materials. Thus, teff appears to be an interesting alternative food for celiac disease patients and consumers allergic to gluten. This research finding is good news for the food industries and their customers.

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2.4 Effect of teff (*Eragrostis tef*) variety and storage on malt quality attributes

Research article

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Effect of teff (*Eragrostis tef*) variety and storage on malt quality attributes

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Varieties of a cereal may have a considerable influence on malting qualities owing to variations in the physicochemical properties of the grains. This research was aimed at assessing the influence of five teff varieties on malt quality attributes. The teff samples were malted using previously optimized malting conditions and mashed with the congress mashing procedure. In this research, the *Kuncho* teff variety was malted in a one year period after harvesting, whereas the other four varieties were malted after three years of storage. Alpha- and β-amylase, and limit dextrinase activity, were in the ranges 14–68, 10–440 and 375–1072 U/kg, respectively. Extracts ranged from 54% for Dessie to 74% for Ivory teff. Free amino nitrogen, protein content, soluble nitrogen, Kolbach index, viscosity and wort colour were in the ranges 160–364 mg/L, 8.6–13.6%, 532–1048 mg/100 g, 24–50%, 1.441–1.629 mPa s and 5.9–9.0 EBC units, respectively. High-performance liquid chromatographic analysis for individual fermentable sugars revealed that the highest value in all varieties was recorded for glucose followed by maltose. The concentration of glucose ranged from 9.49 g/L in Brown teff, to 19.42 g/L in Ivory teff, whereas maltose ranged from 2.95 g/L in Dessie teff to 16.1 g/L in *Kuncho* teff. All of the malt quality attributes considered in this study were markedly influenced ($p < 0.05$) by the type of teff cultivar. It was concluded that the use of different teff varieties yielded malts with significantly different malt quality attributes.

Keywords: enzyme activity; malt quality; teff

Introduction

Teff (*Eragrostis tef*) is a tropical gluten-free cereal that originated in Ethiopia, between 4000 and 1000 BC *(1)*. It is environmentally more flexible than most other cereals. Teff is a low-risk cereal that grows over a wider ecology and can tolerate harsh environmental conditions where most other cereals are less viable, and it shows better tolerance to many diseases and pests *(2,3)*. Among other applications, teff is mainly used in Ethiopia and Eritrea for making *injera* and *tela*. It is also gaining popularity as a health food in the Western world, notably in the Netherlands and the USA, and its flour is becoming a preferred ingredient in gluten-free diets and sport foods *(4)*. Since teff is a gluten-free cereal with a high nutritional value, researchers are interested in studying it for utilization in different food applications. In addition to its nutritional and agricultural benefits *(2,3)*, it has also shown promising brewing properties *(5)*.

Malt is the major raw material used in the brewing industry. The primary quality criterion for use of cereals in the brewing industry is their potential to produce malt with diastatic power (amylase activity), which is needed to hydrolyse starch molecules and produce fermentable sugars. The brewing industry is reliant on the enzymes of malted cereals in order to ensure the efficient utilization of the raw materials. Hence, the fundamental role of the malting process is the mobilization of these endogenous hydrolytic enzymes (amylases) of the grain that are able to digest starch *(6,7)*. The major targets in malting and brewing comprise obtaining maximum extract, maximum fermentable sugars, enough nutrients for yeast growth, a balanced combination of high molecular weight compounds to have acceptable and stable foam and smooth palate fullness in beer, and avoiding haze formation during storage *(7,8)*. The malting qualities of a cereal are influenced by many factors including variety *(9)*, moisture, temperature, oxygen and other factors required in the malting process *(10)*.

Although literature reports on the effect of storage on enzyme activities of teff are lacking, storing the teff grain for several years is common practice in Ethiopia, as teff has a high tolerance to disease and pests. However, some reports on the effect of storage on enzyme activities of some other cereals have indicated that enzyme activities decrease with increased time of storage *(11)*. Thus the current research was designed to examine the malt qualities of some teff varieties and to assess the influence of storage on malt quality.

Materials and methods

Teff sample collection

Five teff (*Eragrostis tef*) varieties were used for this research. *Kuncho* (DZ-Cr-387) teff variety, which had been harvested in 2010, was obtained from the Debrezeit Agricultural Research Centre, Ethiopia. The other four varieties of teff (Ivory, Brown, Dessie and Sirgynia) were grown in North America and harvested in 2007. These four varieties were obtained from a teff company, in Idaho, USA. The samples were stored at room temperature prior to the malting process.

Malting conditions

The grain samples were manually cleaned by sifting and winnowing to ensure that they were free from chaff, dust and other

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impurities. Portions of these samples were used for analysis of the moisture content, thousand-kernel weight, gelatinization temperature and germination energy; the remaining portions were used for malting. Exactly 500 g aliquots of the teff grains, in duplicate, were placed in nylon bags and steeped in tap water at 24°C to 48% degree of steeping. At the end of the steeping, the samples were germinated at 24°C in a temperature-controlled chamber with 95% relative humidity for 4 days *(5)*. The samples were dried by increasing the temperature of the air flow in the drying stage from 30 to 65°C, that is, for 18 h at 30°C, 1 h at 60°C followed by 3 h at 65°C *(12)*.

Analytical procedures

The raw teff grains were analysed for moisture content, thousand-kernel weight and germination energy using standard methods *(13,14)*. The gelatinization temperatures of the grains were determined by measuring the rheological behaviour of a flour–water suspension, using the rapid visco-analyser RVA Super 4 (Newport Scientific, Australia) *(15)*. All malt samples were analysed for α-amylase, β-amylase and limit dextrinase activities, dimethyl sulphide (DMS) levels, and moisture content, using standard methods *(13,16)*. The malt samples were mashed using the congress mashing procedure, and the worts were analysed for extract, free amino nitrogen (FAN), soluble nitrogen, colour, viscosity and fermentable sugars following standard procedures *(13,14)*.

Statistical analysis

All the analyses were carried out in duplicate. The results are expressed as mean values and standard deviation (SD). The results were analysed with one-way analysis of variance (ANOVA) followed by Tukey's HSD test with $\alpha = 0.05$ using SPSS for Windows.

Results and discussion

Properties of raw samples

Thousand-kernel weights of the five teff varieties ranged from 0.27 to 0.28 g (Table 1). These values are very small when compared with the values for other cereals such as fonio, pearl millet, sorghum and barley *(17–19)*, suggesting that teff has very small kernels. All of the five teff varieties showed almost comparable germination energies in 72 h, except for *Kuncho*, which showed a 100% germination energy within 24 h. The high germination energies were indicative that all of the varieties had a high potential to germinate and to create homogeneous malt. The moisture contents of the grains were also comparable and there were no significant differences ($p > 0.5$) among the varieties.

Table 1. Properties of the raw samples of the teff varieties

Variety	Thousand kernel weight (g)	Germination energy (%)	Gelatinization temperature (°C)	Moisture content (%)
Ivory	0.281	100	71.7	11.43
Kuncho	0.275	100	69.7	11.41
Brown	0.273	98.5	70.6	11.64
Dessie	0.280	100	72.8	11.32
Sirgynia	0.278	99	72.4	11.40

The gelatinization temperature was determined by measuring the rheological behaviour of a flour–water suspension. This is the temperature at which an abrupt increase in viscosity occurs. Even though the gelatinization temperature of the starch granules depends mainly on the degree of cross-linking of the amylopectin, it may also be influenced by many other factors such as the growing environment of the crop and the granule size of the starch *(20–22)*. Cultivars containing a high proportion of large granules are preferred for malting as small granules have a higher gelatinization temperature *(20,23,24)*. The individual starch granules of teff samples are very small in size, ranging from 2–6 μm in diameter *(25)*. Although teff varieties have different amylose contents *(26)*, the gelatinization temperatures of the five varieties were in the same category (69–73°C), the intermediate gelatinization temperature *(27)*. The results of the gelatinization temperatures of the five teff varieties are shown in Table 1, and these results agree with previous reports for other teff varieties *(26)*. The gelatinization temperatures of all studied teff cultivars were high when compared with barley, but lower than rice, sorghum and some other commonly used cereals *(15,28)*. The gelatinization temperature of *Kuncho* teff is comparable to that of proso millet, which is also considered as a good alternative raw material for gluten-free beers and beverages *(29)*. The high gelatinization temperature of teff starch may result in a high concentration of undegraded starch molecules in the mashing stage of the process, because of inactivation of the starch degrading enzymes at such high temperatures. However, this can be solved by developing a suitable mashing programme for teff malt. Improving the thermal stability of the teff malt enzyme could also be an option to solve the possible problems that can be associated with the high gelatinization temperature of teff. The parameters that control the heat stability of enzymes are poorly understood, but some success has been achieved in improving the heat stability of barley β-amylase *(30)*.

Amylolytic enzyme activities

The levels of amylolytic enzymes, which are responsible for degrading starch molecules, are often considered the first limiting factors for the fermentability of malt *(31)*. Only small quantities of the amylolytic enzymes were detected for varieties from the 2007 harvest, mainly owing to the long-term storage of the samples. During the germination process and the first few hours of kilning, α-amylase continuously increased for all varieties (Fig. 1).

The maximum α-amylase activity (71 U/g) was recorded for the *Kuncho* teff variety after the first stage of kilning, that is, kilning for 18 h at 30°C. The α-amylase activities at the end of the drying process were in the range of 14 U/g for Brown teff to 68 U/g for *Kuncho* teff variety. These values were much lower than the 298 U/g of the standard barley malt flour. The *Kuncho* teff had the highest α-amylase activity among the samples tested. The low α-amylase activity, ranging from 14 to 38 U/g for the other four varieties, could be attributed to the long-term storage of the samples rather than varietal influence. In a previous experiment, where the samples were analysed in the one-year period after harvesting, higher values were reported *(5)* for these four varieties ranging from 37 U/g for Brown teff to 84 U/g for Dessie. Some of those values were comparable to the current result for the *Kuncho* teff, even though there were

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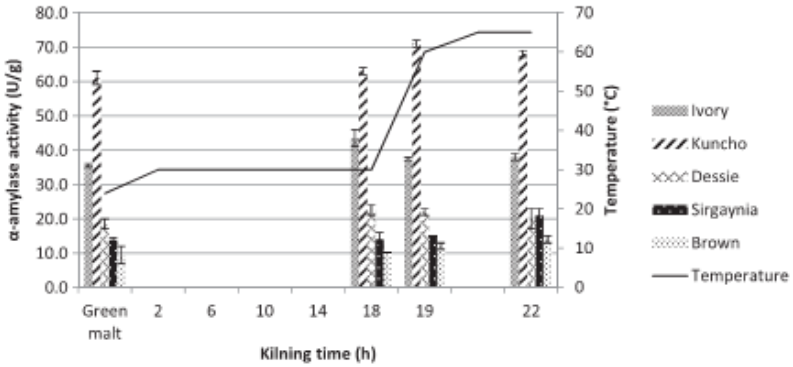


Figure 1. Influence of kilning on α-amylase activity of teff varieties stored for different periods of time.

some variations owing to varietal influence. α-Amylase works by breaking down the starch into high-molecular-weight dextrins, while the β-amylase breaks down these dextrins into simple sugars. In the presence of both α- and β-amylases, starch conversion is more rapid and complete than when either is acting individually *(32)*.

All of the five cultivars had similar trends in the changes in the amylolytic enzyme activity during germination (data not shown) and kilning (Figs 1–3), but had different levels of enzyme activity. For all varieties, the highest values for β-amylase were recorded for the green malts and these started to immediately decline during the kilning process. The decrease in the β-amylase activity during the kilning stage could be attributed to the thermolabile nature of this enzyme *(33)*. β-Amylase plays an important role in the course of mashing as it is responsible for the degradation of the starch, and products resulting from α-amylase hydrolysis of starch from the non-reducing ends of the starch molecules to maltose, the most abundant fermentable sugar in wort *(34)*. β-Amylase activity of the teff varieties is shown in Fig. 2. The β-amylase activity of the four teff varieties, ranging from 10 U/g for Brown teff to 63 U/g for Ivory teff, were quite low when compared with that of barley malt (mean 400 U/g) *(35)*. However,

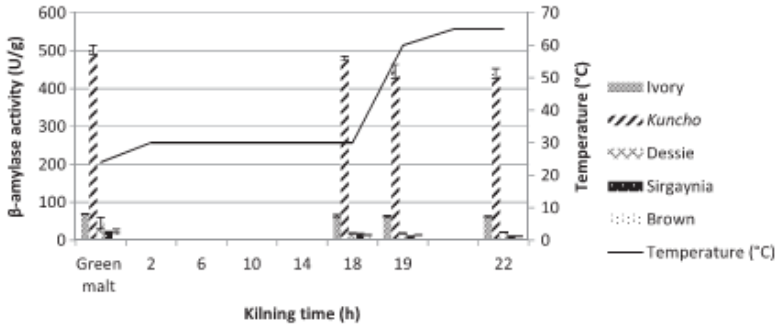


Figure 2. Influence of kilning on β-amylase activity of teff varieties stored for different periods of time.

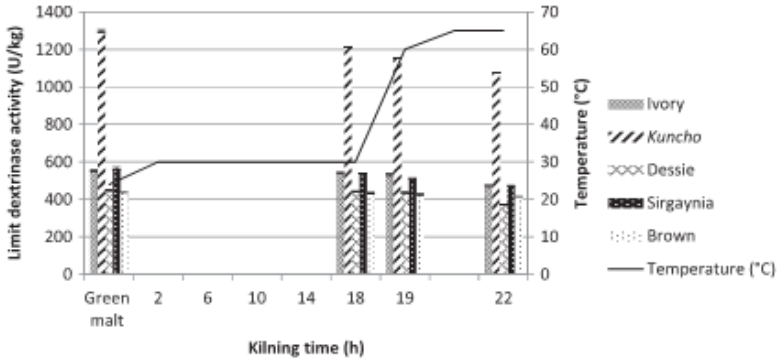


Figure 3. Influence of kilning on limit dextrinase activity of teff varieties stored for different periods of time.

the value recorded for the *Kuncho* teff malt (440 U/g) was comparable to that of barley malt (Table 2). The low values recorded for the other four varieties could be attributed to the influence of long-term storage, as well as variation in cultivars on the enzyme activities. In previous research *(5)* on these four varieties when they were analysed in a one-year period after harvesting, higher β-amylase activities, ranging from 187 U/g to 265 U/g, were reported. There were variations among those values and all the values were also lower than the value recorded for *Kuncho* teff.

Limit dextrinase is also an important enzyme in the brewing industry as it has the potential to convert unfermentable dextrins to fermentable sugars. The limit dextrinase activities of the teff varieties at the end of the kilning process were in the range of 375–1072 U/kg. The highest value was recorded for the *Kuncho* teff variety. The values for the other four varieties ranged from 375 U/kg for Dessie teff to 477 U/kg for the Ivory teff variety (Fig. 3). However, higher values ranging from 792 U/kg for Ivory teff to 1064 for Sirgyina teff *(5)* were reported in a previous experiment for the same varieties malted in a one-year time interval after harvesting. The low limit dextrinase activities determined in the current experiments could be attributed to the long-term storage of these varieties. The current result for the *Kuncho* teff variety and the previous reports for the other four varieties confirmed that the limit dextrinase activity of teff malt was more than twice of that of barley malt *(5)*. This high concentration of limit dextrinase activity in teff malt is responsible for the higher concentration of glucose in all teff varieties (Table 3). At the end of the kilning process, there was about 10% higher α-amylase in the final *Kuncho* teff malt than in the green malt, whereas β-amylase and limit dextrinase were about 12 and 17%, respectively, lower than in the green malts. This indicates that α-amylase in teff malt is more heat stable than β-amylase or limit dextrinase.

Other malt quality attributes

The DMS content of the malt was determined using a gas chromatographic head-space method following the Central European Brewing Commission Technical Analysis (Mitteleuropäische Brautechnische Analysenkommission, MEBAK) procedure 1.3.3 *(13)*. The variety of a cereal and the malting conditions can have a great influence on the dimethyl sulphide precursor (DMSP) of the malt. The growing region, climate and year of cultivation can also have considerable influence. The higher the germination moisture level, the higher the DMSP content of the malt *(7)*.

Neither free DMS nor DMSP was detected in unmalted teff samples. However, the DMS precursor (*S*-methyl methionine) was formed during the germination process, and the highest

Table 2. Malt quality attributes of teff varieties (congress wort) compared with barley malt

Quality attributes	*Kuncho*	Sirgynia		Dessie		Brown		Ivory		
	In 1 year[a]	In 1 year[a,b]	After 3 years[c]	In 1 year[a*]	After 3 years[c]	In 1 year[a,b]	After 3 years[c]	In 1 year[a,b]	After 3 years[c]	Barley malt[d]
α-Amylase (U/g)	68 ± 1.4	43	21 ± 3	84	20 ± 4	37	14 ± 1.4	71	38 ± 1.4	80–210
β-Amylase (U/g)	440 ± 17	196	11 ± 0.7	187	21 ± 0.01	265	10 ± 1.4	261	63 ± 2.1	225–627
Limit dextrinase (U/kg)	1072 ± 20	1064	473 ± 2	1062	375 ± 10	936	416 ± 2	792	472 ± 7.8	ND[e] to 800
FAN (mg/100 g)	182 ± 2.1	172	298 ± 2	285	364 ± 4	99	160 ± 0.1	146	167 ± 2.1	85–237
Soluble nitrogen (mg/100 g)	712 ± 3.5		821 ± 1		1048 ± 2		532 ± 6		968 ± 13	600–900
Kolbach index (%)	45 ± 0.4		37 ± 0.1		42 ± 0.04		24 ± 0.3		50 ± 0.71	35–45
Extract (%, d.m.)	68 ± 3.3	51.5	59 ± 2.8	52.1	54 ± 2.8	60.4	60 ± 1.8	63.6	74 ± 2.1	76–85
Viscosity (mPa s)	1.63		1.44		1.58		1.56		1.44	1.5–1.6
Colour (EBC)	9.0 ± 0.1	5.7	7.0 ± 0.4	5.9	7.6 ± 0.42	4.6	5.9 ± 0.14	5.2	7.8 ± 0.1	2.0–8.0

[a]Samples were malted and analysed in a one year period after harvesting
[b]Source: Zarnkow *et al. (5)*.
[c]Samples were malted and analysed after three years of storage
[d]Source: Hysert *et al. (36)*.
[e]Not dectable.

Table 3. Concentration of fermentable sugars in wort (congress wort) of five teff varieties

Teff variety	Glucose (g/L)	Maltose (g/L)	Maltotriose (g/L)	Fructose (g/L)	Saccharose (g/L)	Total (g/L)
Brown	9.49	3.36	0.75	0.38	0.13	14.11
Dessie	11.29	2.95	0.59	0.92	0.35	16.10
Sirgynia	13.17	3.82	0.76	0.68	0.22	18.65
Kuncho	18.00	16.07	1.68	0.04	0.99	36.78
Ivory	19.42	5.07	0.34	1.85	0.33	27.01

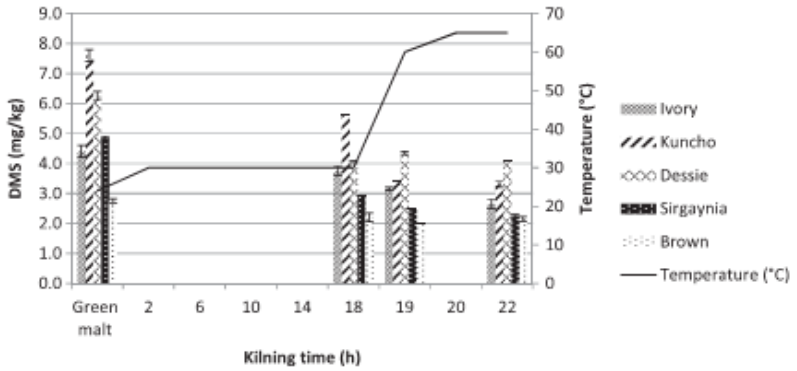


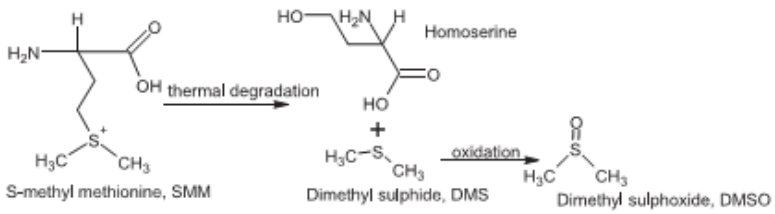
Figure 4. Changes in the total DMS contents of teff malts during kilning.

total DMS values in all of teff varieties were detected for the green malts (Fig. 4). The total DMS, which was determined from DMS precursor and free DMS in the samples, decreased during the kilning process. This is because the DMS precursor is degraded to homoserine, and part of the DMS formed from the precursor is driven off. Also, some parts of the DMS formed from the precursor (*S*-methyl methionine) are oxidized to DMSO in the kilning process *(7,36)*. The degradation of the DMSP is depicted in Scheme 1.

The total DMS levels of the dried malt samples of the five teff varieties ranged from 2.2 mg/kg for Brown teff to 4.1 mg/kg for the Dessie teff variety (Fig. 4). The total DMS levels determined for all varieties were below the upper limit (7 mg/kg) recommended for a good quality malt for brewing *(6,7)*.

The malt quality attributes of the teff varieties were variable (Table 2). The extracts from a congress mashing procedure were lower than those of barley malt. This may be due to the higher gelatinization temperature of teff starch granules, as well as different optimal temperature for the enzyme activities in teff malt. The values of the gelatinization temperatures (Table 1) explain the profiles of the extract yields. The gelatinization temperatures were negatively correlated ($r = -0.5$) with the extract yield regardless of the teff variety. The varieties with high gelatinization temperatures showed low extract yield. However, this was not true for the Ivory teff variety. This may be due to the high degree of modification that the malt had been subjected to in the malting process, which can be explained by the high value of the Kolbach index (50%). The Kolbach index is used to express the quantity of the nitrogenous substances found in malt that go into solution during mashing. It is a measure of the degree of proteolytic modification of the malt, and also provides an indication of the quantity of proteolytic enzymes contained in the malt *(13)*. Cell wall and protein degradation make the starch much more accessible to amylase enzymes, resulting in increased extract content *(37)*. The ratio of the soluble nitrogen and total nitrogen indicates the degree to which the endosperm (the carbohydrate–protein matrix) has been enzymatically unlocked (modified). The Kolbach indices 50, 45 and 42 recorded for Ivory, *Kuncho* and Dessie teff varieties, respectively, indicate that these samples were highly modified (Kolbach index > 41%) *(37,38)*. Amino acids, collectively referred to as FAN, are the principal nitrogen source in wort and are an essential nutritional component of yeast and bacteria during fermentation. FAN is an estimate of the total amount of assimilable free amino acids in wort *(37)*. This is determined based upon the reaction of ninhydrin with the free α-amino nitrogen group *(14)*. Research findings have revealed that brewing yeast is capable of assimilating simple amino acids and peptides, but not proteins. Thus, the amount of free amino nitrogen is important to guarantee a stable fermentation process. For conventional wort (specific gravity of 1.048, 12°P), a FAN level of about 145 mg/L or more is reported to be necessary for a healthy fermentation *(39–44)*. The results of the current research revealed that all of the teff varieties had higher levels of FAN (Table 2). The Dessie teff variety showed a higher nitrogen extract than the other varieties (measured as total soluble nitrogen and FAN). The high value of the nitrogenous material in the extract of Dessie teff (Table 2) may reflect the high nitrogen content of this variety.

The values of the wort colours recorded for most of the studied teff varieties were higher than those of most barley varieties. This could be attributed to Maillard reaction products caused by Maillard precursors produced at high degrees. The high Kolbach indices and FAN values are good indicators of this phenomenon. Higher FAN values were recorded for the long-term stored samples of the four (Ivory, Dessie, Sirgynia and Brown) varieties when compared with the values reported in a previous experiment for samples analysed in a one-year period after harvesting



Scheme 1. Schematic representation of the degradation of dimethyl sulphide precursor during malting.

(Table 2). The viscosities of the worts, ranging from 1.44 to 1.63 mPa s, were comparable to barley wort viscosities and there were no problems with lautering and filtration.

Fermentable sugars

Researchers focus on the amount of fermentable extract, which is directly linked to the potential alcohol yield, instead of dealing only with the total extract yield of raw materials. The fermentable sugars, maltose, maltotriose and glucose, are the main products of starch degrading enzymes such as α- and β-amylases and are considered to be key to fermentation *(34)*. The fermentable sugars in the worts were determined using HPLC and following European Brewery Convention method 8.7 *(14)*. The results of this study showed that the total fermentable sugar levels varied among the varieties and this can be explained by the large differences in enzyme activities. The level of total fermentable sugars in *Kuncho* teff (37 g/L) was significantly higher than in the other four varieties (Table 3). The levels of the individual fermentable sugars also showed high variability.

Kuncho teff had a much higher concentration of maltose (16.1 g/L) compared with the other varieties. This could be attributed to the high β-amylase activities of *Kuncho* teff. Maltose comprised about 44% of the total fermentable sugar of *Kuncho* teff wort. The concentrations of maltose in the other varieties were comparable, except that its concentration in Ivory teff was slightly higher (5.1 g/L) than in the other three varieties. The lowest concentration of maltose (2.95 g/L) was recorded for Dessie teff variety. The glucose concentrations in *Kuncho* (18 g/L) and Ivory (19.4 g/L) teff varieties were much higher than those in the other three varieties. The contributions of the glucose concentrations in *Kuncho* and Ivory teff varieties to their respective total sugar concentrations were about 49 and 72%, respectively. The lowest glucose concentration (9.5 g/L) was recorded for Brown teff variety.

Since the starch granules from malted cereals are attacked by enzymes during the germination process, they can be hydrolysed below the gelatinization temperature *(45,46)* to yield the recorded significant concentration of fermentable sugars in the teff samples. However, the total fermentable sugars of the teff samples were lower than the values reported for barley samples *(47)*, where the values in the four barley varieties ranged from 54.1 to 57.3 g/L. This can be attributed to the unsuitability of the congress mashing procedure for teff samples owing to its high gelatinization temperature. The glucose concentrations in the teff samples (9.5–19.4 g/L) were higher than those in barley samples (5.6–8.1 g/L). This could be explained by the high limit dextrinase activity of teff malt compared with barley malt. However, the maltose concentrations in the teff samples (2.95–16.1 g/L) were much lower than those in barley samples (37.4–38.2 g/L) *(47)*. This could be due to the deactivation of the β-amylases of teff before the starch molecules are gelatinized. β-Amylases are responsible for the production of maltose. Thus, the amount of maltose in the wort is less than the amount expected for gelatinized starch granules hydrolysed by β-amylases. The low production of maltose, in general, is not due to limited concentration of amylolytic enzymes in teff samples; rather it is because of inadequate gelatinization of the starch.

Conclusions

The concentrations of fermentable sugars, the enzyme activities and the other malt quality attributes were markedly influenced by the types of teff cultivars. In general, the *Kuncho* teff variety showed the highest enzyme activity and concentration of fermentable sugar when compared with the other studied cultivars. The malting qualities of samples malted in a one-year period after harvesting indicated that teff had good malting characteristics and brewing potential. This is good news for researchers who are investigating alternative raw materials for gluten-free beverages. The chromatographic analysis showed that maltose and glucose are the principal sugars in the worts of the five studied teff varieties. The large differences in the amylolytic enzyme activities, as well as other malt quality attributes of the five teff varieties considered in this study, can be explained by the differences in the varieties of teff, and storage periods of the samples. In general, it can be concluded that the use of different teff varieties yields malts with significantly different malt quality attributes and long-term storage has a negative influence on the malting qualities of teff samples.

Acknowledgements

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2.5 Thermal stability of starch degrading enzymes of teff (*Eragrostis tef*) malt during isothermal mashing

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Short communication

Thermal stability of starch degrading enzymes of teff (*Eragrostis tef*) malt during isothermal mashing

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ABSTRACT

Thermal stability of starch degrading enzymes varies from one source to another. This research was aimed to study thermal stability of starch degrading enzymes of teff malt. Isothermal mashing at temperatures ranging between 40 and 75 °C with sampling in 15 min interval for a total of 90 min was conducted. The study showed that deactivation rate constants of alpha- and beta-amylases ranged from 0.0003 to 0.0409 min^{-1}, and 0.002 to 0.032 min^{-1}, respectively. Rate of deactivation of limit dextrinase was not significant at temperatures lower than 60 °C but showed high deactivation at higher temperatures with rate constants ranging from 0.02 to 0.1 min^{-1}. The thermal deactivation energies of alpha-amylase, beta-amylase, and limit dextrinase were found to be 148, 82, and 144 kJ/mol, respectively. The present findings have significant applications in commercial processes where determination of the upper temperature limits for these enzymes is required.

1. Introduction

The demand for gluten-free foods is certainly increasing. Interest in teff has increased noticeably due to its very attractive nutritional profile and gluten-free nature [1,2], making it a suitable substitute for wheat and other cereals in their food applications including in beverage production. The capacity of a cereal to produce malt with amylolytic enzymes is a primary quality criterion for the cereal to be used in brewing industries. Our previous findings showed that teff has the potential to produce malt with high amylolytic enzyme activities [2–4]. Starch degrading enzymes are among the most important enzymes used in food and other industries involving starch hydrolysis. It is well known that germination of cereals causes a significant increase in the enzyme activities. On the other hand, kilning and mashing processes may decrease the activities of the enzymes [5–8]. Thermal stability of the enzymes is a very crucial property in designing processes involving starch hydrolysis as it determines the limits for use of the enzymes [9]. The temperature program in mashing, for example, is normally designed to favor starch degrading enzymes in order to maximize extract yield and formation of fermentable sugars. The relationship between thermostability of starch degrading enzymes and the gelatinization temperature of the starch essentially determines the formation of fermentable sugars during mashing [10,11].

The thermal stability profiles of starch degrading enzymes vary from one cereal to another [12,13]. Studies on barley malt showed that limit dextrinase, which hydrolyses α-1,6-linkages of amylopectin, has somewhat more thermal stability when compared to beta-amylase. But it is readily inactivated at temperatures higher than 65 °C. On the other hand, α-amylase has high stability when compared to the other two, and retains significant amount of its activity at relatively higher temperatures (>65 °C) [14–16]. However, the thermal stabilities of starch degrading enzymes of teff malt have not been studied yet. Thus, this research was aimed to study the thermal stability of amylolytic enzymes of teff malt.

2. Materials and methods

2.1. Thermal stability test

We studied the thermal deactivation of alpha-amylase, beta-amylase, and limit dextrinase during isothermal mashing of teff malt in a 1:4 malt to water ratio at temperatures ranging from 40 to 75 °C with sampling in 15 min interval for a total of 90 min. The samples were cooled down to room temperature, and assayed for alpha-amylase, beta-amylase, and limit dextrinase activities using standard methods [17–20]. Since limit dextrinase did not show any deactivation at temperatures lower than 60 °C, additional experiments were conducted at temperatures of 62, 63, 64, 65, and 70 °C. The malt samples used in these experiments were prepared using a previously developed condition of malting [3,4]. It is necessary to know the enzyme activities in the malt samples in order to determine the absolute residual enzyme activity after thermal exposure. Thus, preliminary experiments were carried out to determine the enzyme activities that can be achieved under the mashing conditions before exposure of the enzymes to higher thermal energy. The activities of all the three types of enzymes considered in this study showed a significant increment throughout the 90 min of thermal exposure at 30 °C but started to decline after 90 min. Hence, the highest enzyme activities recorded at this temperature were used as initial activities (X_o).

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2.2. Enzyme activity assay

The wort samples were assayed for alpha-amylase, beta-amylase, and limit dextrinase activities. The enzyme activities were determined spectrophotometrically following standard methods [17–20]. According to these procedures, determination of the enzyme activities of malt involves extraction of the enzymes from the malt using extraction buffers followed by incubating the extract with standard substrate. However, in this study the extracts produced at the different mashing temperatures were incubated with the standard substrate.

2.2.1. Alpha-amylase

For the determination of alpha-amylase activity, teff malt extract (0.2 ml) was incubated for 10 min at 40 °C with 0.2 ml of pre-equilibrated substrate mixture consisting of non-reducing-end blocked p-nitrophenyl maltoheptaoside (BPNPG7) as well as excess levels of gamma-amylase and glucoamylase. The substrate is absolutely resistant to hydrolysis by exo-enzymes such as β-amylase, gamma-amylase and glucoamylase. On hydrolysis of the oligosaccharide by endo-acting α-amylase, the excess quantities of glucoamylase present in the mixture give instantaneous and quantitative hydrolysis of the p-nitrophenyl maltosaccharide fragment to glucose and free p-nitrophenol. The reaction was terminated by the addition of trizma base solution. Then the absorbance of p-nitrophenol in the solution was measured at 410 nm against distilled water [17]. One unit of enzyme activity is defined as the amount of enzyme which releases 1 μmole of p-nitrophenol/min under the defined assay conditions.

2.2.2. Beta-amylase

For beta-amylase measurement, teff malt extract (0.2 ml) was incubated with 0.2 ml of pre-equilibrated substrate mixture of β-glucosidase and p-nitrophenyl-β-D-maltotrioside (PNPβ-G3) at 40 °C for 10 min. The substrate (PNPβ-G3) is rapidly hydrolysed by β-amylase, but not cleaved by cereal α-amylase, which requires a longer stretch of α-1,4-linked D-glucosyl residues to satisfy the substrate sub-site binding requirements [21]. The rate of release of p-nitrophenol by the β-glucosidase present in the substrate mixture relates directly to the rate of release of maltose by β-amylase. The reaction was terminated and color developed on addition of a high pH trizma base solution. The absorbance of the solution was determined at 410 nm against distilled water [18].

2.2.3. Limit dextrinase

The substrate in the determination of limit dextrinase was azurine-crosslinked-pullulan, which is hydrolysed by limit dextrinase and pullulanase, but is resistant to attack by other commonly occurring amylolytic enzymes such as α-amylase, β-amylase and amyloglucosidase. A pre-equilibrated teff malt extract at 40 °C for 5 min was incubated with the substrate at 40 °C for 10 min. After terminating the reaction by trizma base solution, the absorbance was determined at 590 nm [19]. Hydrolysis by limit-dextrinase (pullulanase) produces water soluble dyed fragments, and the rate of release of these substances or the increase in absorbance at 590 nm can be related directly to enzyme activity. Enzyme activity in U/ml was determined by reference to standard curve (Absorbance at 590 nm versus activity in U/ml). One Unit of activity is defined as the amount of enzyme required to release 1 μmole of glucose reducing-sugar equivalents per min from pullulan under the defined assay conditions.

2.3. Enzyme kinetics data analysis

At higher temperatures the enzyme stability decreases by thermal denaturation; as a result the enzymatic activity starts to decline. The thermal energy applied for the denaturation process is termed as the energy of activation of thermal denaturation reaction, or as it is usually called energy of deactivation [22]. Since the active enzymes proceed directly to inactive state without providing significant amount of stable intermediates, thermal deactivation of enzymes is considered as a first order reaction [23–26]. Thus, the deactivation rate constants of the enzymes were determined from residual enzyme activities, which represent the ratio of the enzyme activity at time t to the highest enzyme activity determined from a sample before thermal exposure, using a first order reaction expression.

$$\frac{dX}{dt} = -kX \tag{1}$$

where X represents the maximum enzyme activity following thermal exposure of the teff malt samples for a certain time t.

The values of the deactivation rate constants (k) were determined from a plot of residual activity data $\ln(X/X_0)$ as a function of time (t) at a particular temperature [25,27], where X_0 represents the maximum enzyme activity before thermal exposure (in our experiments the maximum activity determined at 30 °C).

The energies for thermal deactivation of the enzymes were determined from linearization of the Arrhenius equation (Eq. (2)), and by plotting the natural logarithm of the deactivation rate constant values against the reciprocal of the absolute temperature.

$$k = k_0 \exp\left(-\frac{E_d}{RT}\right) \tag{2}$$

where R is universal gas constant (8.314 J/mol K); k is rate constant; and T is temperature in Kelvin. The values of the deactivation energies (E_d) and the pre-exponential factors (k_0) were estimated from the slope and intercept of the plot of $\ln k$ versus $1/T$, respectively.

2.4. Statistical analysis

All the experiments were conducted in triplicate, unless otherwise stated. All statistical analyses were performed with one-way analysis of variance (ANOVA) followed by Tukey's HSD test at $\alpha = 0.05$ using SPSS for windows.

3. Results and discussion

Thermal deactivation kinetics of alpha-amylase, beta-amylase, and limit dextrinase from teff malt were determined by incubating the enzymes at different temperatures ranging between 40 and 75 °C for a total of 90 min with sampling in 15 min interval. The time–temperature data were used to determine the residual enzyme activities. The proportions of the residual enzyme activities are related exponentially to both the activation energy and the rate constant. From the plot of semi logarithm of enzyme activity residue as a function of time, the slope tells us the rate at which the enzyme activity is changing with the change in time. The statistical analysis showed that there was no significant decrease in the alpha-amylase activity at 40 and 50 °C during the 90 min of mashing (Fig. 1). However, at higher temperatures it was significantly decreased ($p < 0.05$).

The rate constants for the deactivation of alpha-amylase in the wort samples determined from the plot of residual activity data against time (Fig. 1A) ranged from 0.0003 min^{-1} at 40 °C to 0.04 min^{-1} at 75 °C. The deactivation rate constant (k) increased with an increase in the mashing temperature (Fig. 1B), normally following an Arrhenius relationship (Eq. (2)). The rate constants were used for the determination of Arrhenius energy (E_d) and the pre-exponential factor (k_0). The deactivation energies and the pre-exponential factors of the enzymes considered in this study were determined by fitting the data using linear regression to an Arrhenius-type equation (Eq. (2)). The deactivation energy of alpha-amylase in teff malt was found to be 148 kJ/mol. The result showed that its thermal stability is low when compared to the stability of alpha-amylase in jowar (*Sorghum bicolor*), where higher deactivation energy (189.54 kJ/mol) was reported [6]. Based on the Arrhenius type equation developed in this study, it can be estimated that all the alpha-amylase in teff malt will be deactivated at a mashing temperature of 78 °C.

The deactivation rate constants for beta-amylase were in the range of 0.002–0.032 min^{-1}. The highest value was recorded for samples mashed at 70 °C and the lowest was for samples mashed at 40 °C (Fig. 2A). The high values of the deactivation rate constants for beta-amylase at lower temperatures when compared to those of alpha-amylase are good indicators for its less tolerance to thermal exposure at these temperatures. The statistical analysis also confirmed the low stability of beta-amylase at the studied temperatures when compared to alpha-amylase. Beta-amylase activities were significantly decreased ($p < 0.05$) at all studied temperatures during the 90 min of thermal exposure (Fig. 2). Thermal deactivation of alpha-amylase (Fig. 1A), and beta-amylase (Fig. 2A) after 65 °C displayed very dramatic differences. Other researchers [26] also reported considerable reduction, 35 and 57%, in the alpha-amylase activities of millets within a short time (20 min) of incubation at 65 and 70 °C, respectively. This can be explained by partial unfolding and covalent alterations of the protein structures in the aqueous medium [28]. The Arrhenius plot of the deactivation rate constant for beta-amylase is shown in Fig. 2B. The deactivation energy was found to be 82 kJ/mol.

One of the possible factors, among others, for low levels of limit dextrinase activity in cereals [12,29] is the less thermostability of

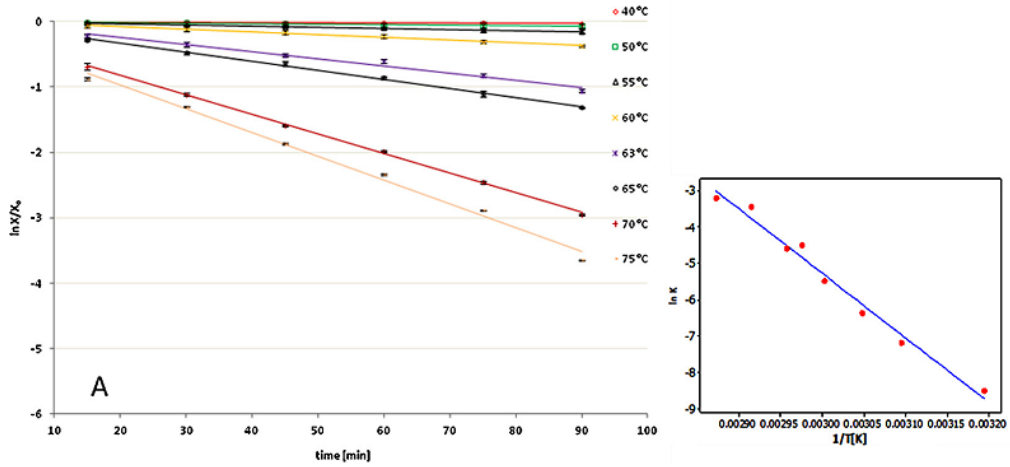

Fig. 1. Thermal deactivation profile of alpha-amylase from teff malt: (A) natural logarithm of the enzyme residual activity against heat treatment time and (B) natural logarithm of the enzyme deactivation rate constants versus reciprocal of absolute temperature.

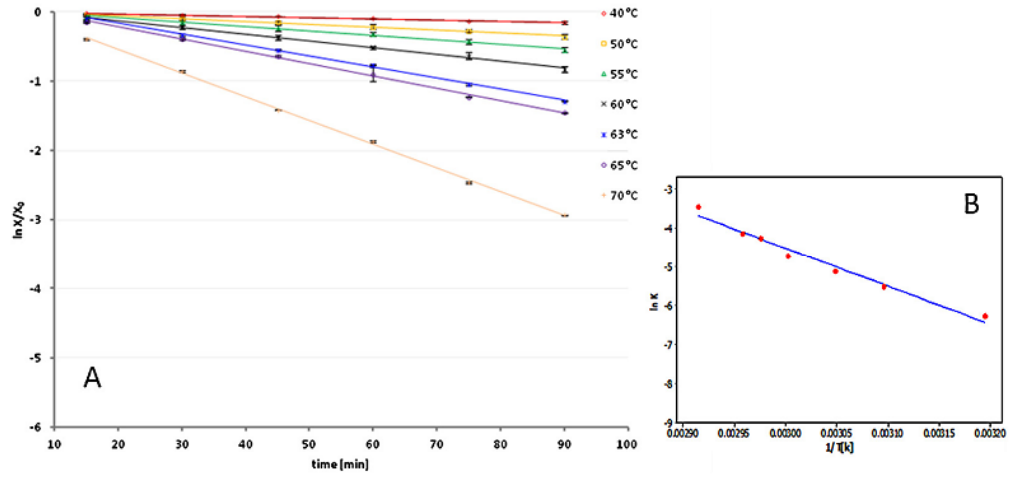

Fig. 2. Thermal deactivation profile of beta-amylase from teff malt: (A) natural logarithm of the enzyme residual activity against heat treatment time and (B) natural logarithm of the enzyme deactivation rate constants versus reciprocal of absolute temperature.

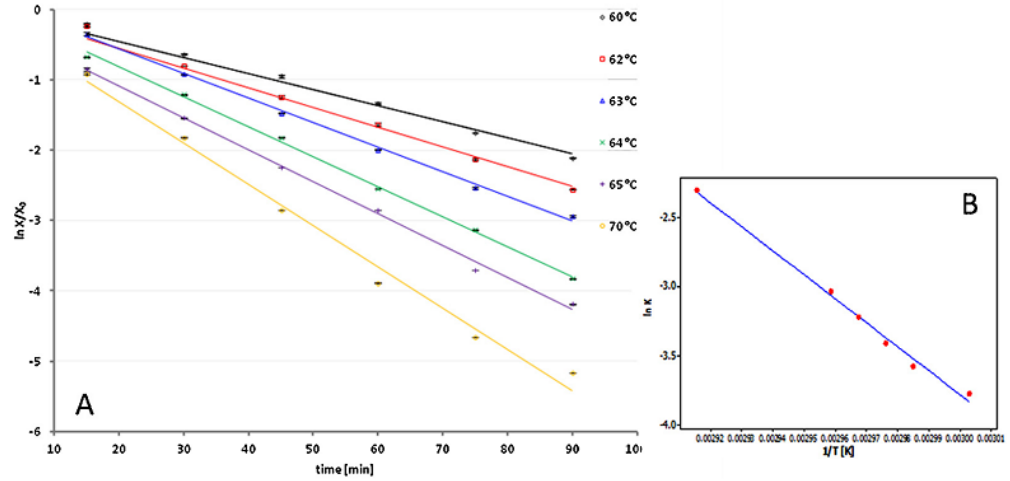

Fig. 3. Thermal deactivation profile of limit dextrinase from teff malt: (A) natural logarithm of the enzyme residual activity against heat treatment time and (B) natural logarithm of the enzyme deactivation rate constants versus reciprocal of absolute temperature.

Table 1
Calculated values of deactivation energies and frequency factors using the Arrhenius model (Eq. (2)).

	Alpha-amylase	Beta-amylase	Limit dextrinase
E_d (kJ/mol)	148	82	144
k_0 (min^{-1})	7.99×10^{20}	7.74×10^{10}	7×10^{20}

the enzyme. In our experiments, no deactivation was observed for limit dextrinase at temperatures lower than 60 °C. However, it was highly deactivated in between 62 and 70 °C, and completely deactivated at 75 °C (Fig. 3). The deactivation rate constants determined from the slope of $\ln X/X_0$ versus time (Fig. 3A) were in the range of 0.02 min^{-1} at 60 °C to 0.1 min^{-1} at 70 °C. The deactivation energy, which was estimated from the slope of ln *k* versus reciprocal of absolute temperature (Fig. 3B), was found to be 144 kJ/mol. Finding of the conditions at which limit dextrinase exhibits more stability would be a potential way of increasing the concentrations of fermentable sugars in the wort that can be resulted from the high efficiency of the enzymes. The deactivation of limit dextrinase was observed only at high temperatures. As a result high frequency factor was determined although small temperature range was considered for its thermal stability study. The experimentally observed values of the deactivation energies and frequency factors for alpha-amylase, beta-amylase and limit dextrinase are shown in Table 1.

High enzyme activities were recorded for alpha amylase at higher temperatures when compared to those of beta-amylase and limit dextrinase. This indicates that alpha-amylase is more stable at higher temperatures than beta-amylase and limit dextrinase. This was confirmed by the deactivation rate constants. It can be seen that alpha-amylase has lost very little of its activity at temperatures lower than 60 °C. At higher temperatures, alpha-amylase was by far the most thermostable among the starch degrading enzymes considered in this study. Some activities of alpha-amylases were detected after 90 min of mashing at 75 °C whereas the activities of beta-amylase and limit dextrinase were undetectable at this condition. The thermal deactivation tests showed that beta-amylase and limit dextrinase lost more than 99% of their activities after 70 °C, whereas small activity was detected for alpha-amylase beyond this temperature. The deactivation of beta-amylase was much more pronounced than those of alpha-amylase and limit dextrinase at low temperatures. This was confirmed by the rate constants. Deactivation of the enzymes at higher temperatures, in general, could be attributed to unfolding of the protein structures. An unfolding of the enzymes gives randomly coiled structure with no enzyme activity [30]. The deactivation usually starts with a reversible conformational change in the proteins followed by secondary irreversible processes such as aggregation or covalent modification resulting in irreversible conformational changes in the protein molecules [31]. As a result most of the enzymes are deactivated at these high temperatures.

4. Conclusion

The knowledge of thermal stability of the enzymes would provide a basis for understanding the interaction between starch degradation and the working temperatures during mashing. The mathematical equations for the deactivation rates of the amylolytic enzymes in the teff malt samples are useful for estimating the activities of the enzymes. The information provided by the equations can be used to design the working time and temperature for the mashing process in order to produce teff wort with the required proportions of the fermentable sugars for specific application in the beverage industries. It is also useful for proper selection of the mashing-in and mashing-off temperatures during optimization of the mashing program. From the Arrhenius type equation developed in this study, it can be estimated that all the alpha-amylase in teff malt will be deactivated at a mashing temperature of 78 °C. Hence, this temperature can be used as a mashing-off temperature in the mashing program for teff malt.

Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.procbio.2013.08.019.

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2.6 Investigation of fermentation conditions for teff malt-wort by *Lactobacillus amylolyticus*

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Abstract

Lactic acid fermentation plays an important role in improving the sensory quality and shelf life of foods. This research was aimed to assess the influences of fermentation factors on some quality attributes of teff malt-wort, and investigate optimal fermentation condition for the teff malt-wort using response surface methodology. The experimental design and statistical analysis were performed using Design Expert statistical software. High lactic acid formation was observed between 42 and 48 °C. An increase and decrease in temperature beyond the stated range caused a decrease in the formation of lactic acid. Temperature was the dominant factor influencing the three response variables viz. lactic acid to sugar ratio, lactic acid, and pH. However, the main factor influencing extract was time followed by cell concentration. The optimum conditions of the factors such as temperature, initial pH, initial cell concentration, and fermentation time determined with RSM were found to be 42 °C, 5.4, 1.86×10^5 cells/ml, and 52 h, respectively. With this set of condition, a beverage with pH of 3.5, lactic acid concentration of 9.5 g/L, and lactic acid to sugar ratio of 0.26 was predicted to be produced. The validation experimental runs demonstrated a good correlation between the experimental and predicted values.

Keywords: Fermentation, *Lactobacillus amylolyticus*, RSM, teff malt

1. Introduction

Teff (*Eragrostis tef*) is a gluten-free cereal with attractive nutritional profile. Although it is a good source of health-benefit nutrients (Bultosa, 2007; Dekking et al., 2005; Gebremariam et al., 2012), further availability of the nutrients can be improved by fermentation (Campbell, 1994). In addition to its many other functions, fermentation is known for improving digestibility and flavour of the final product (Minamiyama et al., 2003; Steinkraus, 1994; Zarnkow et al., 2007a). The use of lactic acid bacteria (LAB) is of great interest as many of these bacteria are generally recognized as safe organisms and play important role as biopreservatives (Klaenhammer, 1993; Settani et al., 2005). During fermentation process, LAB are exposed to various environmental stress conditions such as temperature, pH, acid, high osmotic pressure, and absence of available nutrients (Ahmed et al., 2006). The stress responses of the bacteria are of interest as they may have impact on the final quality of the fermented product. Hence, it is vital to investigate the influences of the main factors affecting the fermentation process, and identify the fermentation condition that leads to have a final product with the required levels of its quality attributes.

The conventional ‘‘one-factor-at-a-time’’ way of optimizing a multivariable system is time consuming, and usually disregards the interactive effects of the process parameters. Besides, it requires carrying out a number of experiments to identify the optimal processing conditions (Bandaru et al., 2006). These drawbacks of single factor optimization technique can be solved by optimizing all the factors collectively by central composite design (CCD) using response surface methodology (RSM). RSM is one of the powerful tools used in bioprocess optimization to investigate the influences of individual process factors and their interactions on the response variables as well as for indentifying the optimal conditions for a multivariable system efficiently (SAS, 2009; Zarnkow et al., 2007b). Application of RSM helps to minimize time and resources for finding the optimal conditions of the different

factors, and allows better understanding of the interaction between the factors. In addition to its application to study the mathematical correlations between the process parameters and the response variables, RSM helps to plan/design the experiments using CCD or any other package of the statistical tool. The objective of this study was to investigate the influences of temperature, initial pH, bacterial cell concentration, and fermentation time on lactic acid formation, change in lactic acid to sugar ratio, pH, and extract during fermentation of teff malt-wort. We also identified the optimal fermentation conditions to produce a beverage with highest possible lactic concentration that leads to have a pH between 3 and 4, and lactic acid to sugar ratio of lower than 0.6. These targets were set based on the results of sensory analysis of fermented teff malt-wort beverage in a preliminary experiment, which has been performed before conducting this experiment.

2. Materials and Methods

2.1. Sample preparation and experimental procedures

Teff malt-wort (Gebremariam et al., 2013a, 2013b) was used as a source of carbon, amino acids, minerals, vitamins and other nutrients for the bacteria during the fermentation process. *Lactobacillus amylolyticus* was obtained from microbiology laboratory of the Institute of Brewing and Beverage Technology, Technische Universität München. The bacterial cells were pre-cultured in a wort for about 16 h at 48 °C. The pre-cultured cells were then centrifuged at 4 °C for 10 min with relative centrifugal force (RCF) of 400×g, and washed with sterile Ringer's solution (Charalampopoulos et al., 2003). The experiment was designed by RSM using central composite design (CCD) and all the fermentations were performed in a 100 ml flask. The pH values were adjusted and the required concentrations of *L. amylolyticus* were inoculated to the wort samples as per the experimental design (Table 1). The samples were incubated at the corresponding temperatures ranging between 42–54 °C for certain period of time ranging between 24–56 h. All the samples fermented under these conditions

were analysed for lactic acid, sugar, pH, and extract content and all the measurements were carried out in triplicate. The lactic acid to sugar ratio was also estimated at each experimental point.

2.2. Experimental design using RSM

In this part of the project, we studied the relationships between the experimental factors and the response variables. Besides, the optimal conditions of the factors affecting the fermentation process were identified using RSM. The experimental design and statistical analysis were performed using Design Expert statistical software (version 8.0.7.1, Stat-Ease, Inc, USA). The factors considered in this study such as temperature (X_1), initial pH (X_2), initial bacterial cell concentration (X_3) and fermentation time (X_4), and their levels are shown in Table 1. The selection of levels of the factors was based on literature on growth conditions of *Lactobacillus amylolyticus* (Bohak et al., 1998) and our prior experience gained in preliminary experiments. The response variables were the changes in lactic acid (Y_1), lactic acid to sugar ratio (Y_2), pH (Y_3) and extract content (Y_4) of the beverage. To evaluate the influences of the factors and their interactive effects on the response surface in the region of investigation, a four-factor three-level central composite experimental design was used. In other words, a 2^k factorial points with 2k axial points and six replications at the center point (n_o = 6) leading to a total of thirty (2^k+2k+n_0) experiments was employed, where k is the number of independent variables. Hence, these thirty experiments with different combination of the independent variables as per the CCD were performed. The CCD matrix with four factors is presented in Table 2. RSM was employed to find the optimum conditions of the factors affecting the fermentation process. Further experiments were also conducted at the optimum values of the factors for confirmation of the predicted values.

Table 1. Levels of the independent variables in the experimental design

Factor	Coded levels		
	-1	0	+1
Temperature (°C)	42	48	54
Initial pH	4.8	5.3	5.8
Cell concentration (cells/ml)[1]	5	6	7
Time (h)	24	40	56

Table 2. The CCD matrix employed for four experimental factors (actual values in Table 1)

Run	X_1	X_2	X_3	X_4
1	-1	+1	+1	-1
2	+1	-1	-1	+1
3	+1	+1	-1	+1
4	0	0	0	0
5	-1	-1	-1	+1
6	-1	-1	+1	+1
7	-1	-1	+1	-1
8	0	0	0	-1
9	0	0	0	+1
10	-1	+1	-1	+1
11	0	-1	0	0
12	+1	0	0	0
13	+1	-1	-1	-1
14	+1	+1	-1	-1
15	-1	+1	-1	-1
16	+1	+1	+1	-1

[1] Cell concentrations indicated as 5, 6, and 7 throughout the document are to mean 10^5, 10^6 and 10^7

17	0	0	0	0
18	-1	0	0	0
19	0	0	0	0
20	+1	-1	+1	+1
21	+1	-1	+1	-1
22	0	0	-1	0
23	0	0	0	0
24	-1	-1	-1	-1
25	0	0	0	0
26	0	0	0	0
27	+1	+1	+1	+1
28	-1	+1	+1	+1
29	0	0	+1	0
30	0	+1	0	0

2.3. Validation of experiments

Once the optimum fermentation conditions were identified with RSM, fermentation of the teff malt-wort was carried out in triplicate using the optimum fermentation conditions in order to validate the accuracy of the model. The beverage was also further assessed for its sensory qualities. Fifteen trained sensory panels from the Chair of Brewing and Beverage Technology, Technische Universität München, participated in the sensory evaluation of the final fermented beverage. The beverage was assessed for its odor, taste, sourness, sweetness, mouthfeel and overall acceptability using a 5-point scale (Lawless and Heymann, 1999). The panellists were instructed to evaluate odor, taste, mouthfeel and overall acceptability of the product using the 5-point scale rated from 1 (dislike extremely) to 5 (like extremely).

Similarly the sourness and sweetness of the beverage were assessed from 1 (extremely sweet/sour) to 5 (not sweet/sour).

2.4. Analytical procedures

2.4.1. Determination of lactic acid

The quantitative determination of lactic acid was carried out by Ion Chromatography System (ICS) with automated eluent generation (Dionex EGC III KOH, Eluent Generator Cartridge). The ICS was operated in external water mode with deionised water as regenerant at a flow rate of 0.5 mL/min. KOH was used as an eluent with a flow rate of 0.38 mL/min. A separation column (IonPac® AS15 analytical 2x250 mm) was employed. After proper dilution, 5 ml of the sample was transferred to sample-vial, and it was placed in AS40 auto sampler. The injection volume was 5µL (Wang et al. 2008).

2.4.2. Change in cell concentration

Cell growth was monitored by measuring the optical density (OD) of the medium at 600 nm wavelength in a 10 x 10 x 45 mm cuvette using Ultrospec 3100 UV/Visible Spectrophotometer (Amersham Biosciences). During growth, the samples were diluted with Ringer solution if the OD readings were higher than 1.0. Standard curves were used to relate OD and cell number (cells per millilitre) whenever required (Charalampopoulos et al. 2003).

2.4.3. Sugar concentration

Luff-Schoorl method was employed for the determination of total soluble sugar. The method involves the inversion of sugars present in the samples with hydrochloric acid. The sugars were extracted by adding 200 ml of 40% ethanol to accurately weighed 5 g of the sample in a volumetric flask, and using a mixer (tumbler) at a speed of 15 rpm for 1 h. Then the solution was clarified with solutions of Carrez I and Carrez II. After eliminating the ethanol by evaporation, 15 ml of 0.1 N HCl was added. The test sample was then heated in the acidic solution to hydrolyse the starch, releasing the sugars. The resulting sugars were determined

by titration using the Luff-Schoorl method after neutralizing the sample with 15 ml of 0.1 N NaOH (ICUMSA, 2005).

2.4.4. pH

The pH of the samples was determined using a digital pH meter (Laboratory pH Meter CG 843, SCHOTT Geräte GmbH, Germany) after calibrating with buffers at pH 4.0 and 7.0

2.4.5. Extract content

The extract level of the wort is the most important quality attribute as extract is an indicator of the level of fermentable sugars in the wort. The change in the extract contract during the fermentation process was determined using the procedure of European Brewery Convention (EBC, 2007).

3. Results and Discussion

The influences of temperature, initial pH, initial bacterial cell concentration, and fermentation time on lactic acid formation, changes in lactic acid to sugar ratio, pH and extract content were investigated using RSM. The model's goodness of fit for all the response variables was checked by the coefficient of determination (R^2). The experimental results for the response variables were well fitted to polynomial regressions. The R^2 values indicated that quadratic model is highly statistically significant for the response variables when compared to the other tested models. The R^2 values of the polynomial regression equations of lactic acid formation, lactic acid to sugar ratio, changes in pH and extract content were 0.975, 0.968, 0.967, and 0.975, respectively, indicating that 97.5, 96.8, 96.7, and 97.5 % of the variations in the corresponding response variable could be explained by the model. A regression model with R^2 higher than 0.9 is considered as a model having very high correlation (Abraham and Ledolter, 2006). The R^2 values of the polynomial regressions for all response variables reflect very good fit between the observed and predicted responses, and imply that the models are

reliable for the response variables. The fitness of the models was further confirmed by high values of the adjusted determination coefficient (adj R^2). The adjusted R^2 values of the model for lactic acid, lactic acid to sugar ratio, pH, and extract were found to be 0.949, 0.937, 0.935, and 0.951, respectively.

The effect of each factor and their interactions were analysed using the analysis of variance (ANOVA). The *P*-value was used as a tool for checking the significance of each factor as well as strengths of their interactions. The smaller the *P*-value the bigger the significance of the corresponding factor on the specific response variable (Rahman et al., 2004). The *F*-statistics was also used to check how big the effect of each factor when compared to the other studied factors.

Table 3. Analysis of variance for the entire quadratic model with factors: temperature (X_1), initial pH (X_2), initial cell concentration (X_3) and time (X_4), and response variables: lactic acid (Y_1), lactic acid to sugar ratio (Y_2), pH (Y_3) and extract content (Y_4)

Source	*F*-value				*p*-value (Prob > *F*)			
	Y_1	Y_2	Y_3	Y_4	Y_1	Y_2	Y_3	Y_4
Model	38.4	30.67	29.51	39.52	<0.0001	<0.0001	<0.0001	<0.0001
X_1	254.72	169.66	150	4.68	<0.0001	<0.0001	<0.0001	0.0483
X_2	0.015	1.34	12.72	0.96	0.9042	0.2656	0.0031	0.3443
X_3	23.44	18.1	21.35	84.58	0.0003	0.0008	0.0004	<0.0001
X_4	62.06	100.6	19.35	271.84	<0.0001	<0.0001	0.0006	<0.0001
X_1X_2	0.28	0.16	10.05	0.81	0.6068	0.6968	0.0068	0.3823
X_1X_3	6.04	6.02	9.33	0.04	0.0277	0.0278	0.0086	0.845
X_1X_4	43.05	62.52	21.42	0.63	<0.0001	<0.0001	0.0004	0.4415
X_2X_3	0.013	1.32	0.68	0.32	0.9116	0.27	0.4246	0.5822
X_2X_4	8.27×10^{-4}	0.33	1.16	0.02	0.9775	0.5771	0.2992	0.8909
X_3X_4	5.7	0.12	10.96	3.68	0.0317	0.7337	0.0052	0.0756
X_1^2	18.95	3.72	28.52	0.028	0.0007	0.0743	0.0001	0.8695
X_2^2	0.8	1.88	1.27	0.52	0.3858	0.1922	0.2794	0.4825
X_3^2	4.49	0.82	0.86	2.59	0.0523	0.3818	0.3691	0.1296
X_4^2	1.28	0.63	4.44	50.29	0.2772	0.4415	0.0536	<0.0001

The Fisher's *F*-test with very low probability values (Table 3) demonstrated that the models for all response variables were highly significant. The values of F_{model}, which is the ratio of mean square regression to mean square residual, for the changes in lactic acid, lactic acid to sugar ratio, pH, and extract content were found to be 38.4, 30.67, 29.51, and 39.52, respectively, with probability of seeing the observed *F* value (Prob > F) <0.0001 in all cases. Among the model terms, temperature, cell concentration, and fermentation time as well as their interactions were significant ($P<0.05$) for lactic acid formation. However, the other model terms didn't show significant effect. A larger (insignificant) *P*-value suggests that changes in the predictor are not associated with changes in the response variables. Thus, the final model equation after cancelling model terms with *P*-values > 0.10 had three linear terms, one quadratic term, and three two-factorial interactions, and it is represented by eq. 1.

$$Y_1 = -53 + 2X_1 + 6.4X_3 + 0.4X_4 - 0.03X_1X_3 - 4.9\times10^{-3}X_1X_4 - 0.01X_3X_4 - 0.02X_1^2 \quad \text{eq. 1}$$

Temperature, initial cell concentration, fermentation time, and interactions between temperature and cell concentration as well as temperature and time were significant model terms for the change in lactic acid to sugar ratio. The final model equation for lactic acid to sugar ratio can be represented by eq. 2.

$$Y_2 = -2.14 + 0.12X_1 + 0.48X_3 + 0.04X_4 - 3.2\times10^{-3}X_1X_3 - 6.5\times10^{-4}X_1X_4 \quad \text{eq. 2}$$

All the four factors considered in this study were significant model terms ($P<0.05$) for the change in pH during the fermentation process. In addition to that the interaction between temperature and pH, temperature and cell concentration, temperature and time as well as cell concentration and time had significant effects on the model. The second-order polynomial model used to express the change in pH (Y_3) during the fermentation process as a function of the independent variables (eq. 3) is shown below:

$$Y_3 = 66 - 2.6X_1 + 5.1X_2 - 3.4X_3 - 0.3X_4 + 0.1X_1X_2 + 0.03X_1X_3 + 3.1X_1X_4 + 0.01X_3X_4 + 0.02X_1^2 \quad \text{eq. 3}$$

Temperature, cell concentration and fermentation time were significant model terms for the change in extract content during the fermentation. However, their interactions as well as other factors didn't show significant influence on the change in the extract content. Thus, the second-order polynomial model for the change in the extract (Y_4) is expressed by eq. 4.

$$Y_4 = 8.45 - 0.04X_1 + 0.48X_3 + 0.06X_4 - 9.35\times10^{-4}X_4^2 \quad \text{eq. 4}$$

Regression coefficients in linear regression equations (both simple and multiple linear regressions) represent the change in a response variable for a unit change in predictor/factor when all other covariates remain constant. However, the model equations for all response variables in our investigation involve quadratic and/or interaction terms. Thus, for proper interpretation it is advisable to consider the equation in combination with the graphs. Otherwise, the interpretation would be a bit less intuitive because the effect of the predictor/factor on the response variable varies depending on the value of the predictor (the case of polynomial term) and the value of the other predictor (the case of interaction terms).

The three-dimensional response surfaces were generated to study the interactions among the factors and visualize their combined effects on the response variables. The resulting response surfaces showing the effects of temperature, initial pH, initial bacterial cell concentration and fermentation time on lactic acid formation, changes in pH, lactic acid to sugar ratio, and extract are depicted in figures below. The interaction effects of the factors on the studied response variables were tested for all possible combinations of the factors by keeping two factors constant at a time. The *F*-value of each factor (Table 3) shows that temperature and time were found to be the factors with highest influence on most of the response variables. In the model equations, the interaction of X_1 and X_4 corresponding to temperature and time, respectively, had negative coefficients in the first two model equations indicating that the combined effect of these two factors causes a decrease in the formation of lactic acid as well as lactic acid to sugar ratio. In addition to the linear and interaction effects, temperature had negative quadratic effect for the formation of lactic acid (eq.1) and positive quadratic effect

for the decrease in pH. On the other hand, fermentation time had positive quadratic effect on the change in extract content during fermentation.

In this study, the dominant factor which controls lactic acid formation, change in lactic acid to sugar ratio, and pH was temperature followed by fermentation time. Whereas the change in the extract content was mainly influenced by fermentation time followed by bacterial cell concentration. As the number of RSM factors increases, it becomes difficult to visualize the response surface. In such cases it is helpful to view a special form of response plot called perturbation for RSM data (SAS, 2009). The perturbation plots show how the predicted responses are caused by changing only one factor at a time from the centre point of the experimental region. By default, the Design-Expert sets the reference point at the midpoint (coded 0) of all the factors in order to see how the response changes as each factor moves to the negative or positive direction from the reference point (Anderson and Whitcomb, 2005; SAS, 2009).

It can be seen from Fig. 1(a) and (b) that the increase as well as an extreme decrease in temperature causes a decrease in the formation of lactic acid. High lactic acid formation was observed in between 42 and 48 °C. This was confirmed by the perturbation plot (Fig. 1c). On the other hand, lactic acid formation increased with cell concentration (Fig. 1b) and fermentation time (Fig. 1a). However, pH caused very small effect on the lactic acid concentration (Fig. 2) as it changed from the reference point (pH 5.3). Temperature was the dominant factor that controls the change in lactic acid concentration. The Fisher's test (P=0.9042), also demonstrated that lactic acid formation was not significantly affected by the initial pH.

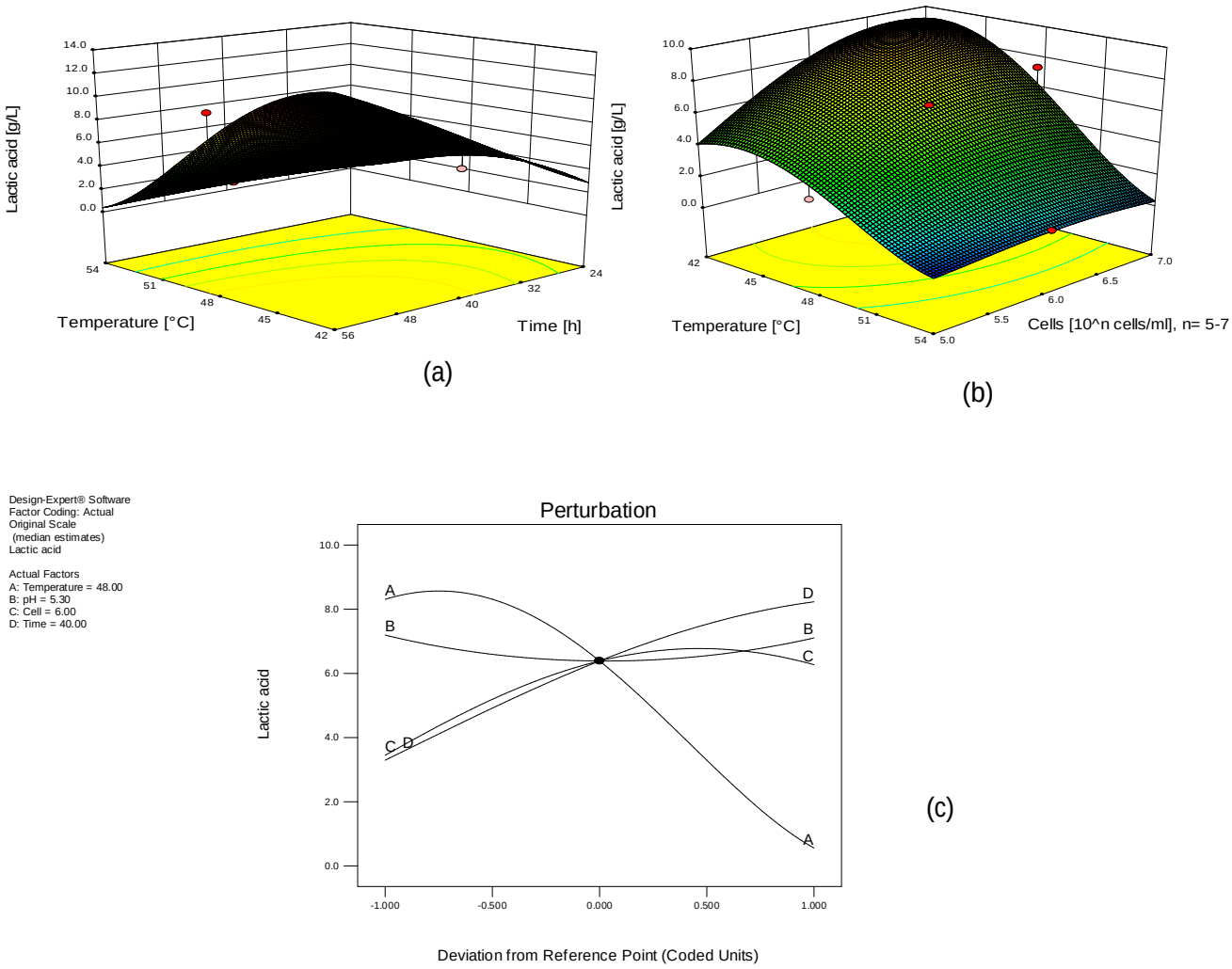

Fig. 1 (a, b) Response surface plots showing the effects of temperature, initial bacterial cell concentration, and fermentation time on lactic acid formation; (c) Perturbation plot showing the change in lactic acid as each factor moves from the chosen reference point (the middle of the design space), where A=temperature, B=initial pH, C=initial cell concentration, and D=fermentation time

The lactic acid to sugar ratio increased with fermentation time (Fig 2a) and cell concentration (Fig. 2b). However, temperature had the opposite effect on the lactic acid to sugar ratio (Figs. 2a, 2b). Fisher's test (Table 3) demonstrated that the influences of temperature (F=169.7) and time (F=100.6) on the change in lactic acid to sugar ratio were much more pronounced when compared to those of initial bacterial cell concentration (F=18.1) and pH (F=1.34). This was further confirmed by the perturbation plot (Fig. 2c). Temperature, fermentation time, and bacterial cell concentration showed significant effect, where as pH had no significant effect on the lactic acid to sugar ratio.

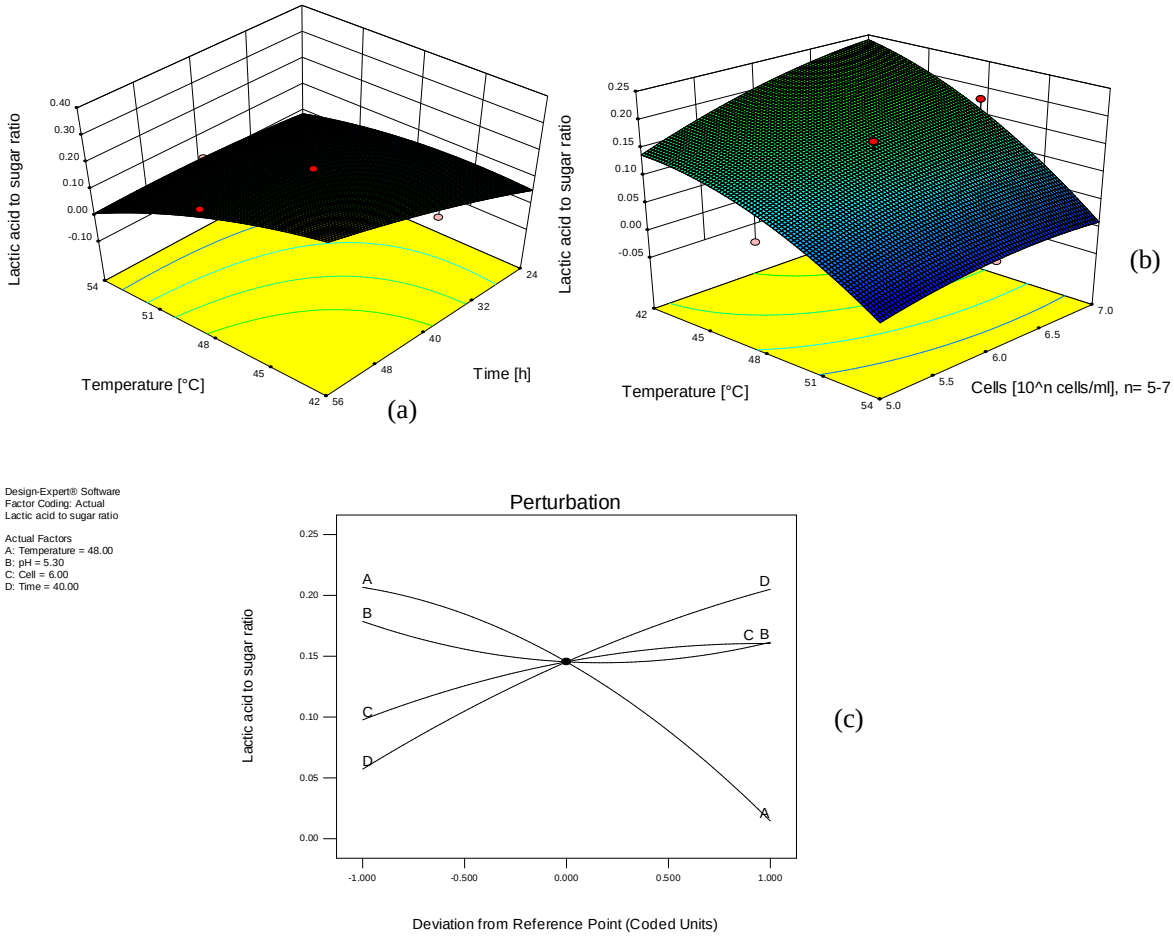

Fig. 2 (a, b) Response surface plots showing the effects of temperature, cell concentration, and fermentation time on lactic acid to sugar ratio; (c) Perturbation plot showing the change in lactic acid to sugar ratio as each factor moves from the chosen reference point (the middle of the design space), where A=temperature, B=initial pH, C=initial cell concentration, and D=fermentation time

In contrast to the change in lactic acid and lactic acid to sugar ratio, the pH showed a constant increment with an increase in temperature (Figs. 3(a), (b)). It was also increased with an increase in the initial pH of the wort (Fig. 3a). On the other hand, fermentation time (Fig. 3b) and bacterial cell concentration (Fig. 3c) had the opposite effect on the pH of the fermentation medium. Although the effect of temperature was much more pronounced (F=150) when compared to the other factors, all the factors tested in this study also showed significant effect (P<0.05) on the change in pH during the fermentation process. The perturbation plot (Fig. 3d) also clearly shows that temperature was the dominant factor influencing the change in pH.

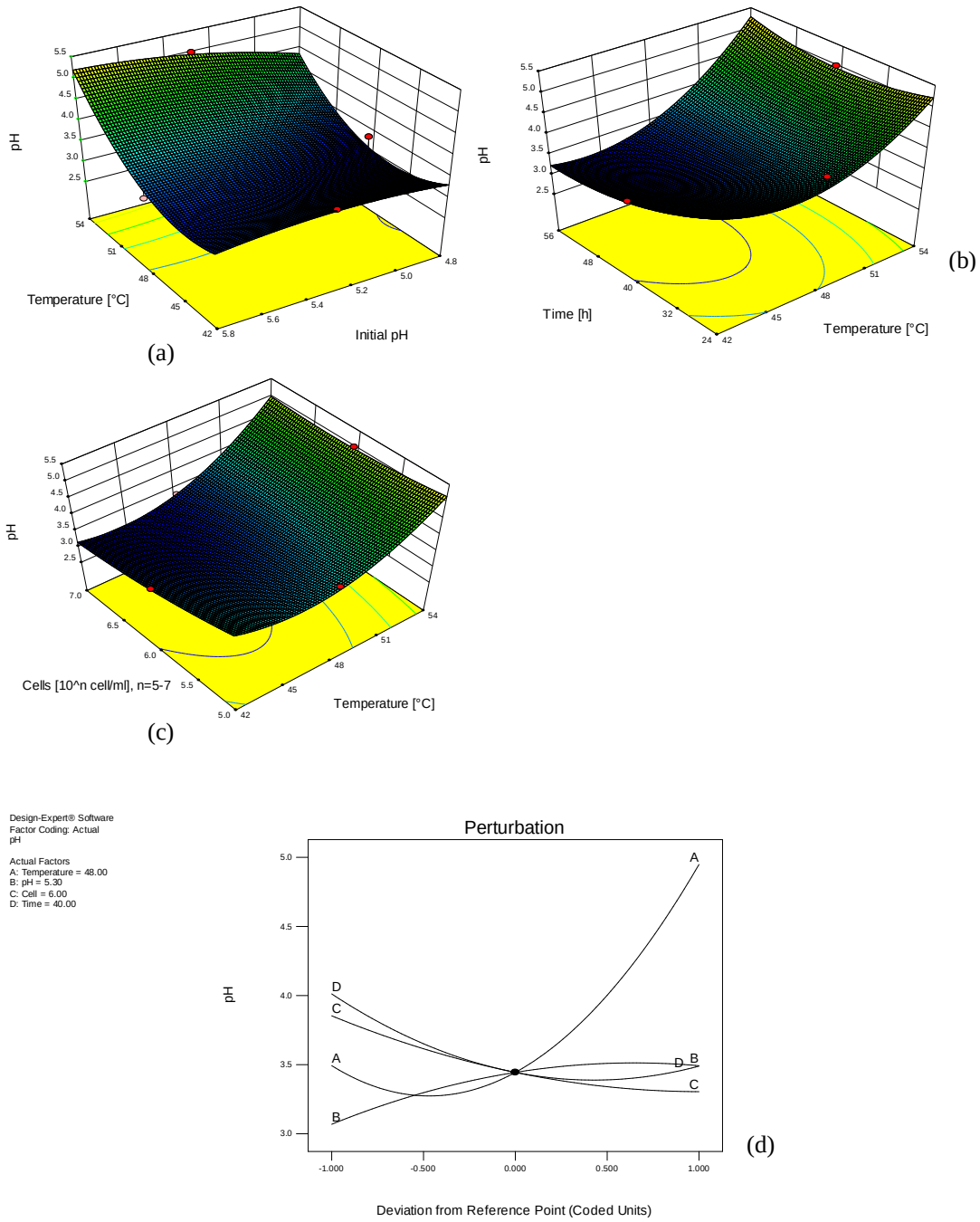

Fig. 3 (a–c) Response surface plots showing the effects of temperature, initial pH, cell concentration, and fermentation time on the change in pH; (d) Perturbation plot showing the change in pH as each factor moves from the chosen reference point (the middle of the design space), where A=temperature, B=initial pH, C=initial cell concentration, and D=fermentation time

The results on extract content showed that it decreased with increase in fermentation time and bacterial cell concentration. But it increased with an increase in temperature (Data not shown). In contrast to the other response variables, where temperature was the dominant influencing factor, the change in the extract content was highly influenced by fermentation time followed by bacterial cell concentration. Among the studied factors, pH didn't show statistically significant effect (P>0.05) on the change in the extract content.

We also investigated the optimum levels of the factors in order to attain the highest possible lactic acid concentration that leads to have a pH between 3 and 4, and lactic acid to sugar ratio of lower than 0.6. Numerical optimization performed by the statistical software pinpointed the most desirable combination of the tested factors based on their predictive models. The desired goals for the response variables were selected by adjusting the weight/importance of the respective response variable that might alter the characteristics of the final beverage. For fermentation of teff malt wort by *L. amylolyticus*, the final optimum fermentation conditions of the factors such as temperature, initial pH, initial cell concentration, and fermentation time determined with RSM were found to be 42 °C, 5.54, 1.86×10^5 cells/ml, and 52 h, respectively. All the optimal points were located inside of the experimental region. With this set of fermentation condition, a beverage with pH of 3.5, lactic acid concentration of 9.5 g/L, lactic acid to sugar ratio of 0.26 and extract content of 11.4 g/100 ml was predicted to be produced.

3.1 Validation of the models

The average values of lactic acid, lactic acid to sugar ratio, pH, and extract content for the teff malt beverage fermented under the optimum conditions were found to be 8.7±0.4 g/L, 0.2±0.01, 3.58±0.03, and 11.43±0.04 g/100 ml, respectively. The confirmation experimental runs demonstrated that the average values from the actual experiment and the predicted values of the response variables were found to be comparable except that the lactic acid

concentration and the lactic acid to sugar ratio from the actual experiment were slightly lower than the predicted values. The deviations in the lactic acid concentration and lactic acid to sugar ratio could be due to experimental error, particularly due to inoculation of less bacterial concentration than the optimum initial cell concentration. But, in general, there was good agreement between the predicted and experimental values. The good correlations between the experimental values under the optimum fermentation conditions and the predicted values justified the validity of the models. Hence, the empirical models were reasonably accurate, and the RSM is indeed a useful tool for predicting and investigating the fermentation conditions.

The teff malt beverage fermented under the optimum condition was further assessed for its sensory qualities and the mean values of the sensory scores for the beverage are summarized in Fig. 4.

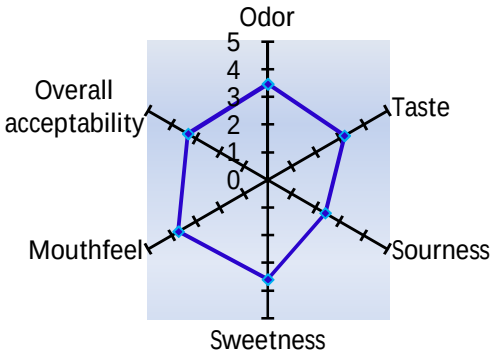

Fig. 4 Graphical chart representing the sensory profile of the product

The sensory scores for odor, taste, mouthfeel and overall acceptability show that the product has mean values greater than 3, indicating that the beverage was well liked by most of the panellists. The sweetness of the beverage was also higher than 3, reflecting that the sugars in the wort were not fully fermented. However, continuation of the fermentation may lead to have negative impact on the sourness of the product. The sourness of the beverage under this

condition was rated to be 2.4 (below average) but the overall acceptability shows that this is not too sour to consume. In addition to its good acceptability by most of the assessors, two assessors clearly described that the sweetness and sourness of the product were well balanced.

4. Conclusion

The present study was focusing on the influence of fermentation factors on the variation in lactic acid, pH, lactic acid to sugar ratio, and extract during fermentation of teff malt-wort, and indentifying the optimum fermentation conditions for the wort by *L. amylolyticus.* Temperature was the dominant factor influencing the three response variables viz. lactic acid to sugar ratio, lactic acid, and pH. However, the main factor influencing the extract content was time followed by cell concentration. The optimal temperature, initial pH, bacterial cell concentration, and fermentation time for fermentation of teff malt-wort were found to be 42 °C, 5.54, 1.86×10^5 cells/ml, and 52 h, respectively. The validation experimental runs demonstrated a good correlation between the experimental and predicted values.

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3. DISCUSSION

This project was designed to study the kilning, mashing, and fermentation conditions of teff malt to be used as a raw material for lactic acid fermented beverage. All experiments were carried out in triplicate unless otherwise stated. Ungerminated teff grain samples had very low activities of alpha-amylase (<3 U/g), and moderate activities of beta-amylase (<25 U/g) and limit dextrinase (<350 U/g). But their activities increased significantly ($P < 0.05$) with germination. The enzyme activities developed during the germination process may be affected by the kilning process, especially when high kilning temperature is applied to the malt with high moisture content (Kunze, 2004). In order to prevent the deactivation of the enzymes, the malt must first be pre-dried before it is subjected to high temperatures. Hence, we considered pre-drying of the teff malt samples at lower temperature for longer period of time before applying high temperatures in developing the kilning program.

The enzyme activities in malted teff samples dried using the two different kilning regimens (R1 and R2) were compared with each other. Both kilning programs involve drying of the germinated samples by increasing the temperature of airflow in stepwise fashion. In the first kilning regimen (R1), the samples were dried for 18 h at 30 °C followed by 1 h at 60 °C and 3 at 65 °C. The second kilning program (R2) involves drying the samples for 18 h at 30 °C followed by 1 h at 60 °C and 3 at 80 °C. Prolonged curing (5 h) in both kilning regimens was also tested, and the results showed that prolonged curing causes an adverse effect on the enzyme activities. The first kilning program (R1) with shorter curing time (3 h) was found to be the best condition in relation to preserving the enzyme activities and it was considered as optimal kilning program for teff malt samples. With regard to alpha-amylase, beta-amylase and limit dextrinase, higher activities were observed for the first kilning regimen (R1). Teff malt was found to have higher limit dextrinase activity (1072 U/kg) than barley malt (≤800 U/kg), and its beta-amylase activity (440 U/g)

was comparable with that of barley malt (225 – 627 U/g) (Narziss and Back, 2009). On the other hand, a significantly lower alpha-amylase activity (68 U/g) was observed for teff malt in comparison with its activity in barley malt (84 – 210 U/g) (Narziss and Back, 2009). However, the observed low level of alpha-amylase may not be a problem in relation to degrading the starch to fermentable sugars. MacGregor et al. (1999) reported that a deficiency in the level of one amylase enzyme (alpha- or beta-amylase) may not affect the amount of total fermentable sugar as far as the other enzyme is available in high concentration. Among the tested levels of alpha-amylase in their experiments, even the lowest level (4 U/g) in the presence of high levels of beta-amylase (e.g. 40 U/g) and no addition of limit dextrinase resulted in comparable levels of fermentable sugars with the other tested levels of alpha-amylase. According to their report, an increase in the level of limit dextrinase in the mash was clearly shown to increase the level of fermentable sugars substantially, particularly in mashes having high levels of beta-amylase. Their findings, in general, suggested that commercial malts contain excess levels of the amylolytic enzymes.

Some increase in amylolytic enzyme activities were reported in the early stages of kilning barley malt due to continuation of the germination process, then a decrease in the enzyme activities was reported during the later stages of kilning (Kunze, 2004). Similarly, in kilning of the teff malt samples, both increment and decrease in the amylolytic enzyme activities were observed. Drying the teff malt samples at different isothermal temperatures showed that the rate of increase in the amylolytic enzyme activities at early stages of kilning and lower drying temperatures was dominating over the deactivation of the enzymes. But beta-amylase didn’t show any increase at all studied temperatures due to its thermolabile characteristics (Kunze, 2004). The increase in the activities of alpha-amylase and limit dextrinase could be attributed to the continuation of the germination process due to high moisture content (>15%) at

such low temperatures. All the amylolytic enzyme activities of teff malt decreased with an increase in the kilning temperature. For the teff malt samples dried using the optimal kilning program (R1), 11% increment in the alpha-amylase activity was observed at the end of the kilning process. However, beta-amylase and limit dextrinase activities decreased by about 12 and 17%, respectively. The significantly higher alpha-amylase activity of teff malt at the end of the kilning process suggests that the overall rate of alpha-amylase development was higher than that of its deactivation, leading to a net increase in the enzyme activity at the end of the kilning process.

Removal of the moisture content during the first phase of the kilning regimen, which also comprises the free drying stage, was very fast. This could be due to the fact that the free drying phase of kilning involves removal of unbound water from the surface of the grain (EBC, 2000). In this part of the project we also studied the rate of drying of teff malt samples at different drying temperatures. The results revealed that the moisture content in teff malt, in general, decreased significantly ($P < 0.05$) in the first 24 h of kilning at all studied temperatures. But no significant change was observed after 24 h of kilning. No DMS was detected for unmalted teff samples. But it increased during the germination process. Although the teff malt samples contained DMS content higher than 7 mg/kg at the end of the germination process, it was significantly decreased to lower than 5 mg/kg for all the samples dried using the different kilning programs.

The amylolytic enzymes surviving the kilning process play great role in the degradation of starch during the mashing process. In the starch degradation, the actions of beta-amylase and limit dextrinase are dependent on the supply of their substrates, which come from the action of alpha-amylase on starch granules and the subsequent release of dextrins (Sun and Henson, 1991). The effective overall action of the enzymes is crucial in yielding high concentration of substrates for the

subsequent fermentation process. Lactic acid bacteria have complex nutritional requirements for growth due to their limited ability to synthesis their own growth factors from available nutrients (Manca de Nadra, 2007). High concentration of easily convertible nitrogen sources such as amino acids or proteins with small molecular weight in the complex medium may stimulate the bacteria to grow faster (Sriphochanart et al., 2011; van Niel and Hahn-Hägerdal, 1999). Research reports showed that a minimal medium containing at least 100 mg/L amino acids (Snell, 1945; Ruiz-Barba and Jimenze-Diaz, 1994; Hebert et al., 2000; Manca de Narda et al., 2003; Sriphochanart et al., 2011) and 40 g/L sugar is required for healthy growth of the bacteria. The extract content of teff malt (68%) obtained by using a congress mashing procedure was lower than that of barley malt (76–85%) (Narziss and Back 2009), and its fermentable sugar content was also lower than 40 g/L, indicating that the congress mashing procedure is unsuitable for mashing teff malt. Hence, we studied the thermal stabilities of amylolytic enzymes of teff malt, and based on the results we also investigated the suitable mashing program for teff malt samples to be used as raw materials for lactic acid fermentation. The target in developing the mashing program was producing teff malt-wort with high concentrations of fermentable sugar (≥40 g/L) and FAN (≥100 mg/L), and pH in the range of 5–6, preferably the lowest pH in this range.

Firstly, the gelatinization temperature of teff malt samples was determined by measuring the rheological behavior of a flour-water suspension, using rapid visco-analyser (RVA) (Keßler et al., 2005). The gelatinization temperature of starch is influenced not only by the type of starch but also by the size and structure of its starch granules. Smaller starch granules have higher gelatinization temperature as compared to higher granules (Whitehurst and Oort, 2010). The gelatinization temperature of *kuncho* teff malt was found to be about 69 °C. The results from thermal stability study of the enzymes in teff malt showed that alpha-amylase is

relatively stable at this temperature whereas the stabilities of beta-amylase and limit dextrinase are highly reduced once the mashing temperature reaches the gelatinization temperature. Thus, there is a very narrow window of time for the starch to be hydrolysed to fermentable sugars during mashing.

Grist to liquor ratio also plays an important role in the contact between enzyme and substrate. The mashing-in concentration should not be too high to avoid lump formation and have suboptimal contact between the enzyme and substrate (Narziss, 1992). High viscosity and lumping may also cause low extract volume due to low filterability (filtering requires overnight) (Wijngaard and Arendt, 2006). In order to identify the proper grist to liquor ratio for mashing teff malt, malt to water ratios of 1:3, 1:3.5, 1:4, 1:4.5 and 1:5 were tested at a temperature (72 °C) slightly higher than the gelatinization temperature of teff malt sample for 90 min.

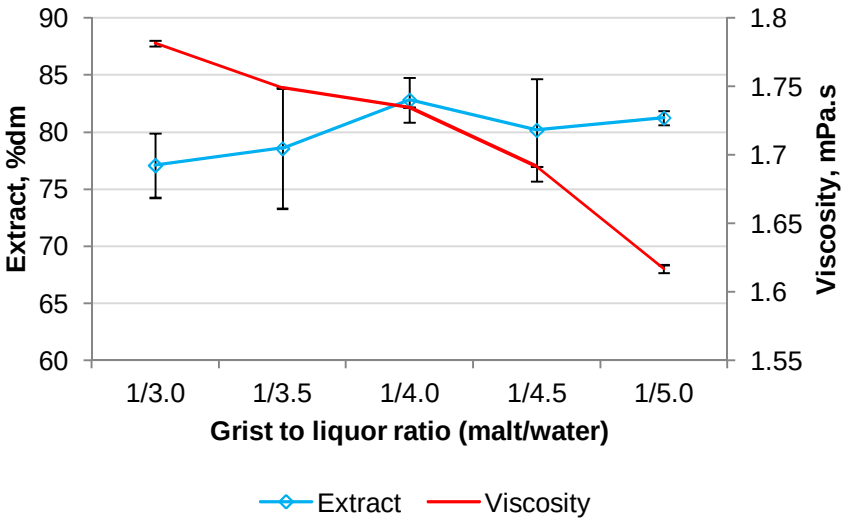

Fig. 1 Extract contents and viscosities of teff wort samples produced using different grist to liquor ratios

The highest grist to liquor ratio (1:3) caused high viscosity and lumping (Fig. 1) when compared to the other ratios, and it resulted in low extract. The extract contents observed for the grist to liquor ratios of 1:4, 1:4.5, and 1:5 were comparable but 1:4 was selected as best ratio because this ratio resulted in thicker mash and slightly

higher extract content. Since heat transfer is inversely proportional to thickness of the material (Janna, 2000; Moran et al., 2003), thicker mash that resulted in the highest possible extract is preferable in relation to protecting the starch degrading enzymes from thermal deactivation. The starch degrading enzymes are more stable in thicker mashes due to the fact that flow of heat will be reduced as the thickness of the mash is increased. As a result the enzymes will be protected from being affected by rapid flow of heat. Briggs (1998) has also stated that enzymes are more protected from thermal deactivation by their substrates and other substances present in the mash. However, as the mashing proceeds the substrates are depleted and the enzymes become more subjected to the heat treatment. On the other hand, thicker mashes may inhibit the enzyme activities. But the highest extract content (>80%) at the 1:4 grist to liquor ratio is an indication that the enzyme activities at this condition were not inhibited when compared to the other thicker mashes (1:3 and 1:3.5 grist to liquor ratios), where lower extract contents were observed. Hence, 1:4 teff malt to water ratio was selected as the optimum ratio to be used in mashing teff malt.

The enzymes surviving the kilning process may also be affected during mashing before they act on the starch molecules. Thus, it is very important to have knowledge about the thermal stability of the starch degrading enzymes of a cereal in order to investigate the optimal mashing program for the specific cereal malt. We studied the thermal stabilities of the three key enzymes namely alpha-amylase, beta-amylase, and limit dextrinase during isothermal mashing of teff malt in a 1:4 malt to water ratio at temperatures ranging from 40 to 75 °C with sampling in 15 min interval for a total of 90 min. Each experiment was conducted in duplicate.

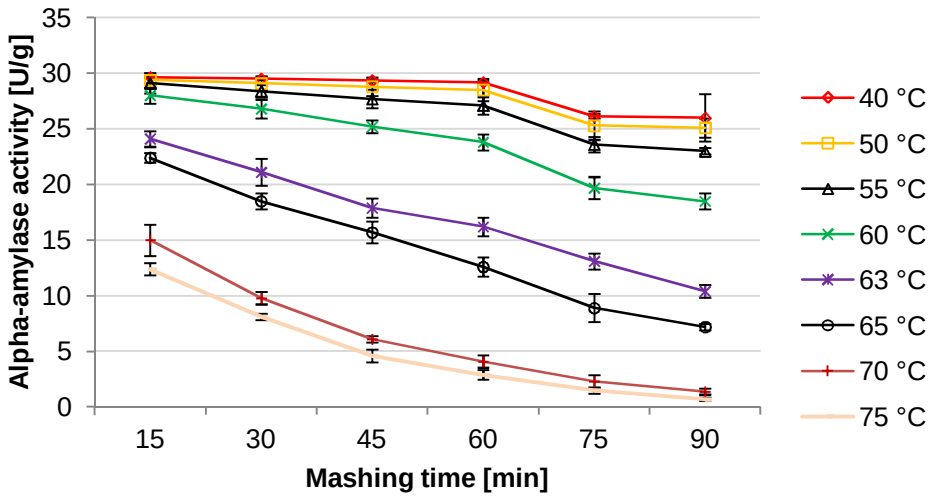

Fig. 2 Thermal deactivation profile of alpha-amylase during mashing of teff malt at different isothermal mashing temperatures

The statistical analysis showed that alpha-amylase activity was stable at lower temperatures (Fig. 2) but significantly decreased ($P < 0.05$) at higher temperatures (>55 °C). On the other hand, beta-amylase activity was significantly affected at all studied temperatures during the 90 min thermal exposure (Fig. 3).

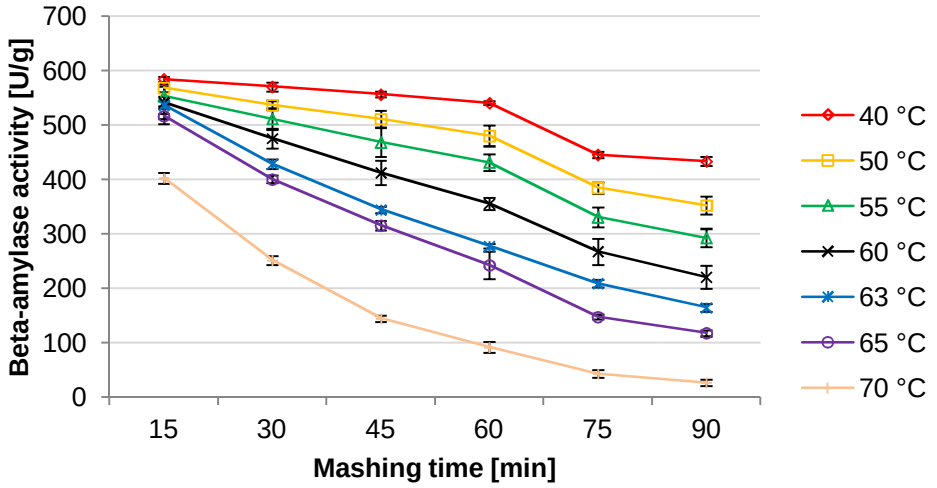

Fig. 3 Thermal deactivation profile of beta-amylase during mashing of teff malt at different isothermal mashing temperatures

In this study, no deactivation of limit dextrinase was observed at temperatures lower than 60 °C in the stated period of mashing time. But it was highly unstable at temperatures higher than 60 °C (Fig. 4). Similar observation was reported for limit

dextrinase in the malt extract of Pokko variety of barley (Stenholm and Home 1999). They noted that the limit dextrinase activity of the malt extract was stable at temperatures up to 62.5 °C, but 40% of the activity was lost in one hour for the sample mashed at 65 °C.

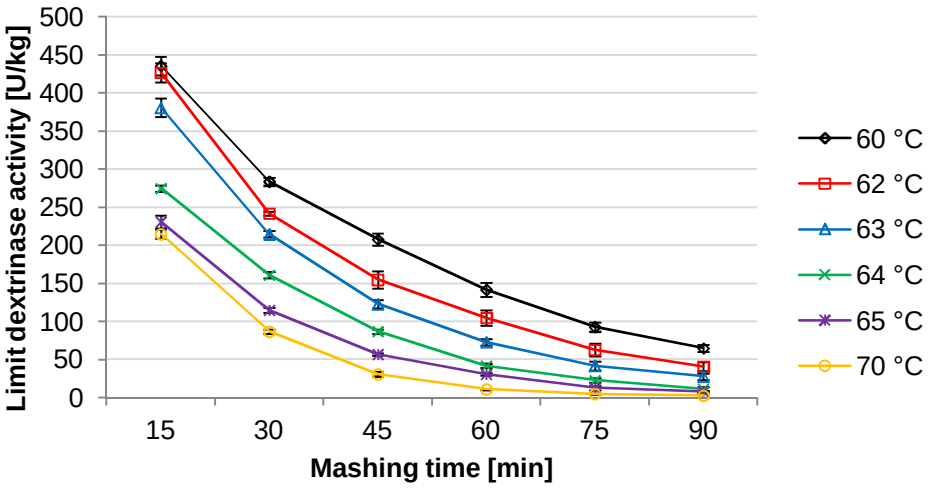

Fig. 4 Thermal deactivation profile of limit dextrinase during mashing of teff malt at different isothermal mashing temperatures

In our study, high activities were recorded for alpha-amylase at higher temperatures when compared to those of beta-amylase and limit dextrinase, indicating that alpha-amylase is more stable than beta-amylase and limit dextrinase. Therefore, in identifying the mashing-off temperature it may be sufficient to find the temperature at which most part of the alpha-amylases are deactivated. The Arrhenius type equation developed in this study was used to estimate the temperature at which almost all the alpha-amylases can be deactivated, and it was found to be 78 °C. At the mashing-off temperature, all beta-amylases and limit dextrinase as well as most part of the alpha-amylases are deactivated and the amount of fermentable sugars present in the wort is fixed (Rehberger and Luther, 1995; Whitehurst and Oort, 2010; Wijngaard and Arendt, 2006).

Isothermal mashing for 60 min at temperatures ranging between 60 and 84 °C with the objective of identifying the mashing-off temperature also led us to conclude that 78 °C can be taken as the mashing-off temperature. The highest extract (85%) was observed for the wort samples produced at 76 °C and higher temperatures (Fig. 5). The extract content remained constant after 76 °C, indicating that the enzymes responsible for formation of fermentable sugars were deactivated.

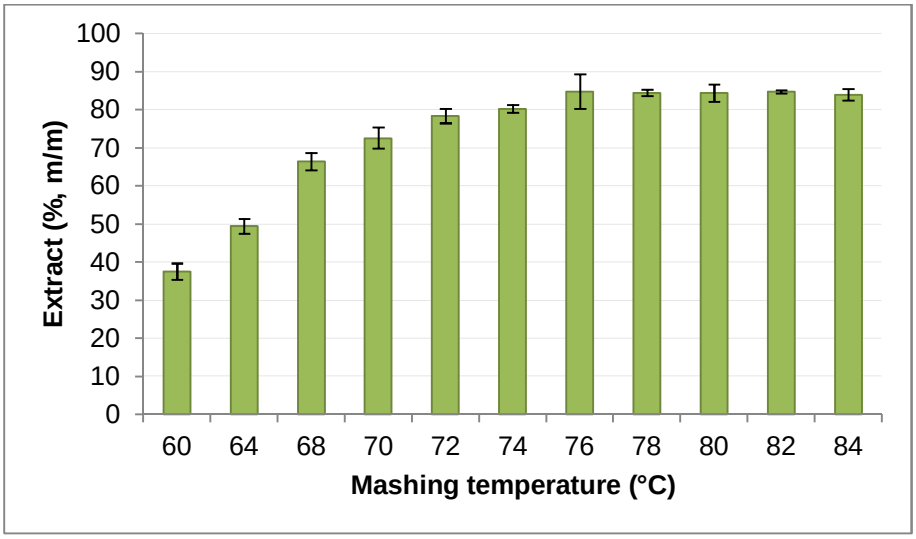

Fig. 5 Extract contents of teff wort samples produced during one hour stand at different isothermal mashing conditions

Wort samples to be used for lactic acid fermentation should contain the highest possible extract and FAN because lactic acid bacteria require some elements such as carbon and nitrogen in the form of carbohydrates and amino acids for growth (van Niel and Hahn-Hägerdal, 1999; Wee et al., 2006). Based on the gelatinization temperature of the teff malt and results from thermal stability studies on the amylolytic enzymes, conversion rest temperatures ranging between 65 and 74 °C were tested to find out the conversion rest temperature that results the highest possible extract and FAN as well as pH in the range of 5 to 6 (Bohak et al., 1998; Muralikrishna and Nirmala, 2005). Hence, mashing trials of 60 min rest at 65 °C

(experiment I), 68 °C (experiment II), 71 °C (experiment III), and 74 °C (experiment IV) followed by 10 min rest at the mashing-off temperature were conducted.

Table 1. Quality attributes of teff wort samples produced by mashing for 60 min at temperatures between 65 and 74 °C followed by 10 min rest at 78 °C

Conversion rest temperature (°C)	Extract (%, dm)	FAN (mg/L)	pH	Viscosity (mPa.s)
65	85±3.0	105±0.2	5.73±0.02	1.62±0.1
68	85±0	96±1.0	5.75±0.01	1.64±0.11
71	86±1.0	98±1.9	5.69±0.04	1.69±0.05
74	84±1.5	72±0.8	5.65±0.07	1.65±0.01

It can be seen from Table 1 that the highest extract content was recorded for the sample mashed at 71 °C conversion rest temperature. Although slightly higher FAN was observed at the conversion rest temperature of 65 °C when compared to other tested temperature, the acidity of the wort sample at this condition was lower. The highest FAN value was recorded at the lower temperature (65 °C), and it was highly decreased at higher temperatures. The slight increase in acidity with temperatures could be attributed to the hydrolysis of some complex organic molecules like lipids, phytin, and proteins to fatty acids, acid phosphates, and amino acids, respectively (Gernah et al., 2011). Once the starch is gelatinized, it is digested rapidly by the amylolytic enzymes present in the malt (Whitehurst and Oort, 2010). The conversion rest temperature represents a compromise between the need for higher temperature to obtain gelatinized starch and lower temperature to preserve enzyme activities. The combined effect of the enzyme activities result in formation of large concentration of fermentable sugars. Although high extract contents were observed

at 65 and 68 °C, the concentrations of the fermentable sugars at these conditions were lower when compared to the values at higher temperatures. This confirms that the FAN and other soluble components of the wort contributed to the high extract contents recorded at 65 and 68 °C. Our results showed that the highest total fermentable sugar was observed at the conversion rest temperature of 71 °C followed by 68 and 74 °C (Fig. 6). In general, glucose was the dominant fermentable sugar followed by maltose. Hence, 71 °C was determined to be the conversion rest temperature for teff malt mashing program. Hence, 71 °C was determined to be the conversion rest temperature for teff malt mashing program.

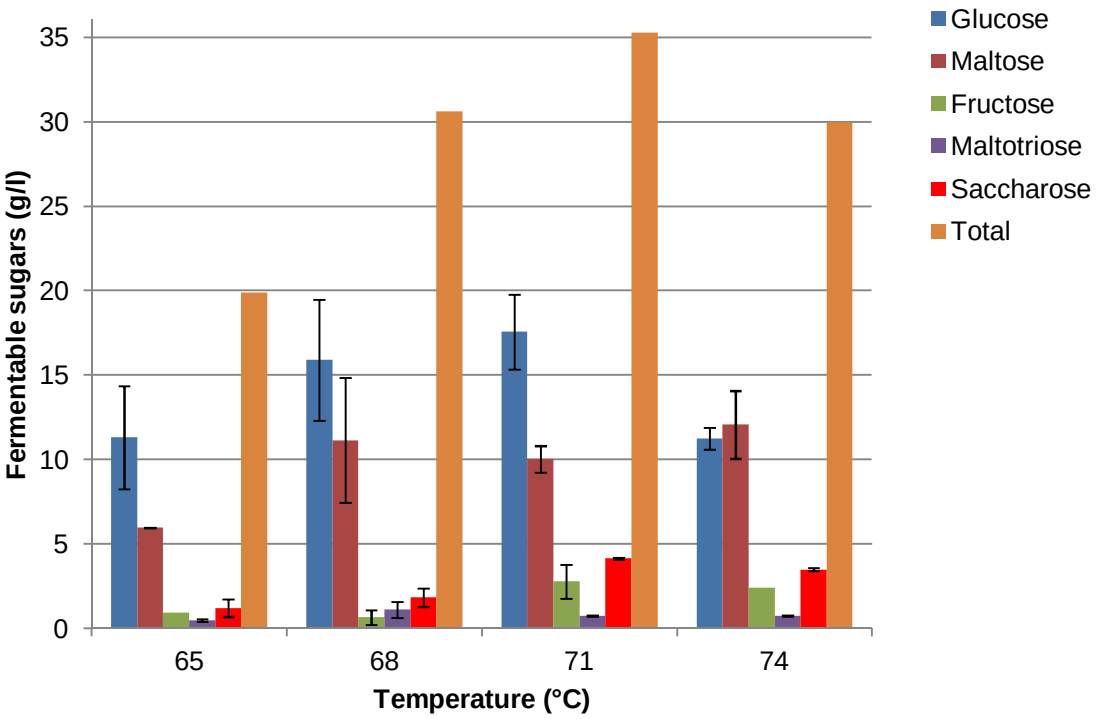

Fig. 6 Fermentable sugars of teff wort samples produced using isothermal mashing of the teff malt for 60 min at temperatures ranging between 65 and 74 °C

Mashing usually starts with the degradation of malt proteins by means of proteases and peptidases. Therefore, lower temperatures are usually applied before applying the conversion rest temperature (Whitehurst and Law, 2002). Some protein degradation has already taken place during the malting process, but rest at low

temperature during the mashing process can also help to increase the free amino nitrogen (FAN), which is required during the fermentation stage as the nitrogen source for the bacteria (Whitehurst and Law, 2002; Sriphochanart et al., 2011). A low mashing-in temperature may also help to break down the starch structure of malts (Taylor and Boyd, 1986), thereby increases the concentration of fermentable sugars. This is particularly important when mashing with malts having low enzyme activities. The effects of different mashing-in temperatures were explored in our experiments. Mashing trials of 30 min rest at 35 °C (experiment I), 40 °C (experiment II), 45 °C (experiment III), 50 °C (experiment IV), and 55 °C (experiment V) followed by 60 min rest at the conversion rest temperature and 10 min rest at the mashing-off temperature were conducted to investigate the mashing-in temperature.

Table 2. Quality attributes of samples produced by mashing for 30 min at temperatures ranging between 35 and 55 °C followed by 60 min rest at 71 °C and 10 min rest at 78 °C

Mashing-in temperature (°C)	Extract (%, dm)	FAN (mg/L)	pH	Viscosity (mPa.s)
35	83±0.1	123±0.05	5.75±0.03	1.71±0.011
40	85±1.0	128±0.1	5.73±0.02	1.70±0.03
45	84±2.6	132±1	5.75±0.01	1.74±0.05
50	79±2.4	130±0.04	5.69±0.04	1.68±0.01
55	78±1.9	119±0.04	5.68±0.03	1.67±0.004

The highest FAN concentration was observed at the mashing-in temperatures between 40 and 50 °C. But the extract content at 50 °C was low when compared to the values at 40 and 45 °C. Although the extract contents of the wort samples mashed at 40 and 45 °C were comparable, slightly higher pH and viscosity were

observed at 45 °C. Taking the advantage of lower pH and viscosity at 40 °C, it was selected as mashing-in temperature. It can be seen from the results (Table 2) that 30 min rest at the mashing-in temperature leads to have higher FAN content when compared with the corresponding mashing program without inclusion of lower mashing-in temperature (Table 1). The large differences in the FAN content observed by adding an extra mashing-in step is due to the action of proteolytic enzymes on the protein structures at low temperatures. The proteolytic enzymes will be deactivated by thermal energy when the malt is subjected to high temperature without including the lower mashing-in temperature rest. Inclusion of the mashing-in temperature also improved the fermentable sugar content of the wort. Therefore, 30 min rest at 40 °C followed by 60 min rest at 71 °C and 10 min rest at 78 °C was taken as a suitable mashing program for teff malt. A total sugar content of about 58 g/L was recorded for the sample mashed with this program. Among the components contributed to the total fermentable sugar, the highest concentration was recorded for glucose (37.7±0.31 g/L) followed by maltose (11.1±1.9). The teff malt-wort prepared using this mashing program was used as a source of carbohydrate and other nutrients for fermentation by *Lactobacillus amylolyticus*.

In beverage production, a well-balanced aroma and flavor of the final product are equally or even more important than the efficient fermentation (Lehnert et al., 2009). A preliminary fermentation experiment was conducted to assess the sensory qualities of the fermented teff malt-wort beverage with different pH, and lactic acid to sugar ratio before carrying out the main experiments. The results from the preliminary experiment showed that the beverage with pH ranging between 3 to 4, and lactic acid to sugar ratio of lower than 0.6 had better acceptability by the assessors. The results from the preliminary experiments showed that the pH decreased rapidly for the first 24 hours of fermentation. A rapid decrease in pH at the beginning of fermentation has great importance for the quality of the end product. A

rapid increase in acidity minimises the influence of spoilage bacteria (Viander et al., 2003). In the slowly acidified environments the lactic acid fermentation can be suppressed by butyric bacteria (Karovicova and Kohajdova, 2005), which may deteriorate the product and lead to have an off-flavour in the final beverage (Vissers et al., 2007).

The optimal fermentation conditions for the factors such as temperature, initial pH, bacterial cell concentration and fermentation time for the fermentation of teff malt-wort were found to be 42 °C, 5.54, 1.86×10^5 cells/ml, and 52 h, respectively. The sensory quality assessment of the beverage produced at this condition indicated that the overall acceptability of the beverage was above average. Another fermentation experiment with pineapple showed that further improvement of the sensory quality of the beverage can be achieved by fermenting with 25% or lower concentration of pineapple juice. The overall acceptability of the beverage fermented with 25% of pineapple was rated to be 4 on the scale of 5 (like extremely), indicating that fermenting with pineapple further improves the sensory quality of the beverage. In general, the beverage produced in our experiment had high acceptance of odour, taste and flavor, and its pH (3.5) was sufficiently low to suppress the growth of food spoilage microorganisms (Karovicova and Kohajdova, 2005; Viander et al., 2003). In this study, the feasibility of developing acceptable gluten-free fermented beverage from teff malt-wort has been demonstrated. The formulated beverage may add to the list of available gluten-free beverages, thus introducing an alternative gluten-free cereal to industries for formulating beverages for celiac disease patients and other people with gluten intolerance.

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5. APPENDIX

Papers presented at national and international conferences as oral and poster presentations during the study period are listed bellow followed by my curriculum vitae.

5.1 Oral Presentations

1. Gebremariam, M. M., Zarnkow, M., Becker, T.: Kinetics of lactic acid formation during fermentation of teff malt wort by *Lactobacillus amylolyticus*. International Conference on Food Science and Nutrition/World Academy of Science, Engineering and Technology, London, United Kingdom, 2013-07-08.
2. Gebremariam, M. M., Zarnkow, M., Becker, T.: Bestimmung der Maischbedingungen von Teffmalz (*Eragrostis tef*), ein alternatives Getreide zur Herstellung von glutenfreien Lebensmitteln und Getränken. 6. Symposium Funktionelle Lebensmittel, Kiel, Deutschland, 2013-06-05.
3. Gebremariam, M. M, Zarnkow, M., Becker, T.: Thermochemical stability of starch degrading enzymes of teff (*Eragrostis tef*) malt during isothermal mashing. International Conference on Food and Biosystems Engineering, Skiathos Island, Greece, 2013-06-02.
4. Gebremariam, M. M., Zarnkow, M., Becker, T.: Characterization of the malting qualities of some teff (*Eragrostis tef*) cultivars. The third International Young Scientists Symposium for the Brewing, Distilling, and Malting Sectors, East Midlands Conference Centre, University of Nottingham, UK, 2012-10-23.
5. Gebremariam, M. M., Zarnkow, M., Becker, T.: Studies on the kilning conditions of teff (*Eragrostis tef*) malt as alternative raw material for gluten free foods and beverages. World Brewing Congress 2012, Portland, OR, USA, 2012-07-29.

5.2 Poster Presentations

1. Gebremariam, M. M., Zarnkow, M., Becker, T.: Varietal effect of teff (*Eragrostis tef*) on the dimethyl sulphide (DMS) content and enzyme activities of teff malt. World Brewing Congress 2012, Portland, OR, USA, 2012-07-30.
2. Gebremariam, M. M., Zarnkow, M., Becker, T.: Influence of kilning on the dimethyl sulphide content and enzyme activities of teff (Eragrostis tef) malt.

Jahrestreffen des Fachausschusses Lebensmittelverfahrenstechnik, Vlaardingen, The Netherlands, 2011-03-22.

5.3 Curriculum Vitae

Name: Mekonnen Melaku Gebremariam

Date of Birth: 07.10.1978

Place of Birth: Ziway, Ethiopia

Work Experiences

01.10.2007 – 30.03.2010	Lecturer, Institute of Nutrition, Food Science and Technology, Hawassa University, Hawassa, Ethiopia
01.09.2006–30.09.2007	Part time Instructor, Department of Chemistry, St. Mary's School, Addis Ababa, Ethiopia.
01.10.2003–30.01.2005	Chemist, Federal Micro and Small Enterprises Development Agency, Addis Ababa, Ethiopia
01.09.2002–30.09.2003	Instructor, Department of Chemistry, Kokeb School, Addis Ababa, Ethiopia
01.02.2001–30.08.2002	Instructor, Department of Chemistry, Don Bosco Catholic School, Ziway, Ethiopia
17.07. 2000 – 30.01. 2001	Instructor, Department of Chemistry, Waka Senior Secondary School, Waka, Ethiopia

Education

03.2005–08.2007 Addis Ababa University, Addis Ababa, Ethiopia

- Field of study: Chemical Engineering (Specialization: Food Engineering)
- Degree: M.Sc in Chemical Engineering (Food Engineering)
- Grade: 3.75 on the scale of 4.0 (Very Great Distinction)

- Eligibility evaluation of Masters Degree by the Technische Universität München (Note: 1.3), February 2010

11.1996–07.2000 Debub University, Ethiopia

- Field of study: Chemistry

- Degree: Bachelor Degree in Chemistry