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TEF AS AN INDUSTRIAL CROP FOR FOOD PROCESSING. EXPLORING ITS LATENT POTENTIAL AND FLOUR HANDLING CHARACTERISTICS.

EL TEF, CULTIVO DE INTERÉS EN EL DESARROLLO DE ALIMENTOS. ESTUDIO DE SUS CARACTERÍSTICAS Y POTENCIAL INDUSTRIAL

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DEDICATION

This thesis is dedicated to the memory of my younger sister, Tigist Abebe, whom I miss every day.
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ABBREVIATIONS

AACC  American Association of Cereal Chemists
µ₀  steady state viscosity
a  exponent from fitting power law to G’ data from a frequency sweep
a*  chromatic color coordinate
a_w  water activity
b  exponent from fitting power law to G” data from a frequency sweep
b*  chromatic color coordinate
BD  bulck density
BET  Brunauer-Emmett-Teller
BV  breakdown viscosity
c  exponent from fitting power law to tan δ data from a frequency sweep
C*  chroma of the color
D_{50}  mean particle diameter
DS  damaged starch
DSC  differential scanning calorimetry
EIAR  Ethiopian institute of agricultural research
FC  foaming capacity
FS  foam stability after 60’
FV  final viscosity
G’  elastic modulus at a frequency of 1 Hz obtained from fitting power law to G’ data from a frequency sweep
G”  viscous modulus at a frequency of 1 Hz obtained from fitting power law to G” data from a frequency sweep
GAB  Guggenheim-Anderson-de Boer
h  hue of the color
H_0  levelling-off value of melting enthalpy
H_o  melting enthalpy at initial time
H_t  melting enthalpy at time t
ΔH  endotherm enthalpy
\( J_0 \) instantaneous compliance
\( J_1 \) retarded elastic compliance or viscoelastic compliances
\( J_c \) creep compliance
\( J_{max} \) maximum creep compliance
\( J_r \) recovery compliance
\( L^* \) luminosity color coordinate
\( \text{LVR} \) linear viscoelastic region
\( m \) elastic modulus, \( G_1' \), at a gel concentration of 1\% obtained from fitting power law to \( G_1' \) versus gel concentration data
\( n \) exponent from fitting power law to \( G_1' \) versus gel concentration data
\( \text{OAC} \) oil absorption capacity
\( \text{OLVR} \) outside the linear viscoelastic region
\( p \) viscous modulus, \( G_1'' \), at a gel concentration of 1\% obtained from fitting power law to \( G_1'' \) versus gel concentration data
\( \text{PFA} \) powder flow analyzer
\( \text{PFSD} \) powder flow speed dependence
\( \text{PT} \) pasting temperature
\( \text{PV} \) pasting viscosity
\( q \) exponent from fitting power law to \( G_1'' \) versus gel concentration data
\( \text{RAG} \) rapidly available glucose
\( \text{RDS} \) rapidly digestible starch
\( \text{RMSE} \) root mean squared error
\( \text{RS} \) resistant starch
\( \text{SDRI} \) starch digestion rate index
\( \text{SDS} \) slowly digestible starch
\( \text{SEM} \) scanning electron microscope
\( \text{SP} \) swelling power
\( \text{SV} \) set back viscosity
\( \text{SV} \) swelling volume
\( (\tan \delta)_1 \) loss tangent at a frequency of 1 Hz obtained from fitting power law to \( \tan \delta \) data from a frequency sweep
\( t_{1/2} \) half life
\( \text{TD} \) true density
\( T_e \) endset temperature
\( T_{g'} \) glass transition temperature of maximally freeze-concentrated system
\( T_{m'} \) onset of ice melting temperature
\( T_o \) onset temperature
\( T_p \) peak temperature of the endotherm
\( TS \) total starch
\( TV \) trough viscosity
\( WAC \) water absorption capacity
\( WAI \) water absorption index
\( WHC \) water holding capacity
\( WSI \) water solubility index
\( \lambda_I \) retardation time
\( \tau_{\text{max}} \) maximum shear stress in the linear viscoelastic region
\( \omega \) oscillation frequency
# TABLE OF CONTENTS

ACKNOWLEDGMENTS ........................................................................................................... i
ABBREVIATIONS.................................................................................................................... iii
TABLE OF CONTENTS........................................................................................................... vii
NOTE TO THE READER......................................................................................................... 1
LIST OF ORIGINAL WORKS................................................................................................. 1
OUTLINE OF THE THESIS..................................................................................................... 2
ABSTRACT............................................................................................................................... 5
RESUMEN................................................................................................................................. 7

1. INTRODUCTION ................................................................................................................ 11
   1.1. Crop general description .............................................................................................. 11
   1.2. Tef grain physical attributes and anatomy ................................................................. 12
      1.2.1. Grain physical attributes ..................................................................................... 12
      1.2.2. Grain anatomy .................................................................................................... 13
   1.3. Composition of tef grain ............................................................................................. 15
   1.4. Health benefits of tef grain food products ................................................................. 19
   1.5. Grain milling and its effects ......................................................................................... 20
   1.6. Flour handling characteristics .................................................................................... 23
      1.6.1. Moisture sorption in flours .................................................................................. 23
      1.6.2. Flour flowability and packing characteristics ..................................................... 24
   1.7. Tef utilization and application for food formulations ................................................... 26
      1.7.1. Flour gel characteristics for application in food gel formulations ....................... 26
      1.7.2. Tef in bread making ............................................................................................ 28
      1.7.3. Other areas of tef application ............................................................................. 30

2. OBJECTIVE OF THE THESIS .......................................................................................... 35
3. WORK PLAN……………………………………………………………………………… 39
   3.1. Study materials…………………………………………………………………… 39
   3.2. Tef grain milling ………………………………………………………………… 39
   3.3. Experimental …………………………………………………………………… 40
4. PRINCIPAL RESULTS AND DISCUSSION ……………………………………… 43
5. GENEREAL CONCLUSIONS …………………………………………………… 57
   CONCLUSIONES GENERALES…………………………………… 59
6. REFERENCES……………………………………………………………………….. 63

STUDY I: Impact of variety type and particle size distribution on starch
enzymatic hydrolysis and functional properties of tef flours ……… 79
Abstract ………………………………………………………………………………… 79
Introduction ……………………………………………………………………………… 80
Materials and methods ………………………………………………………………... 81
Results and discussion ………………………………………………………………... 85
Conclusions ……………………………………………………………………………… 99
References ……………………………………………………………………………… 100

STUDY II: Flowability, moisture sorption and thermal properties of tef
   [Eragrostis tef (Zucc.) Trotter] grain flours ………………………………………… 107
Abstract ………………………………………………………………………………… 107
Introduction ……………………………………………………………………………… 108
Materials and methods ………………………………………………………………... 109
Results and discussion ………………………………………………………………... 115
Conclusions ……………………………………………………………………………… 124
References ……………………………………………………………………………… 125

STUDY III: Rheological and textural properties of tef [Eragrostis tef (Zucc.)
   Trotter] grain flour gels ……………………………………………………………… 131
Abstract ………………………………………………………………………………… 131
Introduction ……………………………………………………………………………… 132
STUDY IV: Viscoelastic properties and stickiness of tef grain flour enriched wheat dough matrices

STUDY V: Suitability of tef varieties in mixed wheat flour bread matrices: a physico-chemical and nutritional approach
NOTE TO THE READER

This thesis is based on five original works which are represented in the text by roman numbers I, II, III, IV and V. Each work is either published or submitted and under revision in different international journals. Each work reported in published articles or manuscript under revision constitutes a study or a chapter in this thesis. The status of the articles or manuscripts on publication process and the authors are presented in the list of original works part.

Before the presentation of each study, the reader will find the outline of the thesis and an abstract written both in English and Spanish. Then, a summary of the whole thesis which includes an introduction with the general and specific objectives, the work plan, core results obtained with discussion and the general conclusion of the thesis are presented. Finally, the reader will find each of the five studies.

LIST OF ORIGINAL WORKS


OUTLINE OF THE THESIS

This thesis investigates the engineering characteristics of tef flours obtained from three varieties of tef and evaluates the effect of mill type on flour techno-functional properties. The thesis further explores the potential of tef in gel like food and bread baking applications. In order to accomplish this general objective five studies have been conducted. Study I evaluated the impacts of variety type and particle size distribution/mill type on techno-functional properties and starch enzymatic hydrolysis tef grain flours. Study II determined the engineering properties (flowability, packing and water sorption characteristics and thermal properties) of tef grain flours (from three grain tef varieties) which are important to understand its powder behavior during storage, conveying and processing. Study III, the suitability of three tef varieties for gel like foods was explored through evaluation of their gelling performance and characterization of the rheological and textural properties of the gels. Study IV assessed viscoelastic properties and stickiness of tef grain flour enriched wheat dough matrices by taking both the effect of tef variety type and dose level into consideration. Finally, Study V physical, sensory and nutritional aspects tef grain flour enriched (with three varieties of tef) breads were studied.
ABSTRACT

Recently, tef [Eragrostis tef (Zucc.) Trotter] grain is attracting the attention of the modern food industry since it is a gluten-free grain encompassing highly appreciated nutritional advantages. However, as a relatively new raw material for most countries other than Ethiopia, a deep study on its inherent characteristics related to processing is still needed for its use in various food applications across the globe. The engineering characteristics and techno-functional properties of tef grain flours from three varieties (DZ-01-99, DZ-Cr-37 and DZ-Cr-387) were studied. Their potential in gel like food and bread making applications was explored. Commercial wheat (whole and refined) and rice flours were included as references.

Tef variety and mill type had important effects on flour granulation, bulk density and starch granule which dictate the hydration and pasting properties. Different granulation of tef grain flour induced different in vitro starch digestibility.

Tef grain flours were less flowable than the reference flours and their flow and packing properties were more sensitive to water activity. Tef grain flours led to sigmoidal sorption isotherms with estimated monolayer water content of 0.053 (BET model) and 0.057 (GAB model) g water/g dry solids. No differences among the three tef varieties were observed on their flour starch gelatinization enthalpies that were higher than that of wheat and similar to rice. The amylopectin recrystallization extent after 7 days of storage at 4°C was significantly higher in tef flour gels than in wheat ones.

The minimum flour concentration required for gel formation from the three tef varieties was 6-8%, similar to wheat flour. The gels formed from the tef cultivars showed solid-like behavior indicating their ability of forming self-supporting gels. The dependence of viscoelastic moduli with concentration fulfilled a power law. Avrami model was successfully fitted to the textural parameters evolution of tef grain flours gels with time. Important differences were observed among tef and rice and wheat flours, probably contributed by their differences in protein, starch, lipid and fiber constituents. The measured gelling properties suggest that tef grain flours would be suitable ingredients in gel food formulations.
Incorporation of tef flours to wheat dough matrices visibly affected the dough structure by reducing viscoelastic moduli values and the maximum stress they can tolerate before its structure is broken, and increasing dough instantaneous and retarded elastic compliances. Effect of dose was not always significant in the parameters measured. On average, the DZ-Cr-37 supplemented doughs exhibited higher elastic and viscous moduli, lower compliances and higher steady state viscosity and led to significantly lower volume breads. Tef incorporation up to 30% gave ciabatta type breads with higher volume than the control bread (100% wheat). Tef incorporation led to breads with enhanced mineral contents and lower starch digestion rate index. DZ-01-99 (brown tef) supplemented breads had higher flavonoid content and larger anti-radical scavenging activity than those blended with the remaining varieties and higher than the control bread.

In general, results revealed the importance of the variety and the mill on flour engineering and techno-functional properties and confirmed the suitability of tef as an ingredient in the formulation of new cereal-based foods.
RESUMEN

Recientemente, el tef [Eragrostis tef (Zucc.) Trotter] está captando la atención de la industria alimentaria moderna, ya que es un grano libre de gluten con ventajas nutricionales altamente apreciables. Sin embargo, su aplicación alimentaria en países no africanos es relativamente nueva, siendo necesario un estudio profundo de sus características relacionadas con su procesado. Por ello, se estudiaron las características ingenieriles y propiedades técnico-funcionales de tres variedades de harina de tef (DZ-01-99, DZ-Cr-37 y DZ-Cr-387). Se estudió su potencial como gelificante y su aplicación en panadería. Se incluyeron como referencias muestras de trigo comercial (integral y refinado) y harina de arroz.

La variedad de tef y el tipo de molino tuvieron un efecto importante en la granulometría, densidad aparente y almidón dañado, influyendo en las propiedades de empastado y de hidratación. La granulometría también afectó a los valores de digestibilidad del almidón in vitro.

Las harinas de tef mostraron peores características de flujo y más sensibilidad a cambios de humedad que las harinas de referencia. Las harinas de tef dieron lugar a isotermas de absorción sigmoidales con un contenido de agua monocapa de 0,053 (modelo BET) y 0,057 (modelo GAB) g agua / g sólidos secos. No se observaron diferencias en las entalpías de gelatinización entre las tres variedades de tef, las cuales fueron más altas que las obtenidas en trigo y similares a las mostradas por arroz. El grado de recristalización de la amilopectina a los 7 días de almacenamiento a 4°C, evaluada mediante su entalpía de fusión, fue significativamente mayor en los geles de harinas de tef que en los de trigo.

La concentración mínima necesaria para la formación de gel en las tres variedades de tef fue de 6-8%, similar a la requerida por la harina de trigo. Los geles formados a partir de tef mostraron una componente elástica marcada y predominante frente a la viscosa, que indica su capacidad para formar geles de la consistencia y características viscoelásticas adecuadas. La dependencia de los módulos viscoelásticos con la concentración de harina en el gel cumplió con la ley de la potencia. El modelo de Avrami se ajustó con éxito a la evolución de los parámetros de textura de los geles de tef frente al tiempo.
observaron diferencias importantes entre las harinas de tef, trigo y arroz, probablemente debidas a los diferentes contenidos en proteínas, almidón, lípidos y fibra que los constituyen. La medida de las propiedades de gelificación sugieren que las harinas de tef serían ingredientes adecuados en la formulación de alimentos en base gel.

La incorporación de harinas de tef a masas panarias de trigo en dosis del 10 al 40% afectó notablemente a su comportamiento reológico. En ensayos dinámicos se observaron reducciones significativas de ambos módulos viscoelásticos, de la tangente de pérdida y del esfuerzo máximo que las masas podían soportar antes de la ruptura de su estructura. En ensayos de deformación progresiva, se obtuvieron incrementos de su capacidad de deformación elástica, tanto instantánea como retardada, ante la aplicación de un esfuerzo constante. El efecto de la dosis sobre los parámetros medidos no siempre fue significativo. Como promedio, las masas enriquecidas con DZ-Cr-37 mostraron mayores módulos elástico y viscoso, menor capacitancia mecánica y mayor viscosidad estacionaria, que condujeron a panes de volúmenes significativamente más bajos. La incorporación de hasta el 30% de harina de tef en panes de trigo tipo *chapata* dio como resultado panes con mayor volumen que el pan control (100% trigo). La incorporación de tef al pan mejoró su contenido en minerales y disminuyó la velocidad de digestión del almidón establecida mediante ensayos *in vitro*. Los panes con DZ-01-99 (tef marrón) mostraron mayor contenido en flavonoides y actividad anti-radical que el resto de variedades y que el pan control.

En general, los resultados obtenidos revelaron la importancia tanto de la variedad como del tipo de molino usado sobre las propiedades ingenieriles y tecno-funcionales de la harina de tef, y confirmaron su idoneidad como ingrediente en la formulación de nuevos alimentos a base de cereales.
1. INTRODUCTION

1.1. Crop general description

Tef [Eragrostis tef (Zucc.) Trotter] crop is an annual grass species (Poaceae) indigenous to Ethiopia (Vavilov, 1951; Costanza et al., 1979) where the major world center for the genetic diversity of tef is found (Ebba, 1975; Ketema, 1993). It is a tropical C4 self-pollinated but chasmogamous tetraploid cereal plant having a chromosom number of $2n = 4X = 40$ (Berehe, 1976; Ketema 1993).

Tef has been cultivated and utilized for human consumption mainly in Ethiopia since time in memorial. The crop was introduced into several other countries like Australia, India and South Africa (Costanza et al., 1979), Argentina (Nicora, 1939), Ukraine (Krasnokutskii & Konstanc, 1939), Malawi, Zaire, Sri Lanka, New Zealand, Mozambique, Uganda, Tanzania, Palestine, Kenya, Canada (Ketema, 1997), USA and parts of Asia (Castellani, 1948) and was grown mainly, but not exclusively, as a forage crop. However, currently with the understanding of the nutritional and health benefits of tef grain, the crop is gaining popularity in the Western world menus and serious efforts are being undertaken to expand its cultivation notably in the Netherlands and United States of America (Evert et al., 2009; van Delden, 2011) and Spain. Tef can grow under wide and diverse agro-ecologies. It grows best between altitudes of 1800 and 2100 meters with an annual rainfall of 750-850 mm and a temperature range of 10-27 degrees centigrade, though in practice in much more varied areas with rainfall up to 1200mm. The length of growing period ranges from 60 to 180 days (depending on the variety and altitude) with an optimum of 90 to 130 days (Ketema, 1997).

Despite tef’s lower yield than other cereals in Ethiopia farmers continued to grow the cereal because Ethiopians have strong preference for consuming tef (Belay et al., 2005) making the grain to receive high market price. In addition, tef performs better than other cereals under moisture stress and waterlogged conditions, has lower disease or insect pest problems, its grain can be stored for a long period of time without being attacked by weevils and other storage pests, and its straw is more a nutritious and preferable animal feed fetching higher income (Ebba, 1969; Ketema, 1997; Amogne et al., 2001).

The recent Central Statistical Authority (CSA) (2014) data show that the tef area coverage, production and yield per hectare are increasing in Ethiopia. Among cereals
produced in the Meher season of the 2013/14 crop year tef ranked first in terms area coverage (taking above 3.0 million hectares) and the production volume (44.2 million quintals) stood second next to maize accounting 20.5% of the total cereal harvest. Compared to the 2012/13 crop year the area allocated, production volume, and productivity per hectares increased by 10.48%, 17.35% and 6.24%. However, due to lower yield potential of tef the total production volume is the second to maize.

Tef cultivars have been distinguished and described based on the color of the grains and inflorescences, ramification of the inflorescences and the size of plants (Tefera et al., 1995). In Ethiopia Debre Zeit Agricultural Research Center (DAZRC) is the center of excellence for tef research within the Ethiopian Agricultural Research Institute (EIAR). From 1970-2011 from tef breeding national research centers 31 tef varieties were released. On experimental station, the yields of these varieties are in the range of 15 to 34 quintals per hectare and on farm level the range was 14 to 20 quintals per hectare (Assefa et al., 2013).

1.2. Tef grain physical attributes and anatomy

1.2.1. Grain physical attributes

Tef grain is a hull less naked grain having oval-shape of size 0.9–1.7 mm (length) and 0.7–1.0 mm (diameter) (Fig. 1). The thousand kernel weight of the improved tef varieties ranged between 0.19 and 0.42g (Assefa et al., 2001; Bultosa, 2007) while the hectoliter weight of the popular tef variety called Quncho (DZ-Cr-387) was 86.42 kg/hL, and this may show perhaps tef grain is the smallest among carbohydrate-rich kernels (Bultosa, 2007; Belay et al., 2009). The great majority of tef consumers in Ethiopia traditionally prefer to buy the tef grain and get the grain milled rather than buying the flour. That means both farmers (planting seeds) and consumers prefer to buy relatively large grain-sized tef (Belay et al., 2005).

Tef grain has a range of colors varying from milky-white to almost dark brown. However, white, creamy-white, light brown and dark brown are the most common. In Ethiopia, tef is classified for marketing purposes based on seed color as: *netch* (white), *key* (brown) and *sergegna* (white and brown mixed) (Tefera et al., 1995). The seed color dictates the product color, mainly the *injera*, and consumers prefer lighter or whiter products which are obtained from white colored grains (Fufa et al., 2011). Participatory
variety selection study by Belay et al. (2005) also indicated seed color is the primary concern for Ethiopian farmers as it fetches the premium price. Recently with a belief that it is more nutritious, brown tef is also gaining popularity among health conscious consumers (Minten, 2013). However, tef quality is also often evaluated by origin in Ethiopia (Minten, 2013), while the quality of tef is also judged by a number of other factors, such as physical appearance, impurities, aroma, texture, and nutritional quality. Of these the impurity level and appearance (color) are used in tef grain grading.

1.2.2. Grain anatomy

Tef grain anatomy study by Parker et al. (1989) and Umeta and Parker (1996) indicate that like other small seeded cereals the embryo, which is rich in protein and lipid, occupies a relatively large proportion of the grain (Fig. 1a and b). Its aleurone layer is one cell thick and rich in protein and lipid bodies. One layer of fused mesocarp and endocarp of pericarp is found next to aleurone layer toward outside of the grain. The testa located within the pericarp and its thickness varies with the color of the grain. The outer pericarp is thin and membranous is equivalent to the beeswing bran of wheat. Tef grain is similar to sorghum and pearl millet by having small size starch granules in its pericarp. The testa of red tef is thicker than white tef and filled with pigmented material, suggested be tannins or polyphenolic compounds (Urga et al, 1997). The cells of the outer endosperm are horny or vitreous contain most of the protein reserves of the endosperm, while the central endosperm is mealy (Parker et al., 1989; Umeta and Parker, 1996). Scanning electron microscopy (SEM) analysis by Parker et al. (1989) showed the thin pericarp, starchy endosperm with the outer horny region and mealy center and a relatively large embryo (Fig. 1a). According to Helbing (2009) and the endosperm cells have an angular, polygonal shape with average size up to 70 microns (Fig. 1b). The central endosperm cells were mealy, and tend to break open during milling, releasing into the flour that bears small sized polygonal shaped individual starch granules from the compound starch grains along with small groups of protein bodies, endosperm cell walls and storage lipidss (Umeta and Parker 1996).
Figure 1. a) Cross-sectional view of by tef grain by scanning electron microscopy (SEM) showing endosperm (en), germ (G) and pericarp (pc) X200 (Source Parker et al., 1989), b) Longitudinal section SEM image of tef grain showing germ and endosperm (Source: Helbing, 2009).

The size of individual tef starch granules estimated using SEM ranged between 2 to 6 μm in diameter which is larger than amaranthus (1–2 μm) and quinoa (1–2 μm) starch granules, equivalent to rice starch granules (2–10 μm) and smaller than A type wheat (20-35 μm), sorgum (20 μm) and maize (20 μm) starch granules (Bultosa et al. 2002; Repo-Carrasco et al., 2003; Lindeboom et al. 2004; Delcour, 2010). According to Bultosa et al. (2002) the starch granules in the different tef varieties appeared morphologically similar to one another. The shape is polygonal, smooth with no surface pores. There is no evidence of strong attachment between adjacent individual starch granules within the compound starch granule and most protein bodies are located outside of the compound starch granules. The starch crude composition is similar to that of normal native cereal starches (Bultosa, 2002).
1.3. Composition of tef grain

Performance of cereal grains during processing and their nutritional value are predominantly dependent on their composition. Cereal grains are the major sources of carbohydrate and protein for the world’s population (Eskin, 1990). They contribute 70% of calorie and 50% of protein consumption in human nutrition. Cereals are also important source of dietary fiber, contributing to about 50% of the fiber intake in western countries (Nyman et al., 1989). Tef grain products are nutritionally well packed because they are always consumed as whole grain (USDA, 2007). This section reviews the chemical composition of tef grain and its nutrients.

Like other cereals starch in tef constitutes 73% of the grain. Amylose was reported to range 25-32% from extracted starch granules (Bultosa et al., 2002) and 20-26% with a mean value of 23% in flour starches (Bultosa, 2007). So far no waxy- or amylo- type starch traits were reported in tef. The X-ray diffraction study on tef starch granules indicated that they are A-type with similar crystallinity to rice (Bultosa and Taylor, 2003). The A-type starches were noted for their good digestibility. Study on 13 Ethiopian tef varieties by Bultosa (2007) revealed the presence of variation in the amylose content of different tef varieties where 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 %). The tef starch granules conglomerate and form starch compound granules of the endosperm (Bultosa et al, 2002; Wolter et al., 2013).

The crude protein contents of 13 tef varieties ranged from 8.7–11.1 % with mean 10.4 % (Bultosa, 2007). Hence, in terms of protein content tef is comparable to that of barley, wheat and maize, and higher than that of rice and sorghum (Table 1). Hager et al. (2012) reported that protein content of a tef flour obtained from a supplier in the Netherland to be 12.8%. However, such comparisons must be treated with caution as cereal grain protein content is strongly affected by cultivar and cultivation conditions. Protein is synthesized during the fruiting period, whereas starch synthesis starts later. If growing conditions in the late fruiting period are good, starch yield will be high but protein content will be relatively low (Lasztity, 1996). Study by Adebowale et al. (2011) and Hager et al. (2012) indicate that prolamins are the major protein storages in tef and, according to Adebowale et al. (2011): prolamins account approximately 40% of...
the total tef protein. Absence of wheat type gluten proteins is another important attribute which is being appreciated in tef (Hopman et al., 2008). Work by Spaenji-Dekking et al. (2005) confirmed the absence of wheat type gluten in pepsin and trypsin digests of 14 tef varieties. This indicates the grain to be a valuable ingredient for functional foods destined for gluten intolerant celiac patients.

In general tef grain can be regarded as a well balanced source of essential amino acids when compared to FAO reference pattern (FAO/WHO, 1973). It contains high amount of lysine that is most often deficient in grain foods. Compared to other cereals, tef grain proteins are known to have higher contents of isoleucine, leucine, valine, tyrosine, threonine, methionine, phenylalanine, arginine, alanine, and histidine (Table 1).

Despite the large proportion of germ in tef grain the fat content is known to be not as such high. The crude fat content of tef is higher than that of wheat and rice, but lower than maize and sorghum (Table 1). The crude fat content in 13 Ethiopian tef varieties ranged 3.0-2.0% with mean of 2.3% (Bultosa, 2007) and among these cultivars the highest crude fat was for DZ-Cr-82 and the lowest was among DZ-01-354, DZ-01-99, DZ-Cr-37, DZ-01-974 and DZ-01-1681 (p < 0.05). El-Alfy et al. (2012) reported that tef grains are rich in unsaturated fatty acids, predominantly oleic acid (32.4 percent) and lino-leic acids (23.8 percent). Compared with tef grain the common cereal grains like rice, wheat and maize contain negligible amount of linoleic acid (LA) and only traces of α-linolenic acid (ALA) (El-Alfy et al., 2012). Furthermore, as tef is consumed as whole grain it can maintain the amount of crude fat and n-6 and n-3 poly-unsaturated fatty acids and this makes it a good source of fatty acids than refined ones. El-Alfy et al. (2012) reported that tef grains are rich in unsaturated fatty acids, predominantly oleic acid (32.4 percent) and lino-leic acids (23.8 percent).

The crude fiber, total and soluble dietary fiber, content of tef is much higher than those of wheat, sorghum, rice and maize (Table 1). The reasons are tef is a minute sized grain with higher proportion of bran versus endosperm and germ (Bultosa, 2007). In contrast to most common cereals, the amount of uronic acid in tef grain is high (Umeta, 1986). Among the Ethiopian tef cultivars the crude fiber contents of the brown tef varieties DZ-01-99 (3.8 %) and DZ-01-1681 (3.7 %) were the highest while those of DZ-Cr-44 (2.7 %) and DZ-Cr-255 (2.6 %) were the lowest.
Minerals are important for various physiological functions in the human body and cereal grains are important sources of minerals. The daily requirement of the major minerals (Na, Mg, K, Ca, Ph, and Cl) is estimated to be more than 100 mg while that of the trace elements (Fe, Cu and Zn) is less than 100 mg (Insel et al., 2004). Even though, there are high variability in the mineral content reported in different studies, tef is more rich in such minerals like Ca, Zn, Mg, Fe, Ph and Cu as compared to the contents found in other cereal grains (Table 1) compared to the other cereals. Hence, tef can be one of the best options in meeting the daily mineral requirement.

Study by McDonough et al. (2000) indicates that ferulic acid (285.9 μg/g) is the major constituent of phenolic acids in tef grains and Vanillic (54.8 μg/g), cinnamic (46 μg/g), coumaric (36.9 μg/g), gentistic (15 μg/g), syringe (14.9 μg/g) acids are also important constituents of tef phenolic acids. These major constituents of phenolic acids in tef do not have galloyl and catechol functional groups and thus are less likely to hamper iron absorption (Baye et al., 2013). Hence, there is a possibility that the anti-oxidative properties of the polyphenols in tef can be exploited while not compromising on iron bioavailability.

In general the nutrient profile of tef grain indicates its potential ingredient to in formulation of nutritious and healthy for food formulations. However, as most of these studies are not variety-based or undertaken on one or two tef types usually described as white and brown tef, the variation available between the different cultivars of tef including the recently released ones have not been explored yet (Baye, 2014).
Table 1. Proximate compositions and amino acid and microelement contents of tef grain compared with sorghum, brown rice and wheat.

<table>
<thead>
<tr>
<th>Component</th>
<th>Tef</th>
<th>Sorghum</th>
<th>Brown Rice</th>
<th>Wheat</th>
</tr>
</thead>
<tbody>
<tr>
<td>Starch (%)</td>
<td>73</td>
<td>62.9</td>
<td>64.3</td>
<td>71</td>
</tr>
<tr>
<td>Crude protein (%)</td>
<td>11.0</td>
<td>8.3</td>
<td>7.3</td>
<td>11.7</td>
</tr>
<tr>
<td><strong>Amino acid (g/16g N)</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Lysine</td>
<td>3.7</td>
<td>0.3</td>
<td>3.7</td>
<td>2.1</td>
</tr>
<tr>
<td>Isoleucine</td>
<td>4.1</td>
<td>0.7</td>
<td>4.5</td>
<td>3.7</td>
</tr>
<tr>
<td>Leucine</td>
<td>8.5</td>
<td>2.1</td>
<td>8.2</td>
<td>7.0</td>
</tr>
<tr>
<td>Valine</td>
<td>5.5</td>
<td>0.8</td>
<td>6.0</td>
<td>4.1</td>
</tr>
<tr>
<td>Phenylalanine</td>
<td>5.7</td>
<td>0.9</td>
<td>5.5</td>
<td>4.9</td>
</tr>
<tr>
<td>Tyrosine</td>
<td>3.8</td>
<td>0.7</td>
<td>5.2</td>
<td>2.3</td>
</tr>
<tr>
<td>Tryptophan</td>
<td>1.3</td>
<td>0.2</td>
<td>1.2</td>
<td>1.1</td>
</tr>
<tr>
<td>Threonine</td>
<td>4.3</td>
<td>0.5</td>
<td>3.7</td>
<td>2.7</td>
</tr>
<tr>
<td>Histidine</td>
<td>3.2</td>
<td>0.4</td>
<td>2.3</td>
<td>2.1</td>
</tr>
<tr>
<td>Arginine</td>
<td>5.2</td>
<td>0.6</td>
<td>8.5</td>
<td>3.5</td>
</tr>
<tr>
<td>Methionine</td>
<td>4.1</td>
<td>0.3</td>
<td>2.7</td>
<td>1.5</td>
</tr>
<tr>
<td>Cystine</td>
<td>2.5</td>
<td>0.3</td>
<td>1.8</td>
<td>2.4</td>
</tr>
<tr>
<td>Asparagine + Aspartic acid</td>
<td>6.4</td>
<td>5.1</td>
<td></td>
<td>9.0</td>
</tr>
<tr>
<td>Serine</td>
<td>4.1</td>
<td>0.8</td>
<td>5.0</td>
<td>5.0</td>
</tr>
<tr>
<td>Glutamine + Glutamic Acid</td>
<td>21.8</td>
<td>29.5</td>
<td></td>
<td>17.0</td>
</tr>
<tr>
<td>Proline</td>
<td>8.2</td>
<td>1.3</td>
<td>5.0</td>
<td>10.2</td>
</tr>
<tr>
<td>Glycine</td>
<td>3.1</td>
<td>0.5</td>
<td>4.5</td>
<td>4.0</td>
</tr>
<tr>
<td>Alanine</td>
<td>10.1</td>
<td>1.6</td>
<td>5.5</td>
<td>3.6</td>
</tr>
<tr>
<td>Crude fat (%)</td>
<td>2.5</td>
<td>3.9</td>
<td>14.0</td>
<td>3.2</td>
</tr>
<tr>
<td>Crude fibre (%)</td>
<td>3.0</td>
<td>0.6</td>
<td>0.6-1.0</td>
<td>2.0</td>
</tr>
<tr>
<td>Ash (%)</td>
<td>2.8</td>
<td>1.6</td>
<td>1.4</td>
<td>1.6</td>
</tr>
<tr>
<td><strong>Minerals (mg/100g)</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Calcium</td>
<td>165.2</td>
<td>50</td>
<td>6.9</td>
<td>39.5</td>
</tr>
<tr>
<td>Copper</td>
<td>2.6</td>
<td>0.4</td>
<td>0.2</td>
<td>0.2</td>
</tr>
<tr>
<td>Iron</td>
<td>15.7</td>
<td>6.0</td>
<td>0.57</td>
<td>3.5</td>
</tr>
<tr>
<td>Magnesium</td>
<td>181.0</td>
<td>180.0</td>
<td>16.9</td>
<td>103.5</td>
</tr>
<tr>
<td>Manganese</td>
<td>3.8</td>
<td></td>
<td>0.4</td>
<td>1.0</td>
</tr>
<tr>
<td>Phosphorus</td>
<td>425.4</td>
<td>263.3</td>
<td>61.7</td>
<td></td>
</tr>
<tr>
<td>Potassium</td>
<td>380.0</td>
<td>225.2</td>
<td>181.7</td>
<td></td>
</tr>
<tr>
<td>Sodium</td>
<td>15.9</td>
<td>6.2</td>
<td>0.5</td>
<td></td>
</tr>
<tr>
<td>Zinc</td>
<td>4.8</td>
<td>2.0</td>
<td>2.0</td>
<td>1.94</td>
</tr>
</tbody>
</table>

Sources: Bultosa, 2007; Bultosa and Taylor, 2004; FAO, 1992; FAO, 1993; Jansen et al., 1962; Kashlan et al., 1991; Khoi et al., 1987; Gebremariam et al., 2013a; Mengesha, 1966; Mosse et al., 1985; Obilana, 2003; Saturni et al., 2010; Seyfu, 1997; Shoup et al., 1969.
1.4. Health benefits of tef grain food products

The nutrient profiles of tef stated above indicate tef grain is a good contender for a functional food in health promotion and disease prevention. Iron deficiency is one of the frequent micronutrient deficiencies which can be prevented by food fortification and nutritional supplement (Stoltzfus, 2011). Hence, tef grain products can serve as a good option in combating this problem (Alaunyte et al., 2012; Umeta et al., 2005). The low incidence of anemia in tef consuming society of Ethiopia confirms the advantage of tef in this regard (Umeta et al., 2005).

It is known that celiac patients suffer from an immune mediated disease, triggered by the ingestion of gluten proteins from grain wheat, barley, rye, triticale and also from oats because of the possibility of contamination. The estimated number of celiac patients range 0.6-1% of the world population (Guiral et al., 2012). The increasing numbers of diagnosed cases and growing awareness makes the availability of gluten-free foods an important socioeconomic issue. The only effective treatment is the complete avoidance of this protein, i.e. the adherence to a gluten-free diet (Vader et al., 2003). Hence, products of tef grain are becoming popular globally mainly due to the absence of gluten (Bergamo et al., 2011; Dekking et al., 2005; Hopman et al., 2008) and because it is always consumed as whole grain and in many respects tef grain provides more nutritious than common cereal grains. A systematic review and dose-response meta-analysis of cohort studies by Aune et al. (2013) suggests that a high intake of whole grains, but not refined grains, is associated with reduced type 2 diabetes risk. The higher total and soluble dietary fiber, content in tef grain described earlier may indicate its potential in prevention and control of diabetes mellitus. Carbohydrate type and its digestibility are also critical in determining the glucose levels after eating and thereby diabetes risks (Sheard et al., 2004). In this regard also tef grain can serve as an option as it has a lower glycemic index when compared to common cereal grains like wheat and rice (Wolter et al., 2013). Phytates and phenols available in tef grain could also make the grain beneficial for antioxidant activity which could contribute in prevention of cardiovascular diseases and cancers (Boka et al., 2014).
1.5. Grain milling and its effects

Cereal grain milling or grinding is an important unit operation performed to reduce particle size and obtain flour. In Ethiopia almost all tef grain milling operation is dry milling and using Danish type stone mill/ disc mill. In comparison to other milling operations, power use efficiency in these types of mills is generally low and yet they are preferred in many developing areas because of low costs and high durability (Bayram and Oner, 2005; Singh et al., 2005). After the grain is cleaned by winnowing and sieving from its impurities, it is whole floured to the fitness level used traditionally for making injera at the cottage grain milling houses.

Different milling or grinding processes produce flours with different particle sizes and consequently the resulting in to different surface area available for reactions like water binding, solubility, heat transfer, and swelling that dictate flour quality and functionality (Brou et al., 2013; Chen et al., 1999) and end use. Particle size also affects the physical properties of a flour such as density, flowability and packing characteristics which influence the flour handling characteristics (Landillon et al., 2008).

As starch is the major component in most cereal grains, milling process inevitably affects starch component. Tran et al. (2011) simplified the complex structure of starch in grains by grouping them in six stratified levels (Fig. 2). Individual linear and branche of starch molecules (level 1) where anhydroglucose units are linked together by α-(1→4) glycosidic bonds, macromolecular branched structure (level 2) where the linear glucan branches are joined together by α-(1→6) glycosidic bonds to form amylopectin and amylose (levels 1 and 2 are “molecular”), semicrystalline structure (level 3) formed by double helices of amylopectin branches, growth ring structure (level 4) formed by several alternating crystalline and amorphous lamellae, granular structure (level 5) consisting of several growth rings, and whole grain structure (level 6) where starch granules interact with protein, lipid, non-starch polysaccharides, and other components in the grains.

Damage to starch granules (usually termed “starch damage”), the breaking of level 5 starch structure, inevitably occurs during grinding (milling) of cereal grains (level 6 starch structure). The starch granules are broken and sometimes into smaller fragments with hila exposed (Fig. 3). The damage to the starch granules extends further to
disruption of starch crystalline structure (level 3 structure) and degradation of starch molecules (levels 1 and 2) (Li et al., 2014).

Figure 2. Six levels of starch structures in cereal grains. (Source: Tran et al., 2011)
Figure 3. Intact individual and damaged starch granules together with broken fragments of DZ-01-99 (brown tef). Disruption of starch granules exposed growth rings and hila (Own source).

The degree of damage to the starch structures depends on various factors. The type and severity of the mechanical force applied and the grinding time are among the factors related to milling operation while temperature and moisture level could be considered as mill conditions (Li et al., 2014). Grain hardness and the starch granular type are the intrinsic grain properties which determine the starch damage extent. Cereal grains with greater hardness, particularly those with higher protein contents, require greater amount of mechanical energy to break the grain structure and are associated with greater damage to their starches than softer grains (Hasjim et al., 2009). Hence, in addition to the milling process, starch damage is dependent up on the botanical source and variety type of the cereal grain. The visible effect milling method on starch damage, flour physical and functional properties of common cereal grains like wheat and rice are well studied (Bindrani and Rao, 1992; Prasad, et al., 2012). Despite peculiarities of tef grain such information is missing and there is no universal milling method or system developed or selected for milling of tef grains.
1.6. Flour handling characteristics

1.6.1. Moisture sorption in flours

Flour handling which includes conveying, packing/packaging and storage is among the important operations in cereal grain processing. Controlling moisture content during flour handling is a critical activity for facilitation of the operations and preservation. This is a challenging activity because food materials are heterogeneous mixtures of soluble organic and inorganic materials. Moisture sorption is a characteristic of a given material which is temperature specific and it helps to predict product condition and stability over time in different handling conditions (Al-Muhtaseb et al., 2002). Several mathematical models have been proposed to describe water sorption isotherm in food materials. These models are based on thermodynamic concepts - Brunauer, Emmett and Teller (BET) (Al-Muhtaseb et al., 2002) and Guggenheim, Anderson and de Boer (GAB) (Van den Berg and Bruin, 1981) models, or semiempirical and empirical models - Peleg (1993) and Viollaz and Rovedo (1999). The monolayer moisture content is of significant importance to the physical and chemical stability of dehydrated materials with regard to lipid oxidation, enzyme activity, non-enzymatic browning and structural characteristics. The monolayer water content, which gives information about the minimal water content conferring food stability, is valuable additional information that can be obtained from isothermal data determined from BET and GAB models (Labuza, 1980). Although not well defined, the monolayer is often stated to represent the moisture content at which water attached to each polar and ionic group starts to behave as a liquid-like phase and corresponds with the optimal moisture content for stability of low-moisture foods.

As described in earlier section, starch is quantitatively the main component of cereal grain flours and it has crystalline and amorphous layers arranged in an onion-like structure. Although parts of the amylose molecules are also present in them most of the crystalline regions are formed by amyllopectin while amorphous zones are more water-accessible than crystalline regions in which the intermolecular interactions between the chains are too strong to allow solvent penetration (Al-Muhtaseb et al., 2004). Hence, water influences the structure by acting as plasticizer of the amorphous regions. Water sorption isotherm data for different types of cereal flours and starches can be found extensively. These materials exhibited sigmoidal shape characteristic isotherms and
according to the IUPAC classification they can be grouped as type II isotherms (Al-Muhstaseb et al., 2002; Hébrard, et al., 2003). Type II isotherm curve, which is a characteristic of finely divided non-porous solids or macro-porous materials indicating the occurrence of multi-layer adsorption, has three zones I, II and III covering 0-20%, 20-80% and 80-95% relative humidity ranges (Hébrard et al., 2003). Zone I represents the monolayer formation; zone II corresponds to the linear portion of the isotherm where the added water will bind with the components of the material forming layers. In zone III, water presents weak binding and in this state the water is mobile. In general the monolayer moisture contents reported for cereal products range between 0.051 and 0.086 g water/g dry solids. Differences between in the moisture sorption isotherms of different flour types are due to their variation in their damaged starch level, proteins and pentosans (Hébrard, et al., 2003). Furthermore, particle size is found mainly to influence sorption kinetics: the finer the particles, the faster their sorption kinetics. Increasing temperature accelerates sorption kinetics. Thus, absorption kinetics seems to be influenced by physical mechanisms, while the biochemical composition determines the amount of water sorbed. Despite the presence of extensive sorption data for the flours from different types of cereals and starches, such essential information is lacking regarding tef grain flours.

1.6.2. Flour flowability and packing characteristics

Cereal industries utilize cereal powders obtained by milling of the grains as main raw materials for a large number of food applications. The final quality of cereal grain products can be dependent on the initial mixing and handling behavior of the cereal grain powders. Powder flow properties intrinsically affect its behavior during storage, handling and processing operations, such as flow from hoppers and silos, transportation, mixing, compression and packaging (Knowlton et al., 1994; Peleg, 1978). Many powders and bulk solids do not flow reliably or uniformly through bins, hoppers, feeders, or chutes. Hence, getting a reliable and consistent flow out of hoppers and feeders without excessive spillage and dust generation is one of the major challenges in industrial powder processing. Flow problems lead to lost production, plant downtime, extra labor, poor quality control, spoiled material, and inefficient use of capital (Marinelli and Carson, 1992; Thomson, 1997). Therefore, understanding of powder flow properties and avoiding flow problems is mandatory.
Flow and packing properties of a flour system are known to be dependent on particle properties (density, packing properties, moisture content, particle size, surface roughness, chemical composition …) and external factors like temperature, exposure to humidity of air, storage time, and consolidation (Abu-hardan & Hill, 2010; Domian and Fitzpatrick 2005; Fitzpatrick et al., 2004b; Teunou and Fitzpatrick, 1999). The bulk density of food powders is a major physical attribute of considerable concern to the food industry as it determines the filled weight of containers and plays a major role in variety of process and handling operations. Bulk density depends not only on the powder’s chemical composition, particle size and moisture but also on its processing and handling history. Powders are known to be compressible and a considerable increase in density can be caused by static pressure, mechanical compaction or the exposure to mechanical vibration (Malav et al., 1985). Moisture content usually has a significant impact on powder flowability. Increasing moisture content leads to reduced flowability due to the increase in liquid bridges and capillary forces acting between the powder particles. Even a free flowing powder can develop flow problems after an extended period of storage. This effect is due to time-consolidation, where a powder consolidates under its own weight over time (Teunou and Fitzpatrick, 2000). The compaction behavior of a given powder obviously determines its flowing characteristics. Stressed powders can be more or less expanded or contracted leading to highly different inter-particle forces. Particle size, shape, and surface morphology are among the important factors associated with the packing ability of powders (Teunou et al., 1999). In studying powder flow properties, in addition to factors determining packing ability of the powder, particle forces associated with these factors should also be taken into consideration (Deleuil et al., 1994). This means powder must be considered as a whole medium that sums up all these interactions at the particle contacts and this will help to consider every factor that can have an effect on these interactions.

A number of studies have been undertaken on flow properties of different cereal flour types. Differences in flow properties of wheat flours due to cultivar type, milling parameters and storage conditions have been reported (Neel and Hoseney, 1984b; Landlillon et al., 2008). Abu-hardan & Hill (2010) studied the flow behavior of maize and wheat grain flours and starches while powder rheology and compaction behavior of rice grain flour was studied by Mukherjee and Bhattachayara (2006). However, this information lacks in grain tef flours.
1.7. Tef utilization and application for food formulations

1.7.1. Flour gel characteristics for application in food gel formulations

Food gels are intermediate between solids and liquids formed from dispersion under various conditions, such as temperature change, chemical modification of gelling agent and adjustment of pH (Kapri and Bhattacharya, 2008). Moreover, the addition of salt and a water-competitive compound, namely sugar, can also be used. The common gelling agents are polysaccharides and proteins obtained from various cereal and pulse grain flours and their combinations. These gelling agents are used to develop several gelled-type traditional food products like porridges throughout the world. They are also used in the preparation of several convenience foods like puddings, custard, pie fillings, confectioneries and sweets along with dairy products. Tef grain flour is also being used as a thickening agent in a range of products, including soups, stews, gravies, and puddings (Seyfu, 1997).

The major macromolecules that contribute to the gelation of cereal grain flour are starch and protein. When starch suspensions are subjected to high temperature gelatinization occurs after the starch granules swell and rupture due to disruption of hydrogen bonds within the starch granules that involves starch granules crystalline melts and amylose leaching. Subsequently, when gelatinized starch is cooled down while the foods stored at low temperature starch gel is formed and then progressed toward starch retrogradation that occurs because of re-crystallization of the polymer (dispersed amylose and amylopectin) chains (Biliaderis, 2009). It is important to distinguish between the short-term development of a gel structure via amylose crystallization and long term reordering of amylopectin which is a much slower process involving recrystallization of the outer branches of this polymer (Kalichevsky et al., 1990). On the other hand, protein gelation is a process involving the initial denaturation of proteins into unfolded polypeptides that under attractive forces and thermodynamic conditions are gradually associated to form the gel matrix. Upon cooling, the uncoiled polypeptides re-associate to make a network. Cross linking may involve multiple hydrogen bonds, ionic attractions, disulfide bonds, hydrophobic associations or a combination of them (Wang and Damoradan, 1991). When all these changes occur, important variations of viscoelasticity and texture of gel can be measured. This helps characterize the gel
formed and predict the suitability of the flour or the proposed formulation for development of products.

Series of studies are usually performed in order to understand the gelling characteristics of starches and flours according to the nature of the gel formed. The assays include determination of thermal and pasting characteristics, least gelation concentration, rheological and textural properties of the gels formed from cereals like rice and wheat grain flours (Kapri and Bhattacharya, 2008; Singh et al., 2011). The structure of a gel or paste formed is dictated by the concentration, the configuration of the swollen starch granule, the amounts and types of amylose and amyllopectin leached out from the granule and the interaction among amylose, amyllopectin and the granule. Effect of the protein type available in the flours could also be visible, i.e., the presence of gluten in the wheat flours which can hinder or partly reduces the contact between the starch granules or leached amylose entanglements affecting gel properties (Rao, 2007). Lipids and non-starch polysaccharides make important contributions to the formation and viscoelasticity of the flour pastes (Blazek and Copeland, 2008). The conditions of heating such as temperature, heating period and rate, are also important. Available information regarding gel from tef is the report by Bultosa et al. (2004) which focused on the texture, syneresis and freeze-thaw stability of tef starch gels from five tef varieties (four Ethiopian grain tef varieties: DZ-01-196, DZ-01-99, DZ-01-1681 and DZ-Cr-37, and one South African grain tef variety: South African Brown). According to Bultosa et al. (2004), the pastes of all tef starches with 10% starch concentration were short textured like maize starch, and on cooling the pastes became gels. At 10% starch concentration the gel firmness of most tef starch varieties were slightly firmer than that of maize starch gels, while their adhesiveness was significantly lower than that of maize starch indicating the intermolecular forces in tef starches are stronger than those in maize. Bultosa et al. (2004) also observed important variation in the firmness of different varieties of tef starches. The syneresis and freeze-thaw stability study in the same report revealed that the syneresis and retrogradation tendency are lower than that of maize starch, which is beneficial for applications where starch staling is preferred to be reduced. However, the above report is focused on textural and short-term retrogradation tendency of the starch only and lacks holistic understanding on the general viscoelastic properties of the gels obtained from the tef grain flours. Therefore,
in depth study on the gelling characteristics of tef grain flour and the quality of gel obtained from tef grain flour will open its use in the development of nutritionally packed and gluten free gel like food formulation.

1.7.2. Tef in breadmaking

In Ethiopia where tef is a staple food, the grain is whole floured and mainly used for making a popular pancake-like local bread with ‘eyes’ called injera (Yetneberk, 2004). The ‘eyes’ of injera are honeycomb-like holes formed in its top surface, which are produced due to the production and escape of carbon dioxide during fermentation and baking. Sometimes the flour is also used for making porridge, kita (unleavened bread), and amit or muk (gruel) (Selinus, 1971). Studies by Zegeye (1997) and Yetneberk et al. (2004) tef grain appeared superior among other cereal grains showed that in its injera making and keeping quality features. In general, white tef types are preferred for injera, but consumption of injera from red- or brown-grained types is also increasing in Ethiopia, especially by health-conscious urban people (Fufa et al., 2011). Kitta, a sweet unleavened flat bread, is also a cultural bread baked from tef (Ketema, 1993).

Application of tef in bread baking can be categorized under wheat based supplementation and gluten free breads. The current consumers’ awareness for healthy goods to get a healthy life has changed their preferences considerably regarding cereal products. Obesity, type-2-diabetes, coronary heart disease and colo-rectal cancer are among the rising challenges of western population, due to changes in both life style and eating behavior (WHO, 2005). Accordingly, the interest of breads for special dietary requirements and increased nutritional value is rising. Tef incorporations in wheat based tef supplemented breads targeted for exploiting nutritional and health benefits described earlier has been evaluated by different authors (Alaunyte et al., 2012; Ben-Fayed et al., 2008; Mohammed et al., 2009). The studies indicate that tef incorporation higher percentages led to deterioration of bread quality. Breads produced with tef flour up to the level of 30% in Ben-Fayed et al. (2008) and up to 20% in Mohammed et al. (2009) studies exhibited low specific volumes and high crumb firmness. In addition, tef breads had significantly lower sensory scores, as only 10% and 5% tef breads had comparable acceptability scores to wheat bread in Ben-Fayed et al. (2008) and Mohammed et al. (2009) studies, respectively. More recently, a combination of enzymes has been used to improve the quality of tef-enriched breads. Significant improvements were achieved in
terms of loaf volume and crumb firmness during storage in both straight dough and sourdough breadmaking processes (Alaunyte et al., 2012).

Potential of tef for gluten free bread development was also evaluated and possibility of bread quality improvements through promoting their protein networks by using enzymes and hydrocolloids (Hager and Arendet, 2013; Renzetti et al., 2008) and sourdough technology (Arendt et al., 2007).

Replacement of wheat in bakery products is a major technological challenge, as the wheat protein gluten is essential for structure-formation. The gluten matrix is a major determinant of the important rheological characteristics of dough, such as elasticity, extensibility, resistance to stretch, mixing tolerance, and gas holding ability. Consequently, dilution or removal of wheat gluten during supplementation and/or substitution at higher amounts in the dough system impairs proper dough development capacity during kneading, leavening and baking (Dubois, 1978). Poorer gluten hydration during mixing due to the fiber components’ ability to bind water (Lai et al., 1989) and physical disruption of the starch-gluten matrix by the bran components in whole meal flours result in lower bread volumes (Gan et al., 1992). Hence, changing the formulation and process condition might be necessary. In line with this reduced gluten hydration due to higher water binding by fibers in whole meal flours affecting dough gas holding capacity and sensory characteristics of the final bread, such as texture, visual appearance and staling behavior could be tackled by adjustment of water level. Studying the rheological properties of wheat doughs supplemented with tef flours can assist much in predicting the machinability, elasticity, extensibility, resistance to stretch, mixing tolerance and the gas holding capacity of the dough and eventually the quality of the baked bread (Dobraszczyk and Morgenstern (2003). Dough rheology studies undertaken in tef incorporated wheat based breadmaking were limited to empirical or descriptive mechanical testing of doughs (Mohammed et al., 2009; Alaunyte et al., 2012). Performing such test by fundamental or basic mechanical methods provides provision for careful control of the geometry of the measured sample and exact measurements of the stress and strain rate, the results obtained in absolute physical units allow direct comparison of results attained by various testing instruments and researchers (Weipert, 1990). Dough stickiness is a major problem in the industry, particularly in large mechanized bakeries, as sticky doughs with poor machinablity, lead
to process disruption and product loss. It is advisable for proper bread making to keep dough stickiness at low levels (Armero & Collar, 1997). However, such important information is missing in tef supplemented wheat based dough matrices.

1.7.3. Other areas of tef applications

The tef grain, owing to its high mineral content has started to be used in mixtures with legumes and other grains in the baby food industry (Seyfu, 1997). Srawdink et al. (2011) demonstrated the good potential grain tef for making good extruded products by developing a protein rich extruded product with desirable attributes from a formula containing 20% tef, 60% corn, and 20% soy protein isolates. Coleman et al. (2013) studied the use of tef flours in biscuits and cake making. Similarly, Hager et al. (2013) demonstrated the possibility of using tef in pasta formulations. The other area of tef utilization is in traditional local alcoholic beverages such as opaque beer called tella, a sprit called katikala/arake, and shameta which are being prepared at household level (Gebremariam et al., 2013b). Tef has also shown good malting properties to be a promising raw material for gluten free brewing (Gebremariam et al, 2013a and b).

In general most grain tef physicochemical characterization and study in product formulation were done either on commercially available grain tef flours or singly on flours obtained from laboratory mill or disc mills (Bultosa, 2007; Hager et al., 2012). Hence, factors associated with different milling methods or mill type utilized on different tef variety type dictating the flour physical and techno-functional properties need to be studied. Unlike the earlier house hold level processing, the growing utilization of tef by the western world where cereal processing is undertaken dominantly in industries and the emerging of companies specializing in tef flour supply and injera baking in Ethiopia (Fufa et al., 2011) will probably lead to tef flour bulk handling. Therefore, availing the information on tef flour bulk handling and storage properties which include flour moisture sorption, flowability and packing characteristics is important.

The majority studies undertaken on application of tef in food formulations so far were not considerate of the available different tef varieties (Alauyante et al., 2012; Coleman et al., 2013, Hager et al., 2013; Srawdink et al., 2011). In addition, the potential of tef in application for gel-like food formulation is not yet explored. The above studies have
confirmed the potential of tef in formulating tef supplemented nutritious and healthy bread making which addresses the current trend of consumers’ demand (Mohammed et al., 2009; Alauyante et al., 2012). However, the major challenge encountered was producing tef supplemented bread with good volume, textural and sensory attributes at higher tef incorporation levels. Hence, further research is required to overcome this challenge via exploiting the techno-functional variation that could be available across tef varieties and exploring different formulations. This should be assisted by studying the viscoelastic and stickiness properties the tef enriched wheat based dough matrices that will help predict the performance of different formulations in advance.
2. OBJECTIVES OF THE THESIS

The general objective of this thesis is studying the engineering characteristics and techno-functional properties of grain tef with special emphasis at exploring its potential in gel like food and bread making applications. In order to accomplish the general goal, the following five studies were developed to fulfill specific objectives given under each study:

2.1. Impact of variety type and particle size distribution on starch enzymatic hydrolysis and functional properties of tef flours: Study I

Effect of the mill type utilized/flour granulation on the flour physical and techno-functional characteristics and starch hydrolysis has not been reported yet. Therefore, this study tried to address this gap by studying impact of variety type mill type on: i) flour particle size distribution, density and color, ii) grain flour structure and starch damage, iii) flour techno-functional properties, iv) in vitro starch digestibility, and (V) proteins characterization by assessing their molecular weight distribution for three grain tef varieties were studied.

2.2. Flowability, moisture sorption and thermal properties of tef [Eragrostis tef (Zucc.) Trotter] grain flours: Study II

The growing demand for products from grain tef due to its nutritional and health benefits is raising the interest of modern food industries and bulk milling, flour handling and processing operations of this cereal grain are inevitable. Therefore, availing the relevant engineering properties necessary for flour bulk handling is important. The specific objectives of this study were: i) Determining the moisture sorption behavior of grain tef flours, ii) Studying the flowability and packing characteristics of tef flours as affected by ambient moisture, and iii) Undertaking differential scanning calorimetry studies to determine the thermal characteristics of tef grain flours.

2.3. Rheological and textural properties of tef [Eragrostis tef (Zucc.) Trotter] grain flour gels: Study III

Studying the gel rheology and its characteristics of tef grain flour from different tef varieties and the properties of the gels formed helps know the potential of tef as an
ingredient in the development of nutritious and healthy gel type products. The specific objectives of this study were: i) Determination of minimum concentration at which gel can be formed from tef grain flours ii) Evaluation of the viscoelastic properties of tef grain flours as a function of concentration and temperature, and iii) Determination of the kinetics of gel textural evolution and color properties of tef grain flour gels with storage time.

2.4. Viscoelastic properties and stickiness of tef grain flour enriched wheat dough matrices: Study IV

Significance of tef variety type and effect of tef incorporation level on the fundamental viscoelastic properties and handling characteristics (stickiness) of tef enriched wheat grain flour dough matrices were assessed and the results were correlated to the bread volume obtained. The specific objectives set under this study were: i) Evaluation of the effect of tef variety type and dose level on the dynamic oscillatory rheological behavior of tef enriched dough, ii) measuring the creep-recovery characteristics of tef enriched flours both in and outside viscoelastic region, and iii) assessing the evolution of dough stickiness with respect to tef dose level and relating the results to the bread volume.

2.5. Suitability of tef varieties in mixed wheat flour bread matrices: a physicochemical and nutritional approach: Study V

Optimal and variety selective incorporation of tef grain flour in wheat could help the exploitation of the nutritional and health benefits obtained from tef grain, without significantly affecting the anticipated bread quality. Thus, specific objectives of this study related the effect of tef incorporation level and variety type on i) bread physical properties, ii) the nutritional composition, iii) starch in vitro digestibility (bread glycemic index), and iv) sensorial quality of breads thereof.
3. WORK PLAN

3.1. Study materials

The studies were undertaken on selected tef grain varieties obtained from the National Tef Improvement Program of the Ethiopian Institute of Agricultural Research (EIAR). The three tef varieties (Fig. 4 and Table 2) were selected based on the year of release, seed color, popularity among the Ethiopian tef grain consumers and the tef farming community (Fufa et al., 2011; Assefa et al., 2013). Rice, whole and refined wheat flours studied as references were supplied by Emilio Esteban SA (Valladolid, Spain).

![DZ-01-99-brown tef, DZ-Cr-37-white tef, DZ-Cr-387-white tef]

Figure 4. Selected grain tef varieties

Table 2. Specifications of the grain tef varieties studied (Source: Assefa et al., 2013)

<table>
<thead>
<tr>
<th>Item No.</th>
<th>Variety name</th>
<th>Common name</th>
<th>Seed color</th>
<th>Year of release</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>DZ-01-99</td>
<td>Asgori</td>
<td>brown</td>
<td>1970</td>
</tr>
<tr>
<td>2</td>
<td>DZ-01-37</td>
<td>Tsedey</td>
<td>white</td>
<td>1983</td>
</tr>
<tr>
<td>3</td>
<td>DZ-01-387</td>
<td>Quncho</td>
<td>white</td>
<td>2006</td>
</tr>
</tbody>
</table>

3.2. Tef grain milling

Tef grains obtained from tef improvement program of EIAR were manually cleaned by siftings and winnowing before milling. Two types of mills were used to mill the three
tef grain varieties in to whole flours. The first one was Cyclotech Sample mill (Foss Tecator, Häganäs Sweden) fitted with a 0.5 mm opening screen size (represented as Mill 1). The Cyclone Sample Mill uses a high velocity air-flow, an abrasive surface, and centrifugal forces to grind material. The impeller rotates at a high speed creating the high velocity flow of air to propel particles against the abrasive surface. The second mill was disc attrition mill (represented as Mill 2) (Danish type stone mill / disc mill) which is the traditionally used in cottage tef grain-milling house (Bishoftu, Ethiopia) to mill tef grain to the fineness level suitable for *injera* making in Ethiopia. Then flours were stored at 4°C after packing in moisture tight polyethylene bags.

### 3.3. Experimental

The five studies were set and conducted in a manner that findings of earlier study are to be used as input for the next. The techniques and equipments employed and analytical approaches followed in the five different studies undertaken are presented in respective material and methods section of each study. Statistical analysis and analytical data modeling undertaken are also described in the material and methods sections of each study where applied.
PRINCIPAL RESULTS AND DISCUSSION
4. PRINCIPAL RESULTS AND DISCUSSION

In this section the main results of the studies and the general discussion are presented. The tables and figures in this section refer to the figures and tables of each particular study where specified.

4.1. Impact of variety type and particle size distribution on starch enzymatic hydrolysis and functional properties of tef flours (Study I)

The protein profiles of the three grain tef cultivars were similar, but different from wheat and rice grain flours analyzed as reference (study I Fig. 1). Flour particle size analysis indicates result the effect of both tef variety and mill type are significant on the granulation and uniformity of flour particle sizes (study I Table 2). Mill 1 (Cyclotech Sample mill fitted with a 0.5 mm opening screen size) gave tef flours particles with significantly higher mean diameter (D$_{50}$) and size dispersion and bulk density (BD) and lower starch damage (SD) than mill 2 (disc attrition mill which is the traditionally used in cottage tef grain-milling house to mill tef grain to the fineness level suitable for injera making in Ethiopia). Tef flours from both mills had higher size dispersions than refined wheat and rice flours indicating the lack in uniformity of their flour particles and this could be due to the continuous sieving processes during industrial milling of the reference flours. SEM results (study I Fig. 2) also verified the presence of a more starch granule pulverization in flours from mill 2 than mill1. The significant effect of variety type on the flour particle sizes has been reported on wheat (Landillon et al, 2008) and related to grain components. DZ-01-99 grain flour exhibited the darkest and most red flour that could be due to polyphenol compounds available in the grain flour (study V Table 5) (Umeta & Parker, 1996).

The BD was affected by both and tef grain variety and mill type because both factors dictated flour particle size corroborating the statement by Brown and Richards (1970) fine particles give loosely packed structure while samples with larger particle size form denser. True density (TD) was more dependent on variety type because it is governed by chemical composition rather particle size. Important variation in DS level was observed within tef cultivars and their DS level negatively correlated with their D$_{50}$ ($r = 0.6, p < 0.05$) agreeing with the conclusion by Lijuan et al. (2007) grinding grains to more finer particle size lead to higher SD. The foaming capacity (FC) of tef flours were
stood between the foaming capacity of refined wheat and rice flour. Foaming stability (FS) tef was much higher than both wheat and rice flours showing their ability to maintain the foam. The FC score of tef flours could indicate their better suitability than rice in gluten-free food systems that require aeration for textural and leavening properties.

Flour hydration properties were significantly affected by both type of tef cultivar and mill type (study I Table 3) where among the tef cultivars flour from DZ-Cr-387 and flours from mill 2 were superior in most hydration properties and oil absorption capacity (OAC) measured. The could be probably the smaller particle size gave higher surface area exposed for water and oil binding with higher level of damaged starch granules that can hydrate readily and also susceptible to amylolytic hydrolysis (Yetneberk et al., 2005). The higher water holding capacity and swelling volume in tef grain flours than refined wheat and rice grain flours could also be could be in part contributed by higher fiber contents in tef flours and variations on the level of starch granules damage and protein contents in tef flours (Santos et al., 2008).

Tef cultivar and mill type markedly influenced the pasting property indices also (study I Table 4). Because differences in flour particle size, hydration properties and the amount of amylase leached out and dissolved flour components visibly affected these indices. Significant correlations (p < 0.05) were obtained between the mean particle size of tef flours and pasting property indices, mainly final and set back viscosities, pasting time and temperature (in all cases r > 0.6). Tef flours had higher water absorption and solubility indexes and swelling power this probably induced higher pasting and break down viscosities (p < 0.05, r ≥ 0.6), and lower final and setback viscosities and pasting time and temperature (p < 0.05, r ≥ 0.6). The remarkably lower setback viscosity in tef flours than in wheat and rice flours indicates the presence of lower amyllose retrogradation in tef confirming the potential of tef in formulation of different food products (Bultosa 2007).

The three grain tef varieties had similar contents of free sugar glucose (FGS), starch fractions, rapidly available glucose (RAG) and starch digestion rate index (SDRI) (study I Table 5). Tef flours from mill 2 had higher starch vulnerability to the attack of digestive enzymes. This could be because of their smaller particle size (giving larger surface area of contact with digestive enzymes) and the higher amount of damaged
starch in them (Li et al. 2014). The lower rapidly digestible starch (RDS) content in tef flour versus rice makes this cereal particularly interesting for celiac patients that frequently suffer diabetes of type I besides the celiac disease. The FSG content of the three tef cultivar flours (1.5% dry basis) was more than three and seven times higher than those of wheat and rice flours respectively. Higher FSG available in tef could probably be the reason why cooked tef grain tends to have molasses-like sweet taste.

4.2. Flowability, moisture sorption and thermal properties of tef [Eragrostis tef (Zucc.) Trotter] grain flours (Study II)

As expected, the flours conditioned at the two water activity (a\textsubscript{w}) levels, a\textsubscript{w} =0.59 and 0.85, gave flowers with a highly significant (p<0.001) different flow and packing properties with concomitant flour moisture content increase (study II Table 2). Because at increased surrounding relative humidity, flours absorb more water molecules which form liquid bridges between powder particles and result in greater powder cohesion and reduced flowability (Gröger et al., 2003). Variation in the flow and packing properties due to a\textsubscript{w} were more pronounced on the tef flours than the reference flours. At both a\textsubscript{w} levels DZ-Cr-387 flour was the most cohesive flour while rice flour was the least. Here effect of the particle size (D\textsubscript{50}) of the flours (before conditioning) was significant (r=−0.67; p<0.05 and r=−0.8; p<0.01, for cohesion coefficient and cohesion index respectively) because smaller flour particle have higher degree of contact area giving the stronger the intermolecular forces (Landillon et al., 2008). At a\textsubscript{w}= 0.59 tef flours had equivalent cake strength among themselves and with refined wheat, while they exhibited lower cake strength than whole wheat and rice flours. However, at a\textsubscript{w}= 0.85, DZ-Cr-387 flour showed again the higher cake strength that the other two varieties and the rice flour while the wheat flours had the highest. On average the compaction coefficient of tef flours was 11% and 36% at a\textsubscript{w} = 0.59 and a\textsubscript{w} = 0.85, respectively higher than the reference flours. Among the tef grain variety flours from DZ-Cr-387 had higher compaction coefficients. This indicates tef flours have higher flow resistance than the reference flours and among the tef cultivars flour higher flow resistance was shown by from DZ-Cr-387.

All flour types had type II sorption isotherm curve, according to the IUPAC classification, which is characteristic of finely divided non-porous solids indicating the occurrence of multi-layer adsorption (Hébrard et al., 2003). The flours of the three tef
varieties led to identical isotherms (study II Fig. 1a) and closer to the reference flours (study II Fig. 1b). Monolayer moisture contents the tef and the reference grain flours were very close. Similar to the findings of Timmermann et al. (2001), monolayer moisture contents calculated by BET model (0.052–0.053 g water/dry solids) were lower than those estimated by GAB (0.057 – 0.058 g water/dry solids) and modified GAB (0.69 – 0.71 g water/dry solids).

Both the gelatinization (of the native flour suspensions) and retrogradation scans (previously gelatinized and stored 7 days at 4 °C) all grain flours showed DSC thermograms with two peaks. The first and second peaks were between 50 - 90 °C and 90 - 115 °C respectively. The first peak could be due to the starch gelatinization/retrogradation, while the second peak could be related to fusion of amylose-lipid complexes formed in the course of the starch gelatinization respectively (Eliasson, 1994). The mean gelatinization temperature of all tef flours was 4 °C below that of rice flour and 8 °C above the wheat flour (study II Table 3 and Fig. 2). The widest gelatinization peak, quantified from the difference \( \Delta T = T_{\text{right}} - T_{\text{left}} \), was obtained for rice flour. DZ-Cr-387 tef flour starches gelatinized had \( \Delta T \) than the rest of tef varieties. The melting enthalpies of the recrystallized amylopectin by retrogradation during storage at 4 °C for 7 days in tef flour starches were about 30% the gelatinization enthalpy with insignificant variations among varieties. However, amylopectin in wheat flour gel retrograded significantly to less extent than that of tef. This was also concluded from gel hardening kinetics studies in the previous work (Abebe & Ronda, 2014) where tef flour gels hardening was faster and the final hardness after 9 storage days at 4°C was markedly higher than wheat flour gels.

4.3. Rheological and textural properties of tef [Eragrostis tef (Zucc.) Trotter] grain flour gels (Study III)

The proximate composition of the tef grain flours were distinct from the reference flours (study III Table I). Significantly higher ash content in tef flours could be because tef grains are whole flours with higher fiber content (Bultosa, 2007, Hager et al., 2012). Among the tef varieties ash content in DZ-Cr.37 was 30% higher than the rest of tef varieties while its starch content was the least. Crude fat in DZ-Cr-384 was significantly higher than the remaining two varieties. Amylose contents in the starches of the three
tef varieties were similar and equivalent with rice flour starch but markedly lower than the wheat flour starch.

Results of the oscillatory measurements on gels (study III, Table 2) indicate that the $\tau_{\text{max}}$, $G'_1$ and $G''_1$ increased with flour concentration and $\tau_{\text{max}}$, $G'_1$ and $G''_1$ values at 25°C were higher than those at 90°C. In addition, the dependence of viscoelastic moduli with concentration fulfilled the power law (study III Table 3). The reason why gels at 25°C were stiffer could be due to amylose retrogradation during cooling down to 25°C (Biliaderis and Juliano, 1993) and formation of hydrogen bonds at lower temperature that stabilize and strengthen the matrix (Acevedo et al., 2013). Except for rice flour values of $(\tan \delta)_1$ decreased with increasing flour concentrations revealing the formation of more strong and elastic the gels. At equivalent concentrations, $G'_1$ and $G''_1$ of the gels from the flours of the three tef varieties and wheat and rice flours exhibited marked differences. This could be attributed to their differences in starch content, shape and size of their starch granules, protein content and types, lipid content and profiles, non-starch polysaccharide types and their contents. Although refined wheat flour had higher starch and protein contents than tef flours, its gels showed lower viscoelastic moduli. This is probably because of the presence of gluten in the wheat flours which hinders or partly reduces the contact between the starch granules or leached amylose entanglements (Rao, 2007). Variation in viscoelastic moduli of tef flours mainly the highest score by DZ-01-99 could be attributed to the presence of higher starch amyllopectin molecular weights (AP-Mw) and radii of gyration (Rg) in DZ-01-99 (Bultosa et al., 2008) where lower AP-Mw was shown to impart low paste viscosity in some wheat starches (Shibanuma et al., 1996). Except for rice the gels the critical gelation concentration of the flours fall between 6-8%.

The gel texture study was performed only on tef and wheat grain flours because of the difficulty in getting undisturbed rice flour gel samples out of RVA canisters. The Avrami model was successfully fitted to the textural evolution of tef gels (study III Fig. 1 and Table 4). The firmness of the gels varied in the order DZ-01-99 < DZ-Cr-387 < DZ-Cr-37 < refined wheat < whole wheat. Lower initial firmness and the lower change during storage in firmness of wheat flour gels could be because of gluten-starch interactions that hinder starch molecules (mainly amyllopectin) reorganize and retrograde (Rao, 2007). The tef gels had closer initial springiness, cohesiveness,
resilience, adhesiveness and gumminess to wheat gels but the values markedly decreased with storage time for tef gels. The calculated half life time ($t_{1/2}$) of firmness and gumminess for refined wheat gel (6 and 9 h respectively) was lower than the tef gels (which ranged 48-58 h and 16-99 h respectively). This shows tef gel textural properties could take longer time to stabilize than wheat gels.

The chromatic coordinates ($a^*-b^*$) and the Lightness ($L^*$) of the gels are depicted in study III Fig. 2. The hue angle (h) of the fresh flour gels varied from the more reddish to the yellow in the order: DZ-01-99 < whole wheat < DZ-Cr-37 < DZ-Cr- 387 < refined wheat. The $L^*$ of the gels followed approximately the same order. The high pigment content in DZ-01-99 led to the darkest and the most reddish gel. DZ-Cr-387 gels showed very similar hue and lightness to refined wheat gel. Though could not be visibly differentiated $L^*$ and $C^*$ of tef gels decreased with storage time and this requires complementary study to chemically quantify the pigment evolution.

4.4. Viscoelastic properties and stickiness of tef grain flour enriched wheat dough matrices: Study IV

The higher amount of water in dough due to dough preparation for ciabatta bread type formulation led to dough matrices with lower $\tau_{\text{max}}$, $G_1'$ and $G_1''$. The probable reason for this the higher amount of water in the doughs. Water acts as inert filler causing the dynamic properties to reduce proportionally to moisture content and also water behaves as a lubricant enhancing the relaxation phenomena (Masi et al., 1998). The $\tau_{\text{max}}$, $G_1'$ and $G_1''$ of tef enriched doughs were significantly ($p<0.05$) lower than the control (study IV Table 2). This may be due to the dilution and breaking of the strong network formed during dough development from the gluten found in wheat. Tef grain flours (10%) tef addition was enough to exert the gluten dilution and the weakening effect of the gluten network. The additional increase of tef dose did not lead to the concomitant decrease of viscoelastic moduli. For whole range of frequencies $G_1'$ was greater than $G_1''$ making $(\tan\delta)_1 < 1$ suggesting a solid elastic-like behavior of the dough formulations. The $c$ exponent, as was reported for $a$ and $b$, also decreased constantly with tef addition levels, meaning the ratio $G_1''/G_1'$ tend to be less dependent on frequency (Ronda et al., 2011). This also showed the lower effect of frequency on the tef-supplemented doughs.
structure (Sivaramakrishanan et al., 2004) on than control. Tef variety type also had significant \((p > 0.05)\) effect on \(G'_1\) and \(G''_1\).

The doughs had typical viscoelastic creep-recovery curves combining both viscous and elastic components (Study IV, Table 3). Both tef incorporation and variety affected creep-recovery parameters. The incorporation of 10\% tef flour to replace wheat made creep phase instantaneous \((J_{0c})\) and retarded \((J_{1c})\) elastic compliances to increase significantly \((28\% \text{ and } 33\% \text{ in LVR}; 53\% \text{ and } 46\% \text{ OLVR})\) with respect to control dough values. At the same tef dose level the increase of compliances in the recovery phase was 66\% and 38\% for \(J_{0r}\) and \(J_{1r}\) respectively with respect to the control dough values. This indicates that tef grain flour enriched doughs had higher instant and retarded deformations when subjected to a constant stress and higher recoveries when the stress was removed. The higher compliances in tef incorporated doughs demonstrate their lower viscous characteristics corroborating the lower \(\tan \delta\) values already reported in the oscillatory tests. However, here also the change in elastic compliances to tef grain flour addition levels was discrete. Higher tef grain flour incorporation \(\geq 20\%\) led to higher adhesive force (stickiness) than the control. However, stickiness did not overpass the 100 g value above which dough handling is a problem (Chen & Hoseney 1995).

Tef grain flour supplemented bread formulations up to 30\% level led breads with significantly higher specific volume than the control wheat flour bread (study IV Fig. 1). The highest effect on volume was obtained with 10\% or 20\% additions \((+12\% \text{ on average})\) depending on the tef variety. The breads loaf volumes with 40\% tef grain flour showed a 2\% loaf volume reduction than the control. Paerson correlation results showed a significant interdependence among the oscillatory and creep-recovery parameters (study IV Table 4). The creep compliances parameters showed strongly significant correlations with recovery phase counterparts \((r > 0.92; \ p<0.01)\). The change in dough rheological properties due to tef incorporation somewhat revealed on the bread volume obtained. Bread volume showed also a highly negative correlation with dough stickiness (adhesive force) \((r=-0.87; \ p<0.01)\).

Dough incorporated with DZ-Cr-37 tef cultivar flour exhibited different rheological characteristics because they had the highest \(G'_1\) and \(G''_1\) average moduli, 14\% higher and significantly lower average elastic compliances \((-23\% \text{ for } J_{nc} \text{ and } J_{or}, -30\% \text{ for } J_{1c}\)}
and $J_{ir}, 33\%$ for $J_{\text{max}}$ in the creep phase, and -23\% for $J_{\text{steady}}$ from recovery phase) than those from the remaining two tef varieties. In agreement with dough characteristics bread volume varied with the incorporated tef variety type in a descending order of DZ-01-99 > Quncho DZ-Cr-387 > DZ-Cr-37.

4.5. Suitability of tef varieties in mixed wheat flour bread matrices: a physico-chemical and nutritional approach: Study V

Incorporation of tef grain flours mainly up to 20\% decreased bread crumb firmness of the breads, while increasing further doses to 30 - 40\% tended to increase the firmness (Study V Table 1). Incorporation of DZ-Cr-37 led to relatively higher bread firmness than DZ-01-99 and DZ-Cr-387. Opposite trend of evolution in the firmness and specific volume of breads was observed with increasing dose tef flour. Because more developed breads enclose a larger amount of air and provide a lower resistance to deformations as the applied texture tests. Tef variety type and amount incorporated did not affect the springiness of crumbs of the breads and the average score ranged between 0.94-0.97. In general tef grain flour incorporated breads had higher baking loss than the 100\% wheat counterpart and baking loss showed increasing tendency with tef dose level. Similar trend was observed by other authors (Ezpeleta and Callejo, 2010) and attributed to the fiber constituents in tef. Bread crumb and crust colors were dependent up on both the incorporated tef variety type and dose level (study V Table 1 and Fig. 1). Bread sensory evaluation revealed that breads with 20\% tef doses had equivalent ratings for organoleptic characteristics closer to the regular wheat bread (100\% wheat) and all types of breads lied in the range of above average rating, i.e., “I somewhat like it” (study V Table 2).

The 40\% tef grain flour enriched breads had significantly higher concentration of all the mineral elements tested than the 100\% wheat control bread (study V Table 3). The average increases observed in Ca, K, P and Zn were of 35\%, 70\%, 48\% and 43\% respectively. The Fe content was much higher in tef-enriched breads than in the control breads, in agreement with the earlier report of Alauyante et al. (2012). In addition, Mg and Mn contents in 40\% tef-enriched breads were 2 and 3 times respectively higher. Except for Fe and Mn, no significant differences were observed in bread micro-element contents due to the variety used. The different contents in the original tef flours from these varieties can explain these results.
Contents of extractable, hydrolyzable and total polyphenols of tef-enriched breads were systematically higher than those of wheat flour breads counterparts (0% tef), regardless of the tef variety and the percent of wheat flour replacement achieved (study V Table 4). In general, increasing dosages of tef flour (from 10 to 40%) promoted the level of extractable polyphenols leading to a concomitant decrease of hydrolyzable polyphenol content except for DZ-01-99 enriched samples. Effect of tef variety on the content of polyphenols was in general discrete. The average ratio between hydrolyzable and extractable phenolic content in the present samples was very similar to the one obtained by Saura-Calixto et al. (2007), for cereal grain flours. The amounts of phenolics fraction and sub-fractions found were substantially higher than expected additive values from the respective flours. This fact can be ascribed to the breadmaking process, mainly through the mixing and baking stages that encompass mechanical and thermal input, respectively that leads some phenolics to be leached out. Both breadmaking steps may favor either depolymerization/unfolding and linkage breaking of insoluble, bound forms and further release, or may increase the accessibility of soluble free compounds and soluble conjugates. The total flavonoids (mg CE/100 g sample, d. b.) contents were higher in bread samples enriched with DZ-01-99 (brown grain tef) (115-155 mg) than the white grain tef varieties (DZ-Cr-37 and DZ-Cr-387) enriched breads (80-100 mg) which are almost similar to the control. No significant change was noted with tef grain flour dose in the incorporation range studied. Health effects of polyphenols depend on both their respective intakes and their bioavailability which can vary greatly. Replacement of wheat flour by increasing amounts of tef flour resulted in either a decline in the absolute level of bio-accessible polyphenols (DZ-Cr-37, DZ-Cr-387) or a reduction in the percentage of bioaccessible compounds with respect to total polyphenol content (DZ-Cr-37, DZ-01-99). This fact can be attributed to a physical/sterical interference by tef flour constituents, particularly dietary fibre, that may hinder the accessibility of pepsin and pancreatin enzymes to achieve gastric and intestinal digestion. It has been stated that other compounds of proven resistance to the action of digestive enzymes, such as resistant starch, resistant protein, Maillard reaction compounds and other associated compounds, may reduce the bread phenol bioaccessibility (Saura-Calixto et al., 2000).
The anti-radical scavenging activity in DZ-01-99 flour (32%) was superior than that of DZ-Cr-37 and/or DZ-Cr-387 (27%) because of its higher flavonoid contents which are well known as good free radical scavengers (study V Table 4). This resulted in a concomitant higher anti-radical scavenging activity in DZ-01-99 blended breads (32-36%) regardless the dose of wheat flour replacement, compared to control wheat flour breads (29%) and DZ-Cr-37 and/or DZ-Cr-387 blended breads (24-32%). For white tef grain flour blended samples, incorporation of tef flour into formulations did not induce/contribute to enhance the anti-radical activity of breads thereof, irrespective of the dose of addition. The observation, can be ascribed, to the changes occurring over breadmaking steps in terms of oxidation of phenolic compounds (particularly for the cinnamic acid derivatives) by coupled reaction due to substantial incorporation of oxygen in the dough during mixing (Eyoum et al., 2003), and to losses or degradation of phenolic compounds during baking (Angioloni and Collar, 2011) as a result of the known susceptibility of phenolic acids and flavonoids to heat and in part contributed by the leaching out of phenolics during dough processing.

The RAG, RDS and SDRI in 40% tef incorporated breads were significantly (p < 0.05) lower than the control while effect of tef variety type was invisible (study V Table 5). The study showed tef grain flours incorporated bread had slower starch digestibility than the control bread. Similar was found in this work on grain tef flours in study I and in other works on studies conducted on tef flour substituted pasta (Hager et al., 2013). The breads incorporated with the three tef varieties had similar contents of FGS, RDS, slowly digestible starch (SDS) and resistant starch (RS), RAG and SDRI. The melting enthalpies at the initial time (H₀) in tef incorporated breads tend to be lower probably because amylose retrogradation is lower in tef (Bultosa, 2007) (study V Table 5). However, at 40% tef grain flour incorporation level the leveling-off melting enthalpy (Hₜ) of DZ-Cr-99 incorporated bread was significantly higher than those of DZ-Cr-37 and DZ-Cr-387 incorporated and the control breads. This may indicate that amylopectin recrystallization in DZ-Cr-99 incorporated breads was higher than the rest of the breads.

Future recommendations
In this thesis some technological characteristics, that are essential to use tef in food processing industries, have been studied on three different grain tef varieties. The flour
handling characteristics studied were limited to a single temperature (20ºC) and granulometry. Effects of temperature and granulometry on handling properties of tef grain flours need to be explored in the future. Tef grain flour physical and techno-functional properties evaluated reveal the importance of grain tef variety and mill type. The characteristics of tef heat-set gels, tef-wheat composite doughs and breads were markedly dependent upon the grain tef variety used. Hence, future studies on the rest of available grain tef varieties could give better understanding on the variations available among them and will help to exploit the diversity. Further investigation on exploring the possible areas of application of tef grain for the development of gluten-free products and nutritious food formulations should also be considered.
GENERAL CONCLUSIONS
5. GENERAL CONCLUSIONS

Based on the results found in the studies undertaken on three tef varieties (DZ-01-99, DZ-Cr-37 and DZ-Cr-387), the following conclusions can be drawn:

- Tef variety and mill type have important effect on flour granulation, bulk density and starch damage, affecting the processing performance of the flours and determining the hydration and pasting properties. Different granulation of grain tef flour induced different in vitro starch digestibility. The disc attrition mill led to higher starch digestibility rate index and rapidly available glucose.

- Tef grain flours are less flowable and their flow and packing properties are more sensitive to water activity changes. Hence, caution must be taken during tef grain flour handling. Tef flours led to sigmoidal sorption isotherms with estimated monolayer water content of 0.053 (BET model) and 0.057 (GAB model) g water/g dry solids.

- Flour starch gelatinization enthalpies of the three tef varieties are equivalent, higher than wheat and similar to rice. The amylopectin recrystallization extent after 7 days of storage at 4°C was significantly higher in tef flour gels than in wheat ones.

- The three tef varieties could be considered suitable food ingredients for the formulation of gel-like foods with different consistencies, textural properties and color depending on the concentration and the tef variety selected.

- Incorporation of tef grain flours affects the structure of the wheat based dough matrices visibly by reducing viscoelastic moduli and the maximum stress they can tolerate before its structure is broken, and increasing dough instantaneous and retarded elastic compliances. Tef incorporation up to 30% gives ciabatta type bread with higher volume than the control bread (100% wheat). Tef incorporation led to breads with enhanced mineral contents and lower starch digestibility rate index. The DZ-01-99 supplemented breads exhibited higher flavonoid content and superior anti-radical activity than regular wheat breads, and those blended with either DZ-Cr-37 or DZ-Cr-387 tef varieties.
In general, the suitability of grain tef as an ingredient of nutritional interest and tolerable technological performance in breadmaking applications and in the formulation of innovative cereal-based foods has been addressed. The importance of the variety and the milling technology on flour engineering and techno-functional properties has also been emphasized.
CONCLUSIONES GENERALES

En base a los resultados de los estudios llevados a cabo en las tres variedades de tef (DZ-01-99, DZ-Cr-37 y DZ-Cr-387), se pueden extraer las siguientes conclusiones:

• La variedad de tef y el tipo de molino usado tienen un importante efecto sobre la granulometría de la harina, la densidad aparente y el almidón dañado, lo que afecta al rendimiento de procesado de las harinas, y a las propiedades de hidratación y de empastado. Diferencias en la granulometría dieron como resultado variaciones en los valores de digestibilidad del almidón in vitro. El uso del molino de discos condujo a un mayor índice de digestibilidad del almidón y glucosa rápidamente disponible.

• Las harinas de tef poseen peores propiedades de flujo y mayor sensibilidad a los cambios de actividad de agua que las harinas de arroz y trigo. Por ello, se debe tener especial cuidado en su manipulación. Las harinas de tef dieron lugar a isotermas de absorción sigmoidales, con valores del contenido de agua monocapa de 0,053 (modelo BET) y 0,057 (modelo GAB) g agua/g sólidos secos.

• Las entalpias de gelatinización de las tres variedades de tef son equivalentes entre sí, similares a las de la harina de arroz y superiores a la del trigo. El grado de recristalización de la amilopectina a los 7 días de almacenamiento a 4ºC es significativamente mayor en geles de tef que en los de trigo.

• La harina de tef ha demostrado ser un ingrediente alimentario adecuado para la formulación de alimentos tipo gel con diferente consistencia, textura y color en función de la concentración y la variedad de tef seleccionada.

• La incorporación de harina de tef a masas panarias de trigo afecta a su estructura, reduciendo los módulos viscoelásticos y el esfuerzo máximo que puede soportar su estructura y aumentando la capacitancia mecánica elástica instantánea y retardada. La incorporación de hasta el 30% de harina de tef en panes tipo chapata dio como resultado panes con mayor volumen que el pan control (100% trigo). La incorporación de tef a los panes mejoró su contenido en minerales y disminuyó la digestibilidad del almidón medida in vitro. Los panes suplementados con DZ-01-99
(tef marrón) mostraron mayor contenido en flavonoides y actividad anti-radical que los elaborados con las restantes variedades y mayores que en el pan de trigo.

En general, se ha estudiado la idoneidad del tef como un ingrediente de interés nutricional con un rendimiento tecnológico adecuado en aplicaciones de panificación y en la formulación de nuevos alimentos a base de cereales. Se ha resaltado la importancia de la variedad y la tecnología de molienda sobre las propiedades ingenieriles y técnico-funcionales del tef.
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70


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Impact of variety type and particle size distribution on starch enzymatic hydrolysis and functional properties of tef flours *

Impact of variety type and particle size distribution on starch enzymatic hydrolysis and functional properties of tef flours

Abstract

Tef grain is becoming very attractive in the Western countries since it is a gluten-free grain with appreciated nutritional advantages. However there is little information of its functional properties and starch digestibility and how they are affected by variety type and particle size distribution. This work evaluates the effect of the grain variety and the mill used on tef flour physico-chemical and functional properties, mainly derived from starch behaviour. *In vitro* starch digestibility of the flours by Englyst method was assessed. Two types of mills were used to obtain whole flours of different granulation. Rice and wheat flours were analyzed as references. Protein molecular weight distribution and flour structure by SEM were also analyzed to justify some of the differences found among the cereals studied. Tef cultivar and mill type exhibited important effect on granulation, bulking density and starch damage, affecting the processing performance of the flours and determining the hydration and pasting properties. The colour was darker although one of the white varieties had a lightness near the reference flours. Different granulation of tef flour induced different *in vitro* starch digestibility. The disc attrition mill led to higher starch digestibility rate index and rapidly available glucose, probably as consequence of a higher damaged starch content. The results confirm the adequacy of tef flour as ingredient in the formulation of new cereal based foods and the importance of the variety and the mill on its functional properties.

**Keywords:** tef; *in vitro* starch digestibility; milling; functional properties
1. Introduction

Tef [Eragrostis tef (Zucc.) Trotter] grain, originated from Ethiopia, is becoming a very attractive cereal in the Western world since it is a gluten-free grain encompassing highly appreciated nutritional advantages. Tef grain size is known to be extremely small with mean length ranging 0.61-1.17 mm and mean width ranging 0.13-0.59 mm, that gives an average thousand kernel weight of 0.264 g (Bultosa, 2007). Tef grain anatomy studies by Parker et al. (1989) and Umeta and Parker (1996) indicate that the embryo, rich in protein and lipid, occupies a relatively large part of the grain. The aleurone layer is one cell thick and rich in protein lipid bodies. The testa is located within the pericarp and its thickness varies with the color of the grain. The testa of red tef is thicker than white tef and it is filled with pigmented material, suggested to be tannins or polyphenolic compounds (Umeta and Parker, 1996). Tef grain is consumed as a whole meal and has more iron, calcium and zinc than other cereal grains, including wheat, barley and sorghum (Abebe et al., 2007). The grain proteins offer an excellent balance among the essential amino acids (Yu et al., 2006). Tef has recently been receiving global attention as a “healthy food”, suitable for its employment in novel foods such as baby foods and gluten-free based goods (Dekking et al., 2005).

Different milling or grinding processes have been shown to produce different flours with different particle size and degree of damage of starch granules in flour, depending on the mechanical forces and temperature during the grinding process (Kadan et al., 2008). The kinetics of starch digestion by alpha amylase of barley and sorghum flours were found to be dependent on the particle size of flours (Al-Rabadi et al., 2009). Starch damage encompasses disruption of the granular structure (Level 5) of the starch (Tran et al., 2011), the extent being dependent on the starch size, botanical source and milling condition (Li et al., 2014). The extent of starch damage is known to affect the quality and functionality of the flours.

In Ethiopia tef is mainly processed to injera after milling with disc attrition mills available in cottage grain mill houses. Injera with much and evenly spread eyes, soft texture, easily rollable and bland after taste is rated as excellent. Intrinsic tef flour quality factors which favor these quality aspects include starch granule characteristics and the higher water solubility index of tef flour which positively influence injera quality (Yetneberk et al., 2005).
The effect of milling method on starch damage, flour physical and functional properties and end product quality for common cereals like wheat and rice is well known (Al-Rabadi et al., 2009; Kadan et al., 2008; Tran et al., 2011). However, despite the nutritional interest and peculiarities of tef grain, information available on the functional properties and starch digestibility and its dependence on grain variety and granulation are still lacking. Therefore, the objective of this research was to identify the influence of two types of mills on the physical and functional properties and the starch digestibility of flours from three Ethiopian tef varieties, to properly assess the end use of tef flours thereof. Protein molecular weight distribution and flour structure by SEM were also evaluated to establish their significance on functional properties.

2. Materials and methods

2.1. Material

Three tef varieties DZ-01-99 (brown tef), DZ-Cr-37 (white tef) and DZ-Cr-387 (Qouncho, white tef) were obtained from the Debre Zeit Agricultural Research Center of the Ethiopian Institute of Agricultural Research (EIAR). Rice flour, whole wheat and refined wheat flours used as references were supplied by Emilio Esteban SA (Valladolid, Spain). The proximal composition of the flours from the tef grains and the reference flours are shown in Table 1. Moisture, ash, fat and protein contents of the flours were determined using methods 44-19, 08-01, 30-25 and 46-11A of AACC (AACC, 2000) respectively. Total carbohydrates were determined by difference to 100% (FAO, 2003). Starch content was determined by Fraser et al. (1956) method and amylose and amylopectin with the Megazyme assay kit (Megazyme Bray, Ireland). All the assays were conducted in duplicate.

2.2. Milling process

The tef grains were manually cleaned by sifting and winnowing before milling. Two types of mills were used to obtain the whole flour of the three tef varieties. The first one was Cyclotech Sample mill (Foss Tecator, Häganäs Sweden) fitted with a 0.5 mm opening screen size (Mill 1). The second mill was a disc attrition mill (Mill 2) which is the type traditionally used in cottage tef grain-milling house (Bishoftu, Ethiopia) to mill tef grain for injera making in Ethiopia. The moisture content levels of the three tef
cultivar grains were equivalent (10.3-10.5%, p > 0.05) and in a normal range for field dried tef grains (Bultosa, 2007).

2.3. Protein characterization

All gels were run in minislabs (Bio-Rad Mini Protean II Model). Sodium dodecyl sulphate (SDS)-PAGE was performed according to Laemmli’s method (1970) using continuous gels (12%). Flour samples (1%, w/v) were dissolved in 0.125 M Tris-HCl, pH 6.8 buffer containing 0.02% (v/v) glycerol, 0.1% (w/v) SDS and 0.05% (w/v) bromophenol blue, and centrifuged at 15800 x g for 5 min at 4ºC. Supernatants were loaded onto the gel (30-40 µg of protein per lane). Samples to be run under reducing conditions were boiled for 1 min in 0.005% (v/v) 2-mercaptoethanol (2-ME) buffer before centrifugation. Electrophoresis was conducted for 1 h at a constant voltage of 200 V. The following molecular weight standards were used to estimate the molecular masses of polypeptides: phosphorylase b (94 kDa); bovine serum albumin (67 kDa); ovalbumin (45 kDa); carbonic anhydrase (30 kDa); trypsin inhibitor (20.1 kDa); α-lactalbumin (14.4 kDa), (Pharmacia Hepar Inc, Franklin, OH, U.S.A).

2.4. Granulation and density of flours

Flour particle size distribution was measured using a Sympatec Particle size and shape analyser (Sympatec GmbH, Germany) using defraction of laser light and controlled by HELOS particle size analysis Window 5 software. The particle size distribution was characterized by the mean diameter (D50) and the dispersion ((D90 –D10)/D50) as described in Landillon et al. (2008). Bulk density (BD) of the flours was determined according to Kaushal et al. (2012). Flour samples were gently poured into previously tared 10 ml graduated cylinders. The final volume reading was taken after vibrating the sample until constant value. Flour true density (TD) was determined by liquid displacement method with toluene as described in Deshpande and Poshadri (2011) by using 50ml pycnometers for the determination.

2.5. Flour Color

A Minolta spectrophotometer CN-508i (Minolta, Co.LTD, Japan) was used for flour color measurements. Results were obtained in the CIE L*a*b coordinates using the D65 standard illuminant, and the 2º standard observer. The hue (h) and the chroma (C*) were
calculated from the equations (1) and (2) respectively. The spectrophotometer was programmed to report an average of 5 measurements.

\[
h = \tan^{-1}\left(\frac{b^*}{a^*}\right) \quad (1)
\]

\[
C^* = \left((a^*)^2 + (b^*)^2\right)^{1/2} \quad (2)
\]

2.6. Damaged starch

The damaged starch content in flour samples was determined in accordance with the American Association of Cereal Chemists (AACC) method (AACC, 2012), by using Megazyme starch damage kit (Megazyme International Ireland Ltd, Co. Wicklow, Ireland). Absorbance was read at 510 nm in a microplate reader BIOTEK EPOCH (Izasa, Barcelona, Spain). The damaged starch was determined as percentage of flour weight on a dry basis. Three replicates were made for each sample.

2.7. Technological functional properties

Foaming capacity (FC) and foam stability (FS) were determined as described by Collar and Angioloni (2014a, 2014b) based on the methods used by Alu’datt et al. (2012). Briefly, 2 g of flour sample was mixed with 40 ml distilled water at 30°C in a 100 ml measuring cylinder. The suspension was stirred and shaken manually for 5 min to produce foam. The volume of foam was measured after 0 min (VT) and 60 min (V1). FC was calculated directly from VT while FS was calculated from 100*(V1/VT).

The water holding capacity (WHC), the amount of water retained by the sample without being subjected to any stress, was determined with slight modification of the method used by Nelson (2001). Samples (2,000g ± 0.005g) were mixed with distilled water (20 ml) and kept at room temperature for 24 h. The supernatant was removed and WHC was measured as grams of water retained per gram of solid. The swelling volume (SV) was obtained by dividing the total volume of the swollen sample by the original dry weight of the sample (Nelson, 2001).

Water absorption capacity (WAC) and oil absorption capacity (OAC) of the flours were determined by the centrifugation method described by Beuchat (1977). Two grams of
flour were mixed with 20 mL of distilled water or corn oil in 50 mL centrifuge tubes. The dispersions were occasionally vortexed while they were held at room temperature for 30 min, followed by centrifugation for 30 min at 3000 x g (Orto Alresa, Spain). The supernatant was removed and weighed and results were expressed as grams of water or oil retained per gram of flour.

Water absorption index (WAI) and water solubility index (WSI) of the flours were measured as described in Kaushal et al. (2012). 2.5 g of flour sample (w0) was dispersed in 30 ml of distilled water, using a glass rod, in tared centrifuge tubes; then cooked at 90°C for 10 min, cooled to room temperature and centrifuged at 3000 x g for 10 min. The supernatant was poured into a pre-weighed evaporating dish to determine its solid content and the sediment was weighed (ws). The weight of dry solids was recovered by evaporating the supernatant overnight at 110°C (ws). WAI, WSI and swelling power (SP) were calculated from the equations:

$$WAI(g/g) = \frac{w_{ss}}{w_0}$$  \hspace{1cm} (2)

$$WSI \ (g/100g) = \frac{w_{ds}}{w_0} \times 100$$  \hspace{1cm} (3)

$$SP \ (g/g) = \frac{w_{ss}}{w_0 - w_{ds}}$$  \hspace{1cm} (4)

2.8. Pasting properties of flours

Pasting properties were studied by using Rapid Visco Analyzer (RVA-4, Newport Scientific Pvt. Ltd, Australia) using ICC standard method 162. Parameters recorded were pasting temperature (PT), peak viscosity (PV), trough viscosity (TV), final viscosity (FV), breakdown viscosity (BV), setback viscosity (SV), and peak time (Pt). RVA parameters were calculated from the pasting curve using Thermocline v. 2.2 software. Analysis was done in triplicate.

2.8. Scanning electron microscopy (SEM)

A Scanning Electron Microscope (SEM) model Quanta 200-F (FEI, Oregon, USA) was used to examine the flours. This microscope was equipped with an X-ray detector and
allowed the analysis of samples of low conductivity without prior metallization. The samples were directly mounted on stubs. Observations were made with an accelerating voltage of 1.5 keV.

2.9. Starch fractions analysis

In vitro starch digestibility was measured according to Englyst et al. (1992), including the latest modifications (Englyst et al., 1999; Ennlyst et al., 2000) as previously applied Ronda et al. (2012). The hydrolysed glucose at 20 min (G20) and 120 min (G120) and the total glucose (TG) were determined by glucose oxidase colorimetric method and with six repetitions for each. The free sugar glucose (FGS) content was also determined through a separate test following the procedure proposed by Englyst et al. (2000). From the above results, rapidly digested starch (RDS) = 0.9*(G20 - FGS), slowly digestible starch (SDS) = 0.9*(G120 - G20), resistant starch (RS) = 0.9*(TG - G120), total starch (TS) = 0.9*(TG - FGS) and rapidly available starch (RAG) = G20 were calculated. Starch digestibility rate index (SDRI) was computed from the percentage of RDS in TS in the flours.

2.10. Statistical analysis

Experimental data were analyzed using two-way analysis of variance (MANOVA) and then means were then compared at p<0.05 using Fisher’s least significant difference (LSD) test. Statistical analysis was done by Statgraphics Centurion XVI program (StatPoint Technologies, Inc. 1982-2010).

3. RESULTS AND DISCUSSION

3.1. Protein characterization

The three tef cultivars showed similar protein profiles which were different from the reference flours (Fig. 1). Under non-reducing conditions, polypeptides of 67- 65, 56, 52, 35, 28, 25 and < 20 kDa were observed in the three tef flours. The polypeptide of 52 kDa (Fig. 1a, arrow 1) was dissociated by 2-ME reducing agent in tef flours while an increase in the intensity of bands between 20 and 30 kDa was observed under reducing conditions (Fig. 1b), denoting the presence of disulfide bridges. Rice showed similar protein profile to tef under non-reducing conditions except two new polypeptides at 32 kDa (arrow 2) and 20 kDa (arrow 3). Under reducing conditions the 32 kDa band increased in intensity and a new polypeptide of 25 kDa appeared. As for most other
cereals, prolamins are major storage proteins in tef (Adebowale et al., 2011). However, protein fractions in tef are less complex than those of wheat, in terms of their apparent molecular size differences, and resemble more the pattern found in maize (Hager et al., 2012; Shewry and Tatham, 1990).

**Table 1:** Chemical composition of tef flours (% on dry basis). Wheat and rice flours were included and considered as references

<table>
<thead>
<tr>
<th>Flour</th>
<th>Moisture (%)</th>
<th>Proteins (% w/w)</th>
<th>Ash (% w/w)</th>
<th>Fat (% w/w)</th>
<th>Carbohydrates (% w/w)</th>
<th>Starch (% w/w)</th>
<th>Amylose (% of starch)</th>
</tr>
</thead>
<tbody>
<tr>
<td>DZ-01-99</td>
<td>10.5±0.1a</td>
<td>8.9±0.3b</td>
<td>2.71±0.19c</td>
<td>2.84±0.08d</td>
<td>85.6±0.6c</td>
<td>75.5±0.1c</td>
<td>21.6±0.3a</td>
</tr>
<tr>
<td>DZ-Cr-37</td>
<td>10.3±0.1a</td>
<td>10.5±0.2c</td>
<td>3.52±0.01d</td>
<td>2.63±0.06c</td>
<td>83.4±0.2b</td>
<td>74.0±0.3b</td>
<td>21.8±0.3a</td>
</tr>
<tr>
<td>DZ-Cr-387</td>
<td>10.4±0.1a</td>
<td>8.9±0.2b</td>
<td>2.63±0.09c</td>
<td>3.24±0.06e</td>
<td>85.3±0.3c</td>
<td>75.5±0.4c</td>
<td>21.1±0.4a</td>
</tr>
<tr>
<td>Wheat</td>
<td>12.1±0.1b</td>
<td>12.7±0.2d</td>
<td>0.69±0.01a</td>
<td>1.47±0.06a</td>
<td>85.1±0.2c</td>
<td>78.8±0.4d</td>
<td>23.2±0.5b</td>
</tr>
<tr>
<td>Rice</td>
<td>12.2±0.1b</td>
<td>7.8±0.3a</td>
<td>0.67±0.01a</td>
<td>1.35±0.04a</td>
<td>90.5±0.3d</td>
<td>87.7±0.4e</td>
<td>21.7±0.1a</td>
</tr>
</tbody>
</table>

Data are the mean ± standard deviation. Values with a letter in common in the same column are not significantly different (p<0.05)
3.2. Granulation and density of flours

Granulation and uniformity of particle size have long been assumed to be important factors affecting the processing performance of flours. The mean diameters of flour particles ($D_{50}$) of tef flours varied significantly (Table 2) in the order $D_{Z01}$ - $D_{Cr}$ - $D_{Cr37}$ < $D_{Z01}$ - $D_{Cr387}$ < $D_{Z01}$ - $D_{Cr37}$ (96.6 μm), noting also significantly higher values for mill 1 (96.2 μm) than mill 2 (93.3 μm). The $D_{50}$ of the tef flours was higher than in wheat flour (56.8 μm) and lower than in rice flour (142.4 μm). However, earlier work on three common wheat flours showed $D_{50}$ values ranging from 64 to 99 μm (Landillon et al., 2008) indicating the high dependence of wheat flour particle size on variety type. The size dispersion of tef cultivar flours (2.32 – 2.36) was notably higher than those of wheat and rice flours. This difference could be attributed to continuous sieving processes during industrial milling of the reference flours. Mill 2 led to significantly lower size dispersion (2.13) than mill 1 (2.55) which shows that the discs mill gave flour of more uniform size. For the three tef cultivars mill 1 generated flours

![Figure 1: SDS-PAGE electrophoresis of flours. a) Non-reducing conditions b) Reducing conditions 1) Molecular weight standards; 2) $D_{Z01}$-99; 3) $D_{Cr}$-37; 4) $D_{Cr387}$; 5) Rice; 6) Refined wheat. Arrow 1: 52 KDa; arrow 2: 32 KDa; arrow 3: 20 KDa; arrow 4: high molecular weight aggregates; arrow 5: 94 KDa.](image-url)
with bimodal particle size distribution (4.5- 150 μm and 150-850 μm). In both D<sub>50</sub> and size dispersion significant (p < 0.01) variety x mill interaction was observed and this could be due to the effect of mill type is less pronounced on D<sub>50</sub> of DZ-Cr-387 and more visible on size dispersion of DZ-01-99.

The bulk densities (BD) and true densities (TD) of the tef cultivar flours showed significant (p < 0.01) variations depending on the variety and the mill. DZ-01-99 flour obtained from the mill 2 had the lowest (Table 2). BD can be used to predict packaging requirements of the flours (Akubor, 2007). Tef flours from mill 1 had significantly (p<0.01) higher mean BD (0.86 g/cm<sup>3</sup>) than those from mill 2 (0.80 g/cm<sup>3</sup>) and the mill type influence being more visible on DZ-Cr-387 than on the other tef cultivars. This could be due to the fact that mill 1 led to flours with higher average particle size than mill 2. The observation, in agreement with the statement by Brown and Richards (1970), can be ascribed to the loosely packed structure in fine particles versus denser aggregated granules found in the samples with larger density. As it could be expected, the type of mill did not affect TD as it is mainly dependent on flour composition but not on particle size.

3.3 Flour color

The average lightness (L*) of grain flours from the three tef varieties varied markedly (p<0.01) in the order DZ-01-99 (67.4) < DZ-Cr-37 (78.0) < DZ-Cr-387(82.4) (Table 2). The hue angle (h) of the tef flours also varied from reddish to the yellowish in the order: DZ-01-99< DZ-Cr-37 <DZ-Cr-387. However, compared with wheat and rice flours they all showed lower L* and h. A similar trend of L* and h was recorded on the gels from the three tef cultivars (Abebe and Ronda, 2014). DZ-01-99 grain flour exhibited the darkest and most red flour that could be due to tannin or polyphenol compounds (Umeta and Parker, 1996). The average chroma (C*) of DZ-Cr-387 (15.2) and DZ-Cr-37 (15.2) grain flours obtained from the two mills were significantly higher than that of DZ-01-99 (13.7) indicating more vivid colors. Rice and wheat flours were paler which could be because they are refined flours or with very little amount of bran components. Among the tef cultivars effect of mill type was not significant only on DZ-Cr-37 flour color. Such effect of mill type could probably be related to degree of breaking and pulverisation of the bran of the tef grains. However, although significant, the flour color differences attributed to the mill could hardly be detected by eye.
3.4. Damaged starch

The damaged starch (DS) determined in tef cultivars varied significantly (p<0.001) with the tef variety and the mill used (Table 2). The mean DS varied with variety in the order DZ-Cr-387 (5.33%) > DZ-01-99 (4.14%) = DZ-Cr-37 (4.02%). Notably higher (p < 0.01) DS was exhibited by mill 2 (5.72%) than mill 1 (3.27%). DS in the tef flours increased with decreasing D₅₀ (r = 0.6, p < 0.05). This agrees with report by Lijuan et al. (2007) stating under the same milling conditions milling to smaller flour particle sizes caused higher DS. Tef variety and mill type interaction effect was also significant (p < 0.01). The level of DS in DZ-Cr-387 flour from mill 1 was much higher than the remaining tef cultivars. DS in tef flours from mill 2 were apparently higher than the DS in wheat flour and lower than DS in rice flour evaluated together.

3.5. Technological functional properties

Technological functional properties are summarized in Table 3. Cultivar and mill type did not show significant (p > 0.05) effect on foaming capacity (FC) and foaming stability (FS) of the tef flours. However, FC values exhibited by the flours from tef cultivars were 1.7 times lower than wheat flours and 1.8 times higher than rice flours. Flour foaming occurs mainly due to a continuous cohesive film formed around the air bubbles in the foam. Similarity in the protein type available in the three tef cultivars and their difference with the reference flours discussed earlier may justify the observed FC’s of the flours (Kaushal et al., 2012). The FC score of tef flours could indicate their better suitability than rice in gluten-free food systems that require aeration for textural and leavening properties. The FS of tef flours was much higher than wheat and rice indicating their ability to maintain the foam. Therefore, tef flour could be a better ingredient in gluten-free food system, such as ice-cream, cakes or toppings and confectionary products, which require aeration for textural and leavening properties.
Table 2. Physical properties of the flours and damaged starch level

<table>
<thead>
<tr>
<th>Variety</th>
<th>Mill</th>
<th>Average particle size</th>
<th>Bulk density</th>
<th>True density</th>
<th>Damaged starch (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>D50</td>
<td>Dispersion (D50-D10)/D50</td>
<td>(g/cm³)</td>
<td>(g/cm³)</td>
<td></td>
</tr>
<tr>
<td>DZ-01-99</td>
<td>1</td>
<td>94.1±0.8b</td>
<td>2.51±0.02d</td>
<td>0.85±0.01b</td>
<td>1.43±0.01ab 1.28±0.28a</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>90.7±0.6a</td>
<td>2.17±0.01c</td>
<td>0.79±0.01a</td>
<td>1.42±0.01a 5.56±14c</td>
</tr>
<tr>
<td>DZ-Cr-37</td>
<td>1</td>
<td>98.4±0.9d</td>
<td>2.58±0.01f</td>
<td>0.87±0.01c</td>
<td>1.47±0.04b 2.43±0.16a</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>94.7±0.1bc</td>
<td>2.14±0.01b</td>
<td>0.81±0.01a</td>
<td>1.46±0.01ab 5.85±0.04c</td>
</tr>
<tr>
<td>DZ-Cr-387</td>
<td>1</td>
<td>95.5±0.6c</td>
<td>2.55±0.03e</td>
<td>0.88±0.01c</td>
<td>1.44±0.01ab 4.91±0.04b</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>94.2±0.5b</td>
<td>2.10±0.01a</td>
<td>0.79±0.01a</td>
<td>1.44±0.01ab 5.75±0.01c</td>
</tr>
<tr>
<td>Wheat</td>
<td></td>
<td>56.8±0.1</td>
<td>1.88±0.01</td>
<td>0.76±0.01</td>
<td>1.42±0.01 5.27±0.28</td>
</tr>
<tr>
<td>Rice</td>
<td></td>
<td>142.7±0.3</td>
<td>1.70±0.01</td>
<td>0.84±0.01</td>
<td>1.43±0.01 6.51±0.57</td>
</tr>
<tr>
<td>Variety</td>
<td></td>
<td>**</td>
<td>**</td>
<td>**</td>
<td>**</td>
</tr>
<tr>
<td>Mill</td>
<td></td>
<td>**</td>
<td>ns</td>
<td>**</td>
<td>ns</td>
</tr>
<tr>
<td>Variety x Mill</td>
<td></td>
<td>*</td>
<td>ns</td>
<td>*</td>
<td>*</td>
</tr>
</tbody>
</table>

Data are the mean ± standard deviation. Values with a letter in common in the same column are not significantly different (p>0.05).
* *, ** and ns indicate the level of significance in the effects of tef variety, mill and their interaction. * p<0.05, ** p<0.01 and ns= not significant (p>0.05).
Where: L*, a*, and b* are CIE coordinates, h = hue and C* = chroma (C*).
Flour hydration properties were significantly affected by both type of tef cultivar and mill type (Table 3). Among the tef cultivars, DZ-Cr-387 had relatively higher mean water holding capacity (WHC), swelling volume (SV), water absorption index (WAI), water solubility index (WSI) and swelling power (SP) while it scored lower mean water absorption capacity (WAC). The wheat and rice flours had notably lower WHC and SV than tef flours. The higher fibre content in tef flours, as whole meal (Collar and Angioloni, 2014b), could also explain its higher water binding capacity with respect to refined wheat and rice flours (Santos et al., 2008). Tef flours from mill 2 also had significantly higher WHC, SV, OAC, WAI, WSI and SP. The probable reason for these results could be the smaller flour particle size of flours from mill 2 giving greater surface area for binding water molecules inducing higher water or oil uptake. The significant negative correlation (p < 0.01, r = 0.7) observed between the D50 of tef flours and their WHC confirms the relationship.

The WAC values of tef flours were apparently higher than wheat flour and lower than the rice flour. WAC has fundamental importance in viscous foods such as soups, sauces, doughs and baked products in which good protein-water interaction is required (Granito et al., 2004) making tef to be a more suitable ingredient than rice in gluten free formulation. Effect of mill type on OAC of the tef flours was significant (p<0.05). Flours from mill 1 had lower OAC (0.83g/g) than those from mill 2 (0.86g/g). The tef flours had apparently similar OAC to the reference flours. Higher OAC in DZ-Cr-387 and DZ-01-99 than DZ-01-37 and in mill 2 than mill 1 can partly be attributed to the lower particle size because oil absorption also depends on the physical entrapment of oil. Flours with high OAC are potentially useful in food products for flavour retention, improvement of palatability and extension of shelf life, mainly in bakery and meat products. High OAC makes the flour suitable in facilitating enhancement in mouthfeel when used in food preparations. Therefore, products from DZ-Cr-387 and DZ-01-99 may better have these quality attributes than DZ-01-37.

The water absorption index (WAI) measures the volume occupied by the gelatinized starch and denatured protein and other components after swelling in excess water maintaining the integrity of starch in aqueous dispersion (Marson and Hoseney, 1986). Compared to wheat and rice flours, the mean values of the WAI of the flours from three tef varieties were apparently lower. WSI of the three tef cultivars was apparently higher
than that of wheat and especially that of rice flours indicating the presence of higher soluble matter content in the tef flours. Tef flours from mill 1 had significantly (p < 0.01) lower WAI, WSI and SP (5.71 g/g, 5.21 g/100 g and 6.02 g/g respectively) than from mill 2 (6.20 g/g, 5.83 g/100 g and 6.58 g/g respectively). The value of WSI positively correlated with DS (r = 0.63, p < 0.05) because damaged granules hydrate readily and are susceptible to amylolytic hydrolysis. Similarly the effect of flour mean particle size was important (p < 0.05 and r = -0.5 to -0.6) on gel hydration properties of the tef flours and this could be due to higher surface area being exposed for water binding. Earlier work by Yetneberk et al. (2005) shows that in sorghum and tef composite flours the WSI increased progressively with increasing proportion of tef, giving injera better quality. The increase in WSI agreed with the observation that, during mixing, compared with sorghum, tef dough tended to be stickier and water-soluble components in the tef flour could have modified the dough rheology and the texture of injera positively (Yetneberk, et al., 2005). In evaluating injera making potentials of sorghum varieties higher WSI gave more fluffy, soft and rollable injera (Yetneberk, 2004). In addition, in flat breads superior quality is associated with wheat flours with high damaged starch content and water absorption (Qarooni et al., 1993). Therefore, based on WSI, starch damage level and water absorption injera from DZ-Cr-387 could be more fluffy, soft and rollable followed by DZ-01-99 and then DZ-Cr-37. At the same time mill 2 seems more suitable for preparation of tef flours for injera.
### Table 3. Functional characteristics of flours.

<table>
<thead>
<tr>
<th>Variety</th>
<th>Mill</th>
<th>FC (mL)</th>
<th>FS (%)</th>
<th>WAC (g/g)</th>
<th>OAC (g/g)</th>
<th>WHC (g/g)</th>
<th>SV (ml/g)</th>
<th>WAI (g/g)</th>
<th>WSI (g/100g)</th>
<th>SP (g/g)</th>
</tr>
</thead>
<tbody>
<tr>
<td>DZ-01-99</td>
<td>1</td>
<td>6.5±2.1</td>
<td>28.8±12.4</td>
<td>0.89±0.02a</td>
<td>0.83±0.02abc</td>
<td>2.07±0.12a</td>
<td>3.10±0.03cd</td>
<td>5.57±0.16a</td>
<td>5.37±0.09bc</td>
<td>5.89±0.17a</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>8.0±0.0</td>
<td>7.5±17.8</td>
<td>1.06±0.02e</td>
<td>0.87±0.04cd</td>
<td>2.15±0.31a</td>
<td>3.05±0.36cd</td>
<td>6.18±0.25bc</td>
<td>6.15±0.41d</td>
<td>6.58±0.24bc</td>
</tr>
<tr>
<td>DZ-Cr-37</td>
<td>1</td>
<td>7.0±1.4</td>
<td>43.8±8.8</td>
<td>1.05±0.02de</td>
<td>0.81±0.01a</td>
<td>2.02±0.27a</td>
<td>2.91±0.2c</td>
<td>5.42±0.08a</td>
<td>4.65±0.08a</td>
<td>5.69±0.09a</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>9.0±2.8</td>
<td>49.4±31.2</td>
<td>1.02±0.02cd</td>
<td>0.82±0.02ab</td>
<td>2.31±0.11a</td>
<td>3.19±0.23d</td>
<td>5.96±0.27b</td>
<td>6.27±0.27b</td>
<td>6.58±0.27b</td>
</tr>
<tr>
<td>DZ-Cr-387</td>
<td>1</td>
<td>9.5±2.1</td>
<td>40.3±31.3</td>
<td>0.96±0.01b</td>
<td>0.87±0.01bcd</td>
<td>2.10±0.16a</td>
<td>3.06±0.01cd</td>
<td>6.13±0.13b</td>
<td>6.49±0.13b</td>
<td>6.49±0.13b</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>9.5±0.7</td>
<td>42.2±3.1</td>
<td>0.99±0.01bc</td>
<td>0.89±0.01d</td>
<td>2.65±0.07b</td>
<td>3.50±0.05e</td>
<td>6.46±0.13c</td>
<td>6.40±0.32d</td>
<td>6.70±0.13c</td>
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<tr>
<td>Wheat</td>
<td></td>
<td>14.0±1.4</td>
<td>28.7±2.9</td>
<td>0.70±0.01</td>
<td>0.85±0.01</td>
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<td>2.27±0.11</td>
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<td>4.41±0.07</td>
<td>7.34±0.07</td>
</tr>
<tr>
<td>Rice</td>
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<td>4.5±2.1</td>
<td>0.0±0.0</td>
<td>1.1±0.01</td>
<td>0.84±0.01</td>
<td>1.78±0.05</td>
<td>2.58±0.13</td>
<td>7.21±0.07</td>
<td>1.70±0.09</td>
<td>6.67±0.10</td>
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<td>ns</td>
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</tr>
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<td>Variety X Mill</td>
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<td>ns</td>
<td>**</td>
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<td>ns</td>
</tr>
</tbody>
</table>

Data are the mean ± standard deviation. Values with a letter in common in the same column are not significantly different (p>0.05).

*, ** and ns indicate the level of significance in the effects of wheat variety, mill and their interaction. * p<0.05, ** p<0.01 and ns= not significant (p>0.05).

FC = foaming capacity, FS = Foaming stability after 60', WAC = water absorption capacity, OAC = oil absorption capacity, WHC = water holding capacity, SV = swelling volume, WAI = water absorption index, WSI = water solubility index and SP = swelling power.
Table 4. Pasting properties of hydrated flours.

<table>
<thead>
<tr>
<th>Variety</th>
<th>Mill</th>
<th>PV (mPas)</th>
<th>TV (mPas)</th>
<th>BV (mPas)</th>
<th>FV (mPas)</th>
<th>SV (mPas)</th>
<th>Peak time (min)</th>
<th>PT (°C)</th>
</tr>
</thead>
<tbody>
<tr>
<td>DZ-99-01</td>
<td>1</td>
<td>1336 ± 23a</td>
<td>858 ± 73b</td>
<td>478 ± 71a</td>
<td>1767 ±155a</td>
<td>908 ± 83ab</td>
<td>8.51 ± 0.10b</td>
<td>75.2 ± 0.8b</td>
</tr>
<tr>
<td>DZ-99-01</td>
<td>2</td>
<td>1344 ± 8a</td>
<td>829 ± 72ab</td>
<td>515 ± 70ab</td>
<td>1690 ±128a</td>
<td>861 ± 56a</td>
<td>8.47 ± 0.11a</td>
<td>74.9 ± 1.2b</td>
</tr>
<tr>
<td>DZ-Cr-37</td>
<td>1</td>
<td>1304 ± 37a</td>
<td>844 ± 12b</td>
<td>461 ± 34a</td>
<td>1957 ±22b</td>
<td>1113 ± 23c</td>
<td>8.73 ± 0.07c</td>
<td>83.1 ± 0.9d</td>
</tr>
<tr>
<td>DZ-Cr-37</td>
<td>2</td>
<td>1317 ± 49a</td>
<td>744 ± 44a</td>
<td>574 ± 10b</td>
<td>1713 ±46a</td>
<td>969 ± 7b</td>
<td>8.51 ± 0.03b</td>
<td>79.4 ± 1.0c</td>
</tr>
<tr>
<td>DZ-Cr-387</td>
<td>1</td>
<td>1618 ± 59b</td>
<td>883 ± 35b</td>
<td>735 ± 24c</td>
<td>1840 ±45ab</td>
<td>956 ± 15b</td>
<td>8.49 ± 0.03b</td>
<td>73.1 ± 0.6a</td>
</tr>
<tr>
<td>DZ-Cr-387</td>
<td>2</td>
<td>1676 ± 67b</td>
<td>823 ± 43ab</td>
<td>853 ± 26d</td>
<td>1701 ±47a</td>
<td>878 ± 17a</td>
<td>8.33 ± 0.01a</td>
<td>71.8 ± 0.2a</td>
</tr>
<tr>
<td>Wheat</td>
<td></td>
<td>2060 ± 19</td>
<td>1192 ± 17</td>
<td>868 ± 6</td>
<td>2512 ±30</td>
<td>1319 ± 13</td>
<td>9.25 ± 0.04</td>
<td>84.9 ± 0.3</td>
</tr>
<tr>
<td>Rice</td>
<td></td>
<td>4023 ± 83</td>
<td>1495 ± 95</td>
<td>2528 ±139</td>
<td>3569 ±56</td>
<td>2075 ±129</td>
<td>9.07 ± 0.01</td>
<td>75.3 ± 0.2</td>
</tr>
</tbody>
</table>

Data are the mean ± standard deviation. Values with a letter in common in the same column are not significantly different (p>0.05).
* *, ** and ns indicate the level of significance in the effects of tef variety, mill and their interaction. * p<0.05, ** p<0.01 and ns= not significant (p>0.05).

PV= pasting viscosity, TV = trough viscosity, BV = breakdown viscosity, FV = final viscosity, SV = set back viscosity, and PT = pasting temperature.
3.5. Pasting properties

Among the tef flours the pasting viscosity (PV) of DZ-Cr-387 (1647 mPa.s) was 20% higher than the equivalent PV of DZ-01-99 and DZ-Cr-37 (Table 4). Trough viscosity (TV) was similar for the three tef varieties, with an average value of 830 mPa s. The mill type influenced the TV of the tef flours in which mill 1 led to the higher value, 862 mPa s versus 799 mPa s of mill 2. The breakdown viscosity (BV) of DZ-Cr-387 (794 mPa s) was about 60% higher than that of the other two varieties. This means that this white tef variety showed the highest disintegration degree of the swollen systems and alignment of amyllose and other linear components in the direction of shear.

Mill 2 led to flours with a mean BV value 16% higher than mill 1. Consequently, flour from mill 1 had higher thermostability and lower shear thinning and disintegration of swollen systems than from mill 2. The BV of wheat flour was similar to that of tef; however, the rice flour BV was 3.5–5 times higher. Hence, the result obtained supports the suggestion by Bultosa (2007) indicating the potential of tef to be used under high shear conditions. Final viscosity (FV) shows the ability of the material to form a viscous paste and it is mainly determined by the retrogradation of soluble amylose in the process of cooling and tef cultivar type did not influence it. However, the effect of mill type was significant where flours from Mill 1 had FV 10% higher than mill 2. Setback viscosity (SV) shows how the viscosity of the paste of the flour suspensions recovered during the cooling period. The average SV of DZ-Cr-37 flour was 18% and 10% higher than that of flours from DZ-01-99 and DZ-Cr-387 respectively. The mill used also affected significantly the SV of the flours and mill 1 led to flours with SV values 10% higher than mill 2. The remarkably lower SV of the tef flours with respect to wheat and rice flours is related to amylose retrogradation and confirm that tef flours retrograde to less extent than other cereals. Such lower retrogradation tendency in the tef flours could make them to be advantageous in formulation of different food products.

The peak time (Pt) and pasting temperature (PT) were also dependent on tef variety. Tef flour from DZ-Cr-37 showed the highest Pt (8.62 min) and PT (83.1 °C) and the results lie in the range reported by Bultosa (2007). The Pt of the tef flours were lower than both wheat and rice flours. Mean Pt and PT of tef flours from mill 1 (8.58 min and 77 °C) were also significantly higher than that of mill 2 (8.44 min and 75 °C).
Significant correlations (p < 0.05) were obtained between the mean particle size of tef flours and its pasting properties, mainly FV, SV, Pt and PT (in all cases r > 0.6). A similar trend was reported for PT and FV of rice flours by Hasjim et al. (2013). Tef flours with higher WAI, WSI and SP tend to have higher PV and BV (p <0.05, r ≥ 0.6) and lower FV, SV, PT and Pt (p <0.05, r ≥–0.6).

3.7. Scanning electron microscopy (SEM)

Like the other cereal species, tef starch is organized to form starch compound granules of the endosperm (Fig. 2). The polygonal shaped starch is clearly seen packed together and protein seems to attach outside of the compound starch granule. In both mill types some of these compound granules were pulverized and individual starch granules are released. However, in mill 2 the starch granule pulverization was more pronounced. Hence, compared to the tef flours from mill 1, tef flours from mill 2 had smaller particle size and closer size distribution and this corroborates the results discussed earlier. In addition both large lenticular starch granules (A-granules) and smaller spherical granules (B-granules) can be observed in wheat. Rice flour particles were the larger having very small polyhedral starch granules.

3.8. Starch fractions and in vitro starch digestibility

The three tef varieties had similar contents of free sugar glucose (FGS), starch fractions (RDS, SDS and RS), rapidly available glucose (RAG) and starch digestion rate index (SDRI) (Table 5). However, the effect of mill type on starch vulnerability to the attack of digestive enzymes was significant: mill 2 led to higher RAG, RDS, and RS and lower SDS. As TS was not dependent on milling SDRI was also higher in flours from mill 2. Li et al. (2014) indicate that damaged starch granules in flour (level 6 structure) have greater enzyme digestibility than intact native starch granules and starch digestibility of flours from milled cereal grains increases with the decreasing flour size. Tef flours from mill 2 have the lower mean particle size and higher starch damage (Table 2).
Table 5. Starch fractions, FSG, RAG and SDRI expressed in % referred to dry matter

<table>
<thead>
<tr>
<th>Variety</th>
<th>Mill</th>
<th>FSG (%)</th>
<th>RAG (%)</th>
<th>RDS (%)</th>
<th>SDS (%)</th>
<th>RS (%)</th>
<th>TS (%)</th>
<th>SDRI (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>DZ-01-99</td>
<td>1</td>
<td>1.48 ± 0.08b</td>
<td>34.3 ± 0.9ab</td>
<td>29.5 ±0.8ab</td>
<td>38.5 ±2.2bc</td>
<td>7.7±1.0bc</td>
<td>75.7±1.0a</td>
<td>39.0±1.0bc</td>
</tr>
<tr>
<td>DZ-01-99</td>
<td>2</td>
<td>1.60 ± 0.06b</td>
<td>34.8 ± 0.8abc</td>
<td>29.9 ±0.7ab</td>
<td>36.2±2.2abc</td>
<td>8.0±1.1bcd</td>
<td>74.1±1.1a</td>
<td>40.7±1.5bcd</td>
</tr>
<tr>
<td>DZ-Cr-37</td>
<td>1</td>
<td>1.18 ± 0.06a</td>
<td>34.0 ± 2.4ab</td>
<td>29.5 ±2.2ab</td>
<td>39.5±2.5bc</td>
<td>6.5±1.1ab</td>
<td>75.6±1.1a</td>
<td>39.7±1.6ab</td>
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<tr>
<td>DZ-Cr-37</td>
<td>2</td>
<td>1.86 ± 0.08c</td>
<td>38.5 ± 1.6c</td>
<td>33.0 ±1.5b</td>
<td>33.0±2.9ab</td>
<td>8.9±2.3cd</td>
<td>74.9±2.3a</td>
<td>44.4±1.6cd</td>
</tr>
<tr>
<td>DZ-Cr-387</td>
<td>1</td>
<td>1.43 ± 0.01b</td>
<td>33.8 ± 2.6a</td>
<td>29.1 ±2.3a</td>
<td>40.8±2.5c</td>
<td>5.7±1.6a</td>
<td>75.7±1.6a</td>
<td>38.5±1.6a</td>
</tr>
<tr>
<td>DZ-Cr-387</td>
<td>2</td>
<td>1.49 ± 0.31b</td>
<td>38.0 ± 1.0bc</td>
<td>32.9 ±0.9b</td>
<td>31.1±2.2a</td>
<td>10.5±1.5d</td>
<td>74.5±1.5a</td>
<td>44.1±1.6d</td>
</tr>
<tr>
<td>Wheat</td>
<td></td>
<td>0.46 ± 0.02</td>
<td>39.6 ± 2.2</td>
<td>35.2 ±2.0</td>
<td>44.1±2.5</td>
<td>2.3±1.2</td>
<td>79.0±1.2</td>
<td>41.9±1.3</td>
</tr>
<tr>
<td>Rice</td>
<td></td>
<td>0.20 ± 0.01</td>
<td>47.4 ± 1.9</td>
<td>42.4 ±1.7</td>
<td>37.4±2.9</td>
<td>8.2±2.9</td>
<td>88.0±2.9</td>
<td>48.3±1.3</td>
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</tbody>
</table>

<table>
<thead>
<tr>
<th>Variety</th>
<th>Mill</th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
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<td></td>
<td></td>
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<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Data are on dry basis and the mean ± standard deviation. Values with a letter in common in the same column are not significantly different (p>0.05). * , ** and ns indicate the level of significance in the effects of tef variety, mill and their interaction. * p<0.05, ** p<0.01 and ns= not significant (p>0.05). RDS = rapidly digestible starch, SDS = slowly digestible starch, RS = resistant starch, TS = total starch, RAG = rapidly available glucose, and SDRI = starch digestion rate index.
Figure 2: SEM Pictures of tef flours studied. M1: Mill 1; M2: Mill 2
4. Conclusions

The protein profiles of the three tef cultivars were similar, but different from wheat and rice analyzed as reference. Tef cultivar and mill type used exhibited important effect on flour granulation and uniformity of particle size, starch damage and densities. These parameters were important factors affecting the processing performance of the flours by determining the absorbed water and dissolved flour components and the pasting properties of tef flours. A lighter product could be obtained from DZ-Cr-387 followed by DZ-Cr-37 and then DZ-01-99. This corroborates the report by Fufa et al. (2011) stating the higher preference and value of DZ-Cr-387 than DZ-Cr-37 giving brighter or whiter injera which is more preferable to Ethiopian consumers. Western consumers, more accustomed to white and refined cereals, could also prefer this variety. Based on WSI, starch damage level and water absorption results, injera from DZ-Cr-387 could be more fluffy, soft androllable followed by DZ-01-99 and then DZ-Cr-37. At the same time compared to the Cyclotech Sample mill used in this experiment the disc mill which is currently being used in Ethiopia for milling tef grain seems more suitable for preparation of tef flours for injera. The results confirm the adequacy of tef flours as ingredients in the formulation of new cereal based foods and the importance of the variety and the mill used on its functional properties. Starch fractions available in the three tef cultivars and indices indicating the in vitro starch digestibility of their flours were equivalent. The effect of damaged starch was more important and tef flours from the disc attrition mill had higher RAG and SDRI. Starch digestibility in the tef flours tended to be lower than the reference flours. Extensively higher FSG in tef may indicate its potential to develop products with different taste.

Acknowledgements

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5. References


Kaushal, P., Kumar, V., Sharma, H.K., 2012. Comparative study of physicochemical, functional, antinutritional and pasting properties of taro (Colocasia esculenta), rice
(Oryza sativa) flour, pigeonpea (Cajanus cajan) flour and their blends. LWT - Food Science and Technology 48, 59-68.


Flowability, moisture sorption and thermal properties of tef \([Eragrostis tef\ (Zucc.) Trotter]\) grain flours

Flowability, moisture sorption and thermal properties of tef [Eragrostis tef (Zucc.) Trotter] grain flours

Abstract

Recently tef is attracting the attention of the modern food industry since it is a gluten-free grain encompassing highly appreciated nutritional advantages. As a relatively new raw material little information on its handling and processing properties is available. Flowability, water sorption characteristics and thermal properties of flours from three Ethiopian tef varieties were studied. Wheat (whole and refined) and rice flours were included as references. Tef flours were less flowable than the reference flours and their flow properties were more sensitive to water activity changes. Tef flours led to sigmoidal sorption isotherms with estimated monolayer water content of 0.053 (BET model) and 0.057 (GAB model) g water/g dry solids. No significant differences among the three tef varieties were observed on their gelatinization enthalpies that were higher than that of wheat and similar to rice. The amylopectin recrystallization extent after 7 days of storage at 4°C was significantly higher in tef than in wheat flour.

Key words:

Tef flour; flowability; sorption isotherm; thermal properties
1. Introduction

Tef [*Eragrostis tef* (Zucc.) Trotter] is traditionally processed at household level and consumed as *injera* (fermented flatbread), sweet unleavened bread, local beverage porridges and soup. Recently tef is attracting the attention of the modern food industry since it is a gluten-free grain encompassing highly appreciated nutritional advantages.

As tef is relatively a new raw material for the modern food industry and it is consumed as whole grain, studies should be conducted to determine its powder behavior during storage, handling and processing. Powder flow properties are vital in different unit operations, such as flow from hoppers and silos, transportation, mixing, compression and packaging. The powder flow and packing characteristics are often investigated by calculating indices which help to characterize them via measuring handling angles, tap testing, shear cell measurements, etc. (Freeman, 2007). At present, powder rheometers are being employed giving repeatable, sensitive and quick measurements with high degree of automation (Freeman, 2007; Abu-hardan & Hill, 2010). Flow and packing properties of a flour system are known to be significantly affected by particle properties (moisture content, particle size and surface roughness), chemical composition, and storage conditions as temperature, exposure to humidity of air, storage time, and consolidation (Fitzpatrick et al., 2004; Abu-hardan & Hill, 2010). Differences in flow properties of wheat flours due to cultivar type, milling parameters and storage conditions have been reported (Landlillon et al., 2008). Abu-hardan & Hill (2010) studied the flow behavior of maize and wheat starches and flours while powder rheology and compaction behavior of rice flour was studied by Mukherjee and Bhattachayara (2006). Tef flours have different physical and chemical properties from other cereal flours like wheat and rice (Abebe and Ronda, 2014) which may affect their flowability behaviors.

Moisture sorption behavior of a flour system is an inherent property that indicates its sensitivity to moisture changes and determines its stability. Therefore, it is considered as an important processing and storage parameter. Flour monolayer content, which gives information about the minimal water content conferring the stability, and isotherm equation, compulsory for evaluating the thermodynamic functions of the water sorbed, are determined by studying sorption behavior. Despite the extensive sorption data for the flours from different cereals and starches (Al-Muhstaseb et al., 2002; Brett et al., 2009) such essential information lacks for tef flour.
Thermal treatment of varying severity and moisture content is the most common process step during cereal processing. Hence, understanding the thermal transitions that cereal components undergo as a result of a change in temperature is important. The main concern here is the transitions of starch in the presence of other components of the system, such as proteins, lipids and other nonstarch carbohydrates, which compete for the available moisture. These components are responsible for specific interactions that produce peculiar phase transitions (Schiraldi et al. 2009). Of the different methods available, differential scanning calorimetry (DSC) has become the most frequently used. When starch suspensions are subjected to high temperature in presence of water, its granules swell and rupture due to the disruption of amylopectin double helices (hydrogen bonds dissociation) while amylose preferentially leaches out from the swollen granules (known as gelatinization) (Biliaderis, 2009). Starch retrogradation or staling, caused by re-crystallization of the polymer (dispersed amylose and amylopectin) chains, occurs in foods when gelatinized starch is cooled down and subsequently stored at low temperature (Jang and Pyun, 1997). The crystallization rate approaches a maximum at a temperature between glass transition and equilibrium melting temperature, \( T_m \), of starch crystallites (Slade and Levine, 1989). These properties are dependent on botanical sources, chemical composition and physical conditions of the flour system. So far gelatinization and retrogradation behavior study in different tef varieties using differential scanning calorimetry (DSC) was limited to the starch component isolated from flour (Bultosa and Taylor, 2003). Pasting properties of 13 tef varieties were also reported (Bultosa, 2007). However, as far as we know, thermal properties of whole tef flour determined by DSC were only limited to the gelatinization temperatures of a commercial tef flour (Hager et al., 2013).

Considering the aforementioned information gaps on tef and its potential in modern food industry, the goal of the present work is to investigate the handling (which include flowability, packing and water sorption characteristics) and thermal properties of flours from three Ethiopian tef cultivars.

2. Materials and methods

2.1. Materials

Three tef varieties DZ-01-99 (brown tef), DZ-Cr-37 (white tef) and DZ-Cr-387 (Qouncho, white tef) obtained from the Debre Zeit Agricultural Research Center of the
Ethiopian Institute of Agricultural Research (EIAR) were whole milled using Cyclotech Sample mill (Foss Tecator, Häganäs Sweden) fitted with a 0.5 mm opening screen size. Rice flour, whole and refined wheat flours used as references were supplied by Emilio Esteban SA (Valladolid, Spain). The initial proximal compositions and physical properties of tef, refined wheat and rice flours are reported in Abebe and Ronda (2014) and Abebe et al. (2015). The initial mean particle diameter (D\textsubscript{50}), size dispersion (\((D\textsubscript{90}-D\textsubscript{10})/D\textsubscript{50}\)), bulk density, true density, and damaged starch level of whole wheat flour, not included in earlier reports, were 84.7\(\mu\)m, 8.36, 0.77 g/cm\(^3\), 1.36 g/cm\(^3\), and 5.38 (%) respectively.

### 2.2. Flour flowability

A powder flow analyzer (PFA) coupled to a texture analyzer, TA-XT 2 Plus (Stable Micro Systems, UK) equipped with Texture Expert Exceed™ operating software (v. 2.03) was used to measure the flowability and the compaction properties of the cereals powders. The instrument was fitted with a 5 kg load cell. Before starting the tests the load cell was calibrated using a standard 2 kg weight. The PFA system, as is detailed elsewhere by Landillon et al. (2008) was constituted by a vertical glass container (120 mm height and 25 mm internal diameter) and a rotating specific blade (23 mm diameter and 10 mm height), which is able to go up and down, in right or left rotation. The flowability properties were evaluated during the displacement in a controlled manner of the rotating blade inside the container, filled with the powder sample. The test vessels were filled with the samples to 30ml level. Before each test the flours were conditioned by the blade of the instrument by slicing and then lifting the flour for two cycles. This helped to remove any loading variation and to normalize the flour after filling or effects of the earlier test. The moisture contents of the flours were adjusted by putting them in vacuumed desiccators containing saturated salt solutions of NaBr or KCl, with water activities (a\textsubscript{w}) of 0.59 and 0.85 respectively at 20 °C. For each test four replicates were performed.

Cohesiveness is the tendency for particles of powder to cling together and agglomerate (form larger clusters of particles). The PFA measures this cohesion characteristic by moving the blade in such a way as to lift the powder. A more cohesive powder will cling to itself and to the blade therefore reducing the force exerted on the base of the vessel. The negative area under the force versus distance curve while lifting is recorded as cohesion coefficient (g. mm). The cohesion index (mm) is calculated by dividing the
cohesion coefficient by the initial weight of the sample. Powder flow speed dependence (PFSD) test helps to assess if the flow properties of a powder change with increasing or decreasing flow speeds. The PFA evaluates this characteristic by measuring the work needed to move the blade through the powder at increasing speeds. As described in Janjatović et al. (2012) the PFSD test has 5 sets of 2 cycles each at increasing speeds (10, 20, 50, 100 mm/s) and then the two final cycles at 10 mm/s. The compaction coefficient (g·mm) is the work required to move the blade down through the powder column using a compacting action. The compaction coefficient at each speed is the positive area under the compaction curve averaged over the two cycles at each speed. An increase in the compaction coefficient as the test speed increases indicates increasing resistance to flow while a decrease would mean that the powder becomes more free flowing. Marginal or no change of the compaction coefficient with flow speed would show that the powder is flow speed independent. Flow stability index is calculated by dividing the compaction coefficient of the last 10 mm/s cycles by the compaction coefficient of the first 10 mm/s cycles.

Caking properties, which indicate the potential of powder to form a compact mass, were determined as stated in Abu-hardan and Hill (2010). In this test 5 compression cycles were performed to form a powder bed and finally the blade or the rotor sliced the cake formed and the force required for cutting through the cake or bed was measured. The average force required to cut the cake was recorded as cake strength (g).

2.3. Sorption isotherm

Approximately 3g of flour samples were transferred to glass vials (diameter 23 mm x height 46 mm and volume 10.5 mL) and dried in oven at 50°C for 24 h. Then the flours were further dehydrated in desiccators with P₂O₅ at room temperature until consecutive weight measurements showed a difference less than 0.001g which took 21 days on average. This was taken as the weight of dry sample. The dried samples were equilibrated in evacuated desiccators over saturated salt solutions of LiBr, CH₃COOK, MgCl₂6H₂O, K₂CO₃, NaBr, NaN₂O₂, NaCl, KCl, and K₂SO₄ at corresponding water activity (a_w) of 0.07, 0.23, 0.33, 0.43, 0.59, 0.64, 0.76, 0.85 and 0.98 respectively, at 20±2°C. At high a_w, above 0.7, crystalline thymol was placed in desiccators to prevent microbial spoilage of the flours as described in Penov et al., (2012). The samples were weighed to monitor water
sorption (with balance sensitivity ± 0.0001). Equilibrium was acknowledged when three daily weight measurements varied less than 0.001g. Before each weighing, all vials were closed with caps after the vacuum was released and kept closed until weighing thereafter until vacuum was re applied to the desiccators. Water content at each a_w was determined in triplicate. BET, Peleg, GAB, and modified GAB (Jayas and Mazza, 1993) models (equations 1 to 4 respectively), were fitted to experimental data of moisture content (MC) versus water activity (a_w). The a_w range to which the moisture content data were fitted for BET model was only 0.1–0.5, while those of Peleg, GAB and modified GAB models was 0.1–0.98 . The mathematical models were chosen taking into account their applicability to starchy products and/or their versatility (Brett et al. 2009).

\[ M = \frac{A \times B \times a_w}{[(1-a_w) \times (1 + (B-1) \times a_w)]} \]  
(1)

\[ M = A \times a_w^C + B \times a_w^D \]  
(2)

\[ M = \frac{A \times B \times C \times a_w}{(1-C \times a_w) \times (1-C \times a_w + B \times C \times a_w)} \]  
(3)

\[ M = \frac{A \times B \times C \times a_w}{(1-C \times a_w) \times (1-C \times a_w + B \times C \times a_w)} + \frac{A \times B \times C \times D \times a_w^2}{(1-C \times a_w) \times (1-a_w)} \]  
(4)

Where: A in equation 1, 3, and 4 represents the monolayer moisture content of the BET, GAB and modified GAB models, B in equations 1, 3 and 4 and C in equations 3 and 4 are energy constants related to the temperature. A, B, C and D are constants of Peleg model (Equation 2) as this model is purely empirical. Linear and nonlinear regression statistical analyses were performed using Statgraphics Centurion XVI program (StatPoint Technologies, Inc. 1982-2010). The quality of the fitting was evaluated by comparing the R^2 and the root mean squared error (RMSE) defined as:

\[ RMSE = \sqrt{\frac{\sum_{i=1}^{n} \left( \frac{M_i - \hat{M}_i}{n-p} \right)^2}{n-p}} \]  
(5)
Where $M_i$ and $\hat{M}_i$ are experimentally observed and predicted by the model values of the equilibrium MC respectively, $n$ is the number of data points, and $p$ is the number of fitted coefficients estimated from the model.

### 2.3. Thermal properties and retrogradation

Thermal characteristics of flours were determined using a differential scanning calorimeter (DSC-822e, Mettler Toledo, SAE). Flour samples were weighed into medium pressure steel pans (120 $\mu$L) and distilled water was added using a micropipette to make 70% moisture content to avoid the effects of water scarcity. Sample weights were about 12 mg. Pans were hermetically sealed and samples were equilibrated for 30 min before scanning. The samples were scanned from 25 to 160°C at 5 °C/min using an empty pan as reference. After heating, the samples were cooled at 20°C/min to 25 °C. The glass transition ($T_g'$) of maximally freeze-concentrated solutes and the onset of ice melting ($T_m'$) temperatures were obtained after annealing of samples as reported by Ronda and Roos (2008). The ice melting temperature of the maximally freeze-concentrated system was measured after annealing of samples for 15 min at a temperature slightly below $T_m'$. Samples were cooled at -20°C/min from 25°C to -80°C, heated again until the annealing temperature at 10°C/min and cooled to -80°C at -20°C/min. The final scan was at 5°C/min to 25°C. The annealing temperatures were -17°C for tef and wheat flours and -14 °C for rice. The $T_m'$ values are the average of at least three determinations. The annealing temperatures were chosen for each system at 2 °C below the onset ice melting temperature, $T_m'$, found in preliminary tests. Starch retrogradation was evaluated in the samples previously gelatinized in the DSC oven stored in the pans at (4±2) °C for 7 days. These samples were scanned from 0 to 150 °C at a heating rate of 5 °C/min. The enthalpy ($\Delta H$), the onset and endset temperatures ($T_o$ and $T_e$) and the peak temperature ($T_p$) were established in both scans, at 0 and 7 days. The reported values are at least the means of triplicate measurements.
<table>
<thead>
<tr>
<th>Cereal</th>
<th>Moisture content (%db)</th>
<th>Cohesion coeff. (g.mm)</th>
<th>Cohesion index (mm)</th>
<th>Mean cake strength (g)</th>
<th>Comp. coeff 10mm/s (g.mm)</th>
<th>Comp. coeff 20mm/s (g.mm)</th>
<th>Comp. coeff 50mm/s (g.mm)</th>
<th>Comp. coeff 100mm/s (g.mm)</th>
<th>Flow stability</th>
</tr>
</thead>
<tbody>
<tr>
<td>DZ-01-99</td>
<td>14.2±0.1bc</td>
<td>348±20d</td>
<td>22.1±1.3d</td>
<td>140.9±0.3a</td>
<td>2525± 68cA</td>
<td>2558±25cA</td>
<td>2654±28cA</td>
<td>2598±1dA</td>
<td>1.00±0.04a</td>
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<tr>
<td>DZ-Cr-37</td>
<td>14.1±0.1bc</td>
<td>305±6c</td>
<td>19.3±0.3c</td>
<td>140.9±2.3a</td>
<td>2715± 65dB</td>
<td>2638±29cAB</td>
<td>2641±34cAB</td>
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<tr>
<td>DZ-Cr-387</td>
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<td>390±30e</td>
<td>24.7±1.9e</td>
<td>145.3±4.5a</td>
<td>2692 ± 36dA</td>
<td>2788±13dB</td>
<td>2861±48dB</td>
<td>2815±21eB</td>
<td>0.99±0.03a</td>
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<td>Refined wheat</td>
<td>14.0±0.1 b</td>
<td>389±14e</td>
<td>30.2±1.1f</td>
<td>148.1±5.6a</td>
<td>2213± 48bA</td>
<td>2266±104bA</td>
<td>2358±73bA</td>
<td>2245±13bA</td>
<td>1.03±0.05a</td>
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<tr>
<td>Whole Wheat</td>
<td>13.7±0.1a</td>
<td>232±21b</td>
<td>16.0±1.4b</td>
<td>467.3±30. 1c</td>
<td>1897± 31aA</td>
<td>1919±86aA</td>
<td>2322± 121bB</td>
<td>2373±83cB</td>
<td>1.05±0.07a</td>
</tr>
<tr>
<td>Rice</td>
<td>14.3±0.1c</td>
<td>180±10a</td>
<td>11.1±0.7a</td>
<td>201.7± 7.9b</td>
<td>2149± 16bD</td>
<td>1994±28aC</td>
<td>1856±9aB</td>
<td>1751±18aA</td>
<td>0.94±0.01a</td>
</tr>
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Flours conditioned with saturated solution of NaBr with aw= 0.59 at 20°C

<table>
<thead>
<tr>
<th>Cereal</th>
<th>Moisture content (%db)</th>
<th>Cohesion coeff. (g.mm)</th>
<th>Cohesion index (mm)</th>
<th>Mean cake strength (g)</th>
<th>Comp. coeff 10mm/s (g.mm)</th>
<th>Comp. coeff 20mm/s (g.mm)</th>
<th>Comp. coeff 50mm/s (g.mm)</th>
<th>Comp. coeff 100mm/s (g.mm)</th>
<th>Flow stability</th>
</tr>
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<tbody>
<tr>
<td>DZ-01-99</td>
<td>19.9±0.1c</td>
<td>583±56b</td>
<td>38.6±3.5cd</td>
<td>404.1±11.9b</td>
<td>4123±159cB</td>
<td>4088±98cB</td>
<td>4052±120cB</td>
<td>3692±144dA</td>
<td>0.99±0.03a</td>
</tr>
<tr>
<td>DZ-Cr-37</td>
<td>19.7±0.1b</td>
<td>559±22b</td>
<td>36.0±1.4c</td>
<td>421.8±9.1bc</td>
<td>4843±199dB</td>
<td>4828±146eB</td>
<td>4616±131eB</td>
<td>4193±91eA</td>
<td>0.98±0.02a</td>
</tr>
<tr>
<td>DZ-Cr-387</td>
<td>20.3±0.1d</td>
<td>686±32c</td>
<td>44.8±2.1e</td>
<td>432.6±13.8c</td>
<td>4316±183cAB</td>
<td>4456±122dB</td>
<td>4346±86dAB</td>
<td>4108±60eA</td>
<td>0.98±0.03a</td>
</tr>
<tr>
<td>Refined wheat</td>
<td>19.1±0.1a</td>
<td>536±20b</td>
<td>41.5±1.5d</td>
<td>530.2±14.3d</td>
<td>3254±29bC</td>
<td>3165±139bBC</td>
<td>3047±70bB</td>
<td>2819±73bA</td>
<td>0.98±0.04a</td>
</tr>
<tr>
<td>Whole Wheat</td>
<td>19.6±0.1b</td>
<td>289±36a</td>
<td>19.6±2.3b</td>
<td>667.8±42.5e</td>
<td>2766±144aAB</td>
<td>2502±130aA</td>
<td>2765±142aAB</td>
<td>3035±167cC</td>
<td>1.04±0.09a</td>
</tr>
<tr>
<td>Rice</td>
<td>20.0 ±0.1c</td>
<td>258±8a</td>
<td>16.4±0.5a</td>
<td>260.8±13.3a</td>
<td>3160±44bD</td>
<td>3004±29bC</td>
<td>2845±34bB</td>
<td>2634±34aA</td>
<td>0.98±0.01a</td>
</tr>
</tbody>
</table>

Data are the mean ± standard deviation. Flours conditioned at aw= 0.59 and aw= 0.85 were compared separately and values with the same small letters in a column at each aw level are not significantly different (p>0.05). Compression coefficients of each flour at the four speeds were compared and values with the same capital letters in a row are not significantly different (p>0.05).
3. Results and discussion

3.1. Flour flowability

The flours conditioned at the two \( a_w \) values, 0.59 and 0.85, had moisture contents of 13.7 – 14.3% and 19.1 – 20.3% on dry basis respectively (Table 1). The effect of \( a_w \) was highly significant (\( p<0.001 \)) in almost all flow and packing properties of all the flours studied. This corroborates that at increased surrounding relative humidity, flours absorb water molecules which form liquid bridges between powder particles and result in greater powder cohesion and reduced flowability (Goger et al., 2003). The tef cultivar flours at \( a_w = 0.59 \) showed significant variations in their cohesive properties among themselves while their caking and flowability properties were equivalent. The cohesiveness of the tef flours followed the order DZ-Cr-37< DZ-01-99< DZ-Cr-387. This could be associated with the crude fat content in the tef flours which varied in the same order (Abebe and Ronda, 2014), because fat promotes flour cohesion by providing the necessary sites on individual four particle surfaces for formation of liquid bridges between particles (Neel and Hoseney, 1984). Compared to the reference flours, at \( a_w = 0.59 \) tef flours were more cohesive than the whole wheat and rice flours and less cohesive than refined wheat flour. However, at \( a_w = 0.85 \), DZ-Cr-387 flour was the most cohesive flour of all flours studied, including the refined wheat flour. Among the important flour physicochemical properties determining flour cohesiveness, effect of the particle size (\( D_{50} \)) of the flours (before conditioning) was significant (\( r=-0.67; p<0.05 \) and \( r=-0.8; p<0.01 \), for cohesion coefficient and cohesion index respectively). Such negative relationship between flour cohesiveness and mean particle size could be because of reduction of particle size that increases cohesion behavior because the particle surface area per unit mass increases, favors a greater number of contact points for interparticulate bonding and additional interactions, resulting in more cohesive and less free flowing powders (Landillon et al., 2008). Similar results were concluded for various flours (Landillon et al., 2008). At \( a_w = 0.59 \) the cake strengths exhibited by the tef flours were equivalent among themselves and with refined wheat flour, while they exhibited lower cake strength than whole wheat and rice flours. The initial flour size dispersion (\( (D_{90} -D_{10})/D_{50} \)), was positively correlated with the strength of the cakes formed (\( r=0.82 \) and \( p<0.01 \)). This could be due to the increase in the powder packing where smaller granules filling the gaps between the larger ones. The cake strength
variation among the flours of the tef cultivars became significant at $a_w=0.85$ being DZ-Cr-387 variety which showed again the highest value.

The compaction coefficients of tef flours with $a_w = 0.59$ were significantly higher (at least 11% higher) than that of wheat and rice ones, independently of the blade speed applied during the test (Table 1). This indicates that tef flours have higher resistance to flow than the reference flours. Tef flour flowability was significantly affected by tef variety type. DZ-Cr-387 cultivar flour was less flowable than the remaining cultivars at speeds above 10mm/s. The effect of speed on the compaction coefficients of tef flours and refined wheat flour were marginal which indicates that its flowability was practically independent on flow speed. However, the compaction coefficients, and consequently the flow resistance, of whole wheat flour increased appreciably with increasing speed while rice flour exhibited the opposite behavior. Flow stability gives important information about the changes in the samples during the test. Flow stability close to 1.00 indicates no significantly change in the sample, while values lower or greater than 1.00 reveals the presence of changes. All the tef cultivar flours tested showed and good flow stability.

The compaction coefficients of the tef flours at $a_w=0.85$ were at least 36% higher than the reference flours indicating their higher resistance to flow at this level of moisture content (Table 1). With the exception of whole wheat flour, the compaction coefficient of all the flours decreased with the increase in flow speed. Hence, at this moisture level the flow resistance of these flours decreased with the increasing flow speed while the whole wheat flour showed the opposite behavior. The flow stability of all the flours tested at $a_w=0.85$ was not significantly different than 1.00. This means that flour particles, even in contact with an atmosphere of high relative humidity as 85%, did not aggregate significantly. However, although not significant differences were attained, the flow stability at $a_w=0.85$ showed lower values than at $a_w=0.85$ indicating that probably some changes (disaggregation) took place in flour particles during the powder flow speed dependence tests.

The effect of water activity and moisture content on packing and flow properties was more marked on tef flours than in the reference ones. The cohesion and compaction coefficient at 10 mm/s increased, as result of the moisture increase, around 75% and 67% respectively for tef meanwhile the reference flours increased only 35% and 45%.
The flow resistance increase with the increase in moisture content was always less important at high speeds. The cohesion and compaction coefficients of all the flours increased with moisture content. This could be due to the act of water as an adhesive between particles by creating liquid bridges that increases the powder cohesivity and packing behavior (Groger et al., 2003). Among tef cultivars, the cohesion and compaction coefficients of DZ-Cr-37 were more dependent on moisture content.

3.2. Moisture sorption isotherms of the flours

Experimental sorption isotherms of the flours of the different tef cultivars and the reference flours at 20°C are presented in Fig. 1. All flour types had type II sorption isotherm curves (according to the IUPAC classification) which is characteristic of finely divided non-porous solids or macro-porous materials indicating the occurrence of multi-layer adsorption (Hébrard et al., 2003). This agrees with earlier reports on various types of starchy materials (Al-Muhstaseb et al., 2002). Three zones in the curve are observed I (0–20% RH), II (20–80% RH) and III (80–95% RH). Zone I represents the monolayer formation; zone II corresponds to the linear portion of the isotherm where the added water will bind with the components of the material forming layers. In zone III, water presents weak binding. In this state water is mobile. The flours of the three tef varieties led to identical isotherms (Fig. 1a) and closer to the reference flours (Fig. 1b). The most marked differences among flours were observed at the highest relative humidity (a_w=0.98). At this point sorbed moisture by the tef flours was 32.1%, 31.4% and 32.4% for DZ-01-99, DZ-Cr-37 and DZ-Cr-387 respectively, notably lower than refined wheat (36.2%) and whole wheat (40.2%) and higher than rice (29.5%). These differences could be due to their different compositions and higher hydration capacity of wheat flour which can be mainly attributed to damaged starch, proteins and pentosans (Hebrard et al., 2003).

Calculated Coefficients form BET, Peleg, GAB and modified GAB models, with relative (R^2) and absolute (RMSE) errors, are reported in Table 3. All models presented a good correlation with R^2 values over 0.99 for all the samples although the modified GAB model led to the best fitting. Using the BET model the estimated monolayer water content of the tef flours ranged 0.052 - 0.053 g water/dry solids. With GAB model the flours from the three tef varieties had equivalent monolayer constant (0.057 g water/dry solids). Like earlier reports (Timmermann et al., 2001) monolayer moisture contents
calculated by BET model (0.052-0.053 g water/dry solids) were lower than those estimated by GAB (0.057 – 0.058 g water/dry solids) and modified GAB (0.69 – 0.71 g water/dry solids). The monolayer values were similar for all the tef flours indicating the water content to saturate the monolayer was independent on tef variety. No important differences were observed with reference flours. In both models the monolayer water contents of the tef flours were in the range normally reported for cereal products (Brett et al., 2009). For all powders GAB model has a limit $0 < C \leq 1$ imposed by the theory behind the GAB equation (Equation 3) and values were consistent with previous reports (Hébrard et al., 2003). This GAB constant represent the multilayer moisture capacity and was identical for the three tef flours (0.84) but higher than rice (0.82) and lower than wheat (0.87 and 0.90 refined and whole respectively). These differences mean the different capacity of water uptake of these flours at 20ºC.

**Figure 1:** Isotherms of DZ-01-99 (●) DZ-Cr-37 (■) and DZ-Cr-387 (▲) tef flours and rice (○), refined wheat (△) and whole wheat (□) flours at 20ºC (absorption). Continuous lines correspond to the modified GAB model fittings of a) tef flours and b) reference flours. The points with standard error bars represent the average of three replicates.
Table 2. Fitting parameters for the models applied to sorption data of the flours

<table>
<thead>
<tr>
<th>Model</th>
<th>$a_w$ applicability range</th>
<th>Constants</th>
<th>DZ-01-99</th>
<th>DZ-Cr-37</th>
<th>DZ-Cr-387</th>
<th>Refined wheat</th>
<th>Whole wheat</th>
<th>Rice</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>BET</strong></td>
<td>&lt;0.5</td>
<td>A</td>
<td>0.053±0.001</td>
<td>0.052±0.001</td>
<td>0.054±0.001</td>
<td>0.052±0.001</td>
<td>0.055±0.001</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>B</td>
<td>16.3±1.6</td>
<td>16.4±1.6</td>
<td>15.8±1.7</td>
<td>16.0±1.6</td>
<td>15.3±1.6</td>
<td>17.6±1.5</td>
</tr>
<tr>
<td></td>
<td>&lt;0.5</td>
<td>$R^2$(RSME)</td>
<td>0.992(0.002)</td>
<td>0.992(0.002)</td>
<td>0.993(0.002)</td>
<td>0.992(0.002)</td>
<td>0.994(0.002)</td>
<td></td>
</tr>
<tr>
<td><strong>Peleg</strong></td>
<td>&lt;0.98</td>
<td>A</td>
<td>0.197±0.008</td>
<td>0.192±0.007</td>
<td>0.202±0.007</td>
<td>0.250±0.007</td>
<td>0.311±0.009</td>
<td>0.169±0.007</td>
</tr>
<tr>
<td></td>
<td></td>
<td>B</td>
<td>0.170±0.007</td>
<td>0.165±0.007</td>
<td>0.165±0.008</td>
<td>0.174±0.006</td>
<td>0.171±0.007</td>
<td>0.162±0.007</td>
</tr>
<tr>
<td></td>
<td></td>
<td>C</td>
<td>10.1±1.1</td>
<td>9.7±1.1</td>
<td>9.1±1.0</td>
<td>11.2±1.0</td>
<td>11.7±0.9</td>
<td>9.0±1.0</td>
</tr>
<tr>
<td></td>
<td>&lt;0.98</td>
<td>$R^2$(RSME)</td>
<td>0.994(0.007)</td>
<td>0.994(0.006)</td>
<td>0.995(0.006)</td>
<td>0.996(0.006)</td>
<td>0.994(0.006)</td>
<td></td>
</tr>
<tr>
<td><strong>GAB</strong></td>
<td>&lt;0.98</td>
<td>A</td>
<td>0.057±0.002</td>
<td>0.057±0.002</td>
<td>0.058±0.002</td>
<td>0.055±0.002</td>
<td>0.052±0.001</td>
<td>0.060±0.002</td>
</tr>
<tr>
<td></td>
<td></td>
<td>B</td>
<td>19.5±5.4</td>
<td>19.7±5.2</td>
<td>18.2±4.4</td>
<td>24.9±8.4</td>
<td>27.1±10.6</td>
<td>21.7±5.7</td>
</tr>
<tr>
<td></td>
<td></td>
<td>C</td>
<td>0.842±0.006</td>
<td>0.840±0.006</td>
<td>0.844±0.005</td>
<td>0.869±0.005</td>
<td>0.895±0.004</td>
<td>0.816±0.007</td>
</tr>
<tr>
<td></td>
<td>&lt;0.98</td>
<td>$R^2$(RSME)</td>
<td>0.992(0.008)</td>
<td>0.992(0.007)</td>
<td>0.994(0.007)</td>
<td>0.992(0.008)</td>
<td>0.991(0.009)</td>
<td>0.991(0.007)</td>
</tr>
<tr>
<td><strong>Modified GAB</strong></td>
<td>&lt;0.98</td>
<td>A</td>
<td>0.071±0.005</td>
<td>0.070±0.005</td>
<td>0.069±0.004</td>
<td>0.071±0.004</td>
<td>0.066±0.004</td>
<td>0.071±0.004</td>
</tr>
<tr>
<td></td>
<td></td>
<td>B</td>
<td>10.7±2.2</td>
<td>11.2±2.2</td>
<td>11.2±2.3</td>
<td>11.4±2.2</td>
<td>11.4±2.3</td>
<td>13.6±2.8</td>
</tr>
<tr>
<td></td>
<td></td>
<td>C</td>
<td>0.736±0.032</td>
<td>0.740±0.030</td>
<td>0.756±0.030</td>
<td>0.740±0.029</td>
<td>0.767±0.028</td>
<td>0.726±0.030</td>
</tr>
<tr>
<td></td>
<td>&lt;0.98</td>
<td>$R^2$(RSME)</td>
<td>0.995(0.006)</td>
<td>0.996(0.006)</td>
<td>0.996(0.006)</td>
<td>0.997(0.005)</td>
<td>0.997(0.006)</td>
<td>0.995(0.006)</td>
</tr>
</tbody>
</table>

Where: $A$ represents the monolayer moisture content of the BET, GAB models, while $B$ and $C$ are energy constants related to the temperature. $A$, $B$, $C$ and $D$ are Peleg model constants. RSME is root mean squared error.
3.3. Thermal properties and retrogradation

The DSC thermograms obtained from both above-zero temperature scans, the first applied to the native flour suspensions (gelatinization scan), and the second obtained from samples previously gelatinized and stored 7 days at 4ºC in the DSC pans (retrogradation scan), showed two peaks and similar characteristics for all flours (Fig. 2). The peak that appeared at the lower temperature range (between 60º –85ºC in the first scan and between 40 - 63ºC in the second one) was related to starch gelatinization/retrogradation while the further endothermic signal (between 90º - 115ºC) could be attributed to the fusion of amylose-lipid complexes formed in the course of the starch gelatinization (Eliasson, 1994). Table 3 summarizes the enthalpies and temperatures of the transitions detected in the gelatinization and retrogradation scans. Tef flour starch showed a gelatinization enthalpy, ΔH_gel, of 14.6 ± 0.5 J/g starch, without significant differences among the varieties. It was similar to that of rice and notably higher than that of wheat flour which scored 9.6 ± 0.3 J/g of starch, equivalent to previously reported data (Eliasson, 1994). The values found in literature for rice were very dependent on the variety type and ranged 8.6-17.1 J/g starch (Biliaderis et al., 1986). In tef and wheat flours a single symmetrical endotherm corresponding to the melting starch crystallites was obtained. However, in rice flour a glass transition occurring at the low-temperature side of the gelatinization endotherm at the leading edge of the first melting peak was observed (Biliaderis et al., 1986). In this study, the mean gelatinization temperature of all tef flours was 4ºC below that of rice flour and 8ºC above the wheat flour. DZ-Cr-387 tef flour gelatinized in a wider range of temperatures than the rest of tef varieties (T_g-T_c=18 ºC versus 16ºC for the remaining flours.

The melting peaks of recrystallized amyllopectin obtained after the flour gelatinization and its storage for 7 days at 4ºC appeared at notably lower transition temperatures than initial gelatinization. The transition temperatures for recrystallized amyllopectin are usually 10–26ºC lower than those for gelatinization of starch granules (Ronda and Roos, 2008). The decrease in the melting temperatures suggested that smaller and/or less perfect crystalline regions were formed (Biliaderis et al., 1986). The melting enthalpies of the recrystallized amyllopectin in tef flours were about 30% the gelatinization enthalpy with insignificant variations among varieties. However, amyllopectin in wheat flour gel retrograded significantly to less extent than that of tef. This was also confirmed
in the gel hardening kinetics studies in the previous work (Abebe & Ronda, 2014) where tef flour gels hardening was faster and the final hardness after 9 storage days at 4°C was markedly higher than wheat flour gels. Opposite was confirmed with respect to amylase retrogradation from rapid visco analyzer tests (Abebe et al., 2015). Tef flour gels showed setback viscosities 47% and 130% lower than wheat and rice gels respectively. This indicates a slower amylase re-crystallization and at the same time a higher amylpectin recrystallization extent in 7 days in tef flours in comparison with wheat flours.

The dissociation of amylase-lipid complex was observed in both the gelatinization and retrogradation scans as it is a reversible transition. It had a mean peak temperature of 98 °C for all flours studied independently of tef variety. The temperature of this transition is coherent with data from literature when the water content in the flour suspension is high enough (>70 %) (Eliasson, 1980). The enthalpies of the amylase-lipid transition measured in the gelatinized suspension after 7 days of storage at 4°C (retrogradation scan) are shown in Table 3. The values corresponding to this second scans were preferred as a better resolution of the two peaks of the thermograms (further apart and of more similar sizes) allowed a better integration (Fig. 2a and 2b). The enthalpy of this transition in tef flour gels was not significantly (p > 0.05) affected by the variety and had a mean value of 0.8 J/g starch (3.7 J/g amylose). The amylase-lipid complex dissociation enthalpy in tef was significantly lower than those in wheat and rice flour gels that were 1.9 and 2.3 J/g starch (8.0 and 10.6 J/g amylose) respectively, despite the lower lipids content of wheat and rice flours (Abebe & Ronda 2014). This could be explained by the nature of the lipids available in the flours that dramatically affects the complex formation extent (Eliasson, 1994). The sub-zero temperature scans carried out on gelatinized samples led to the initial melting temperature of the maximally freeze-concentrated system (Tm') values reported in Table 3 and Fig.2c. As expected for high molecular weight systems (Roos and Karel, 1991), the onset temperature of glass transition of the maximally freeze-concentrated system, Tg', coincided with the Tm'. These data inform about the maximum temperature needed to store flour gels without undesirable changes under frozen conditions. The Tm' value of tef (-15.4±0.4 °C) was independent of the variety. Wheat flour gels showed similar Tm' to tef gels, while rice flour gel had a Tm' four degrees above the tef and wheat flours. Probably, the very low content of soluble solids of rice flour, quantified from its water solubility index, with
Table 3. Thermal properties of the flours

<table>
<thead>
<tr>
<th>Tef variety/ Cereal</th>
<th>Tm’ (°C)</th>
<th>ΔHgel (J/g starch)</th>
<th>Tp-gel (°C)</th>
<th>To-gel (°C)</th>
<th>Te-gel (°C)</th>
<th>ΔHret (J/g starch)</th>
<th>Tp-ret (°C)</th>
<th>ΔHamyl-lipid (J/g starch)</th>
<th>Tp-amil (°C)</th>
</tr>
</thead>
<tbody>
<tr>
<td>DZ-99-01</td>
<td>-15.4 ± 0.3b</td>
<td>14.4 ± 0.5b</td>
<td>72.4 ± 0.2d</td>
<td>65.0 ± 0.4c</td>
<td>81.1 ± 0.5b</td>
<td>4.94 ± 0.86b</td>
<td>51 ± 1.4a</td>
<td>0.72 ± 0.20a</td>
<td>97.0 ± 1.1a</td>
</tr>
<tr>
<td>DZ-Cr-37</td>
<td>-15.2 ± 0.4b</td>
<td>14.8 ± 0.4b</td>
<td>71.1 ± 0.2c</td>
<td>64.8 ± 0.1c</td>
<td>80.3 ± 0.1b</td>
<td>4.47 ± 0.61b</td>
<td>52 ± 1.4a</td>
<td>1.11 ± 0.18a</td>
<td>97.3 ± 1.1a</td>
</tr>
<tr>
<td>DZ-Cr-387</td>
<td>-15.4 ± 0.4b</td>
<td>14.4 ± 0.5b</td>
<td>70.0 ± 0.2b</td>
<td>61.5 ± 0.2b</td>
<td>79.9 ± 0.6b</td>
<td>3.64 ± 0.50b</td>
<td>55 ± 1.2a</td>
<td>0.74 ± 0.18a</td>
<td>96.7 ± 0.9a</td>
</tr>
<tr>
<td>Wheat</td>
<td>-15.6 ± 0.4b</td>
<td>9.6 ± 0.3a</td>
<td>63.9 ± 0.2a</td>
<td>56.1 ± 0.6a</td>
<td>71.0± 0.8a</td>
<td>0.99 ± 0.50a</td>
<td>54 ± 1.2a</td>
<td>1.86 ± 0.18b</td>
<td>95.9 ± 1.1a</td>
</tr>
<tr>
<td>Rice</td>
<td>-11.9 ± 0.3a</td>
<td>13.9 ± 0.8b</td>
<td>75.4 ± 0.2c</td>
<td>68.1 ± 0.4d</td>
<td>83.8 ± 0.8c</td>
<td>2.84 ± 0.87ab</td>
<td>55 ± 1.2a</td>
<td>2.30 ± 0.20b</td>
<td>97.5 ± 0.9a</td>
</tr>
</tbody>
</table>

Tm’: onest temperature of the maximally cryo-concentrated system in the gelatinized sample; ΔHgel: Enthalpy associated to gelatinization; T_o-gel and T_e-gel: onset and endset temperatures of gelatinization peak; T_p-gel, T_p-ret, T_p-amil: T_peak of gelatinization retrogradation and amylose-lipid complex dissociation peaks respectively; ΔH_amyl-lipid: Enthalpy of the dissociation of the amylose-lipid complex; ΔH_ret: Melting enthalpy of the recrystalized amylpectin after storage of the gelatinized sample at 4°C for 7 days. Each data is the average of triplicates ± SD.
Figure 2: Thermograms obtained from the first over-zero temperature scan (gelatinization scan) (a); the second over-zero temperature scan (retrogradation scan) after 7 days of storage at 4°C (b) and the sub-zero temperature scan after annealing of gelatinized samples for the determination of the ice melting temperatures of the maximally freeze-concentrated systems (c).
respect to tef and wheat flours could justify this fact (Abebe et al., 2015) as the presence of higher soluble matter decreases the initial temperature of ice melting in starch gels (Ronda and Roos, 2008).

4. Conclusions

In this study engineering properties of tef flours obtained from three Ethiopian tef cultivars were characterized. As the interest in using tef as row material is increasing, these results could be applied in different food processing industries. Powder rheology measurement results reveal the pronounced effect of air relative humidity on handling and packing properties of tef flours, higher than in the reference flours studied. Slight variations were observed among flow and packing properties of the three tef cultivars themselves, while they had important differences with the reference flours. At both $a_w$ levels the tef flours had more cohesive behavior than rice and whole wheat flours and were less flowable than all the reference flours. At $a_w = 0.59$ the flowability of the tef flours was hardly dependent on the bulk flow speed while at $a_w = 0.89$ their flow resistance decreased with increasing bulk flow speed. Among the flours of the tef varieties DZ-Cr-387 was more cohesive and less flowable.

Like other cereal products the tef flours had a sigmoidal shape sorption behavior with type II isotherms. The estimated monolayer water content of the tef flours were closer to reference flours and were in the range normally reported for cereal products. Hence, similar precaution should be undertaken during handling of these flours.

The DSC thermograms of all types of flours during the gelatinization and retrogradation studies had two peaks and the presence of second endothermic could be attributed to the transition of the amylose-lipid complex. The starch gelatinization enthalpies of the three tef cultivar flour water suspensions were higher than wheat flour while they were equivalent among themselves and with rice flour. From DSC study after storing the gels at 4°C for 7 days it could be concluded that retrogradation in tef flours is higher than wheat flour.
Acknowledgements

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5. References


Rheological and textural properties of tef [*Eragrostis tef* (Zucc.) Trotter] grain flour gels

Rheological and textural properties of tef [Eragrostis tef (Zucc.) Trotter] grain flour gels

Abstract
Interest in tef [Eragrostis tef (Zucc.) Trotter] grain in food applications has increased in recent years because of its nutritional merits and the absence of gluten. With the objective of evaluating the suitability of tef for making gel type food products, gel viscoelastic properties of three varieties of tef (one brown and two white) at different concentrations (6, 8, 10, 12 & 14% w/w) were evaluated at 25°C and 90°C. The texture and color evolution for 16% (w/w) gels were evaluated. Proximate compositions of the flours were quantified. Rice, refined and whole wheat flours were analyzed as reference. The minimum flour concentration required for gel formation from the three tef varieties was 6-8%, similar to wheat flour. All tef flour suspensions pre-heated to 95°C led to gels with a solid-like behavior (G’ > G’’), both at 25°C and 90°C, with higher consistency than wheat gels at the same concentration. The dependence of viscoelastic moduli with concentration fulfilled the power law. Avrami model was successfully fitted to the textural evolution of tef gels. Important differences were observed among tef and rice and wheat flours, probably contributed by their differences in protein, starch, lipid and fibre constituents. Gelling properties characterized suggest that tef flours would be suitable ingredients in gel food formulations.

Keywords: Color; gel rheology; tef grain; texture; viscoelastic properties.
1. Introduction

Tef [Eragrostis tef (Zucc.) Trotter] is originated from Ethiopia where it owes major diversity. It is the major staple food grain in the country and also well utilized in the region of North-Eastern Africa (Cruitis et al., 2008). The whole grain is ground to flour for making injera (fermented flat bread), sweet unleavened bread, local beverage porridges and soup (Bultosa and Taylor, 2004).

Tef grain products are nutritionally well packed because they are always consumed as whole grain rich in carbohydrate and fiber (USDA, 2007), with more iron, calcium and zinc than other cereal grains, including wheat, barley and sorghum (Abebe et al., 2007). The grain proteins offer an excellent balance among the essential amino acids (Yu et al., 2006). Tef has recently been receiving global attention particularly as a “healthy food” due to the absence of gluten and gluten-like proteins, making it suitable for celiac disease patients (Dekking et al., 2005), and also due to other dietary advantages such as slow-release of carbohydrate constituents useful for diabetic patients.

Tef flour has been reported to produce high-quality leavened flatbread that stales much slower than if made from other cereals used to produce gluten-free baked goods and traditional flatbreads (Taylor and Emmambux, 2008; Yetneberk et al., 2005). However, as the majority of the gluten free cereals, western type bakery products from tef have different structure, flavor and sensory properties (Mohammed et al., 2009). This effect is more pronounced as the percentage of tef increases. In view of addressing these problems, effects of different enzymes as xylanase, lipase, amylase, glucose oxidase and proteases (Alaunyte et al., 2011; Renzetti and Aredndt, 2009), and microbial tran-glutaminase (Renzinetti et al., 2008) have been studied. However, tef flour-enriched leavened products seem to have still a sensibly decreased quality in giving wheat bread type baked product. Nevertheless, works have shown a high water absorption capacity of tef flour and a slow retrogradation of the starch, which could have positive impact on shelf life of cereal based products (Bultosa, 2007; Bultosa et. al, 2008).

During processing, manufacture, and consumption of foods, gels are formed and the gelled systems are subjected to large deformations that may cause the food either to deform irreversibly or to fail in fracture (Tabilo-Munizaga and Barbosa-Cànovas, 2005). Hence, in order to develop new foods such as gel-like products from tef and/or to
incorporate it to existing formulations for modifying their functional and nutritional quality, an in depth study on gel properties is compulsory. However, studies on tef undertaken so far were more concentrated on nutritional attributes (Hager, et al., 2012), baking quality (Mohammed et al., 2009; Bultosa, 2007) and physico-chemical or functional characterization of starch extracted from the flour (Bultosa and Taylor, 2004; Bultosa et al., 2008). This study was therefore conducted to characterize tef whole flour gelation capacities and to evaluate viscoelastic, textural and color properties of the gels.

2. Materials and methods

2.1. Material

Three tef varieties DZ-01-99 (brown tef), DZ-Cr-37 (white tef) and DZ-Cr-387 (Qouncho, white tef) were obtained from the Debre Zeit Agricultural Research Center of the Ethiopian Institute of Agricultural Research (EIAR). Rice, whole wheat and refined wheat flours were supplied by Emilio Esteban SA (Valladolid, Spain).

2.2. Preparation of tef flours

Grain tef varieties were manually cleaned by siftings and winnowing before milling. Cyclotech Sample mill (Foss Tecator, Häganäs Sweden) fitted with a 0.5 mm opening screen size was utilized for milling.

2.3. Flour proximate composition

Moisture, ash, fat and protein contents of the flours were determined using methods 44-19, 08-01, 30-25 and 46-11A of AACC (AACC, 2000) respectively. Total carbohydrates were determined by difference to 100% (FAO/WHO, 2003). Starch content was determined by Fraser et al. (1956) method and amylose and amylopectin with the Megazyme assay kit (Megazyme Bray, Ireland). All the assays were conducted in duplicate.

2.4. Oscillation measurements

Dynamic oscillatory rheometry of the gels were carried out with a RheoStress 1 rheometer (Thermo Haake, Karlsruhe, Germany) with parallel plate geometry (60 mm diameter) of serrated surface and with 2 mm gap at 90 and 25 °C. Suspensions of different concentrations (6, 8, 10, 12 and 14%, w/w, of flour with 28.5 g total weight)
were prepared by using the Rapid Visco Analyzer (RVA) (RVA-4 Newport Scientific Pty Limited, Warriewood, Australia). The suspensions were stirred with a constant rotating paddle at 160 rpm, heated from 50 to 95 ºC at a rate of 6 ºC/min and held at 95 ºC for 15 min. Hot paste from the RVA was quickly transferred to the parallel plates for 90 ºC measurement while the remaining portion was kept sealed and used for the 25 ºC measurement undertaken after 25 min. The excess of suspension was removed and to prevent drying at the edge vaseline oil was applied to cover the exposed sample surfaces. Before the measurement, the suspension was allowed to rest for 700 s to allow relaxation. Frequency sweeps were carried out in previously established linear viscoelastic zone, from 10 to 0.1 Hz. Stress sweeps were conducted from 0.1 to 1000 Pa at 1 Hz of frequency. The limit of the linear viscoelastic region (LVR), \( \tau_{\text{max}} \), was located by the decrease of G’ modulus above 10%, that coincided with the sudden increase of \( \tan \delta \). The RheoWin 4 software (Thermo Haake, Karlsruhe, Germany) was used for data analysis. Each gel was prepared twice and measured in duplicate. Frequency sweep data were fitted to the power law model as in previous works (Ronda, et al, 2011):

\[
G'(\omega) = G'_1 \cdot \omega^a
\]

\[
G''(\omega) = G''_1 \cdot \omega^b
\]

\[
\tan \delta(\omega) = \frac{G''(\omega)}{G'(\omega)} = \left( \frac{G''}{G'} \right)_1 \cdot \omega^c = (\tan \delta)_1 \cdot \omega^c
\]

The coefficients \( G'_1, G''_1, \) and \( (\tan \delta)_1 \), stand for the elastic modulus, viscous modulus and the loss tangent at a frequency of 1 Hz. Fittings were done in the frequency range (1-10Hz), where a linear double logarithm curve was systematically obtained. The \( a, b \) and \( c \) exponents quantify the degree dependence of these moduli and the loss tangent with the oscillation frequency, \( \omega \) expressed in Hz.

2.5. Gel texture evolution

The texture properties firmness, adhesiveness, springiness, cohesiveness, and resilience and gumminess of flour gels were evaluated using a TA-XT2 Texture Analyser (Stable Microsystems, Surrey, UK) equipped with the software Texture Expert. Gels were prepared from 16% (w/w) suspensions of flours (28.5 g total weight) by using the RVA (RVA-4 Newport Scientific Pty Limited, Warriewood, Australia). This was the
minimum concentration that led to self-standing gels in all flours tested except rice, where was impossible to get it even at concentrations as high as 20%. The suspensions were stirred with a constant rotating paddle at 160 rpm, heated from 50 to 95 °C at a rate of 6 °C/min and held for 15 min at 95 °C, followed by rapid cooling under cold running water (1 min). The canisters were further cooled in a water bath at 4 °C for 2 h. Subsequently, the samples were stored in the same canisters (hermetically closed to prevent moisture loss) for 0, 4, 8, 24, 48, 96 and 192 h at 4 °C and evolution of gel texture properties namely firmness, adhesiveness, springiness, cohesiveness, gumminess and resilience with time were evaluated. The samples in canisters were equilibrated to room temperature for 1 h before analysis. Measurements were done on gels 2 cm diameter and 2 cm of height. A double compression test (TPA) with an aluminium 75 mm diameter (SMSP/75) probe and 1 mm/s speed was used. A 50% deformation was chosen to avoid the total destruction of the gel structure in the first compression of TPA tests. Larger deformations usually applied (75-80%) could crush the samples and lead to invalid textural results (Huang et al., 2007). Results are the average of four replicates carried out on four different gels of each flour.

The data of texture evolution with time was fitted with Avrami equation as described in Acevedo et al. (2013):

\[
\frac{P_\infty - P_t}{P_\infty - P_0} = e^{-kt^n} \tag{4}
\]

Where \(P\) represents the firmness, springiness, cohesiveness, resilience or gumminess at initial time \((P_0)\), at infinite time, leveling-off value, \((P_\infty)\) and the value at any time \(t\), \((P_t)\). \(k\) is a constant of velocity and \(n\) is the Avrami exponent. The values of the constants \(k\) and \(n\) were used to calculate the value of half-life, \(t_{1/2}\), which is defined as the time required to achieve 50% of leveling-off extent of the texture parameter:

\[
t_{1/2} = \left( -\frac{\ln 0.5}{k} \right)^{1/n} \tag{5}
\]

2.6. Gel color

Gel color was measured at 0, 4, 8, 24, 48, 96 and 192 h storages at 4 °C on the remaining samples used to evaluate the gel texture properties described in section 2.5 using Minolta spectrophotometer CN-508i (Minolta, Co. LTD, Japan). Results were
obtained in the CIE L\textsuperscript*a*b* coordinates using the D65 standard illuminant, and the 2°
standard observer. The hue (h) and the chroma (C\text{*}) were calculated from them with the
equations $h = \tan^{-1}(b^*/a^*)$ and $C^* = ((a^*)^2+(b^*)^2)^{1/2}$. L\text{*} ranges from 0 (black) to 100
(white). The hue scale extends from 0° (red), 90° (yellow), 180° (green) to 270° (blue).
The chroma informs about the purity of the colour: a near zero C\text{*} value corresponds to
a colour of low purity, near grey. On the opposite high C\text{*} values mean colors of high
purity near the pure spectral colors. The spectrophotometer was programmed to report
an average of 5 measurements. The difference of color was calculated from
$$\Delta E = ((\Delta a^*)^2 + (\Delta b^*)^2 + (\Delta c^*)^2)^{1/2}.$$ 

2.7. Statistical analysis
Experimental data were analyzed using one-way analysis of variance (ANOVA) and
then means were then compared at p<0.05 using Fisher’s least significant difference
(LSD) test. Statistical analysis and non linear (Avrami model) regression studies were
done by Statgraphics Centurion XVI program (StatPoint Technologies, Inc. 1982-2010).

3. Results and discussion
3.1. Flour proximate composition
The moisture contents among the three varieties of tef flours were not significantly
(p > 0.05) different and were appreciably lower than the moisture contents in wheat and rice
flours (Table 1). A similar range was reported by Bultosa (2007) for thirteen varieties
describing to be in normal range for field dried tef. The protein contents of the three tef
flours were significantly (p < 0.05) lower than the two wheat flours and significantly
higher than rice. Among the tef cultivars, DZ-Cr-37 had the highest (p < 0.05) protein
content.

The tef flours had significantly higher ash content than the other flours including the
whole wheat flour. Among the tef cultivars, DZ-Cr-37 had a 30% markedly higher ash
content than the remaining two. In whole wheat, it is well established that bran is richer
in minerals than endosperm (Fiellet and Dexter, 1996). The higher tef flour ash content
could also be due to its higher fiber content as tef flour comes from whole grain. The
higher contribution of bran to the whole tef grain flour composition could be explained
by the smaller size of tef grain (Bultosa, 2007) which gives higher surface area of bran
per unit amount of grain, in comparison with whole wheat. The fat contents of the three
tef cultivars were also significantly higher than the rest flours. A significant ($p < 0.05$) variation was observed among the crude fat contents of tef varieties in the order DZ-Cr-384 > DZ-01-99 > DZ-Cr-37. The germ, which is totally included in tef flour, could explain its higher fat content. The available total carbohydrate in the tef cultivars was significantly ($p < 0.05$) lower than that of rice and higher than in whole wheat flour. DZ-Cr-37 had significantly lower total carbohydrate than the other tef varieties and refined wheat. The starch content of the cereals ranged between 66.9% (for whole wheat flour) and 87.7% (rice flour). The starch contents of the three types of tef were significantly higher than that of the whole wheat flour and lower than both refined wheat and rice flours. Among the tef cultivars starch content in DZ-Cr-37 flour was significantly ($p < 0.05$) lower than the other two cultivars. The amylose contents in the starches of the three types of tef flours did not differ significantly, resulting closer to rice flour starch. However, starches from the two types of wheat flours had significantly ($p < 0.05$) higher amylose contents than the three tef varieties and rice flours.
**Table 1:** Chemical composition of three tef, wheat (whole and refined) and rice grain flours (% on dry basis, except amylose)

<table>
<thead>
<tr>
<th>Flour</th>
<th>Moisture (%)</th>
<th>Proteins (% w/w)</th>
<th>Ash (% w/w)</th>
<th>Fat (% w/w)</th>
<th>Carbohydrates (% w/w)</th>
<th>Starch (% w/w)</th>
<th>Amylose (% of starch)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tef-brown (DZ-01-99)</td>
<td>10.5±0.1a</td>
<td>8.9±0.3b</td>
<td>2.71±0.19c</td>
<td>2.84±0.08d</td>
<td>85.6±0.6c</td>
<td>75.5±0.1c</td>
<td>21.6±0.3a</td>
</tr>
<tr>
<td>Tef-white (DZ-Cr-37)</td>
<td>10.3±0.1a</td>
<td>10.5±0.2c</td>
<td>3.52±0.01d</td>
<td>2.63±0.06c</td>
<td>83.4±0.2b</td>
<td>74.0±0.3b</td>
<td>21.8±0.3a</td>
</tr>
<tr>
<td>Tef-white (DZ-Cr-387)</td>
<td>10.4±0.1a</td>
<td>8.9±0.2b</td>
<td>2.63±0.09c</td>
<td>3.24±0.06e</td>
<td>85.3±0.3c</td>
<td>75.5±0.4c</td>
<td>21.1±0.4a</td>
</tr>
<tr>
<td>Wheat (refined)</td>
<td>12.1±0.1b</td>
<td>12.7±0.2d</td>
<td>0.69±0.01a</td>
<td>1.47±0.06a</td>
<td>85.1±0.2c</td>
<td>78.8±0.4d</td>
<td>23.2±0.5b</td>
</tr>
<tr>
<td>Wheat (whole)</td>
<td>11.9±0.1b</td>
<td>14.6±0.1e</td>
<td>1.66±0.02b</td>
<td>1.93±0.02b</td>
<td>81.8±0.1a</td>
<td>66.9±0.5a</td>
<td>24.6±0.4b</td>
</tr>
<tr>
<td>Rice</td>
<td>12.2±0.1b</td>
<td>7.8±0.3a</td>
<td>0.67±0.01a</td>
<td>1.35±0.04a</td>
<td>90.5±0.3d</td>
<td>87.7±0.4e</td>
<td>21.7±0.1a</td>
</tr>
</tbody>
</table>

Data are the mean ± standard deviation. Values with the same letter in a column are not significantly different (p > 0.05).
3.2. Viscoelastic Properties of the gels

Viscoelastic behavior of gels formed from the flour suspensions with different concentrations measured at 90°C and 25°C are shown in Table 2. Effects of flour type, concentration and temperature were significant (p < 0.05) on the maximum stress (τ\text{max}) applicable within LVR. τ\text{max} beyond which the gel structure is broken, increased with concentration and decreased with temperature (Table 2). Similar trends were reported for legume flour gels by Acevedo et al. (2013). Cereal type also had significant effect on τ\text{max} at 25°C and concentrations ≥10%. τ\text{max} increased in the order: whole-wheat < rice < refined-wheat = DZ-Cr-37 < DZ-Cr-387 < DZ-01-99. Tef gels showed a firmer and more stable structure than rice and whole wheat gels. At low concentrations, 6-8%, the trend was different and was the rice flour gel which had the highest τ\text{max}. At 90°C, no significant differences were observed in the τ\text{max} of tef flour gels. At this temperature rice and refined wheat flour gels had the highest τ\text{max} and the lowest was for whole-wheat gels. It can be concluded that the effect of temperature and concentration on gel τ\text{max} is highly dependent on the cereal type. Similar trends were observed in G'\text{1}, G''\text{1}, which varied in parallel to τ\text{max}.

The coefficients G'\text{1}, G''\text{1}, and (\tan \delta)\text{1} and the exponents a, b and c were obtained by fitting the power law to the frequency sweep data ranging between 1-10Hz (Table 2). The high R\text{2} values (0.934-0.999) (data not shown) indicate how well the model was adjusted to the system studied. The elastic and viscous moduli were higher at 25 °C than 90 °C. This could be related to the formation of hydrogen bonds at lower temperature that stabilize and strengthen the matrix causing the stiffness of gels (Acevedo et al., 2013). Amylose retrogradation during cooling down to 25 °C could also be a contributing factor (Biliardes and Juliano, 1993). Both elastic and viscous moduli significantly increased (p < 0.05) with the increase of flour concentration (Table 2). However, the rate of increase varied with the type of flour and the temperature. With increasing concentration G'\text{1} and G''\text{1} increased faster at 25 °C than at 90 °C and the moduli increased faster in tef flour gels than wheat and rice flour gels. The rice gels were less dependent on the flour concentration. At equivalent concentrations, G'\text{1} and G''\text{1} of the gels from the three tef varieties and wheat and rice flours exhibited marked differences. This could be attributed to their differences in protein, starch, lipid, non-starch polysaccharide types and their contents (Mariotti, et al, 2005; Rao, 2007; Ronda et al., 2011). In addition to the marked variation in some of these components reported in this study, Hager et al. (2012) observed important differences in
the lipid profiles, fiber, and shape and size of starch granules of refined and whole wheat, rice and tef flours. Although refined wheat flour had higher starch and protein contents than tef flours (Table 1) its gels showed lower viscoelastic moduli. This is probably because of the presence of gluten in the wheat flours which hinders or partly reduces the contact between the starch granules or leached amylose entanglements (Rao, 2007). Though the three tef cultivars have closer proximate composition (section 3.2) and earlier report by Adebowale et al., (2011) indicate similar protein profiles in tef varieties (South African white (Witkop) and brown (Rooiberg) tef varieties and Ethiopian white tef grain), DZ-01-99 (brown tef) scored higher $G'$, $G''$ and $\tau_{\text{max}}$ at 25ºC and concentrations above 10%. This could also be justified by differences in their starch amyllopectin molecular weights (AP-Mw) and radii of gyration (Rg). Bultosa et al. (2008) found higher AP-Mw and Rg in DZ-01-99 starch than in DZ-Cr-37. Lower AP-Mw was shown to impart low paste viscosity in some wheat starches (Shibanuma et al., 1996). The presence of higher fiber in the whole flours should also be considered as it increases the viscoelastic moduli either by reduction of lubrication by water due to competition for water absorption between proteins and fiber or to the fiber acting as a filler in a viscoelastic matrix (Bonnand-Ducasse et al., 2010).

The critical gelation concentration ($C_{\text{crit}}$) or gel point ($G'$ and $G''$ crossover) can be considered as the concentration at which $G'$ begins to diverge from $G''$ (Acevedo et al., 2013; Avanza et al., 2005). Except for rice, structures of low $G'$ ($G' \approx G''$) were formed under 6% concentration both after heating and cooling. Increasing flour concentration over 6-8% made the dispersions to be more elastic ($G' > G''$), confirming the formation of a gel structure. The viscoelastic properties of rice flour suspensions were different from the rest and the rate of increase of their moduli (mainly $G'$) with concentration was much slower. The origin of this fact could be related to their different starch and protein type and content.

All dispersions had $G' > G''$ making $(\tan \delta)_I < 1$ indicating a solid-like behavior of the gels. Similar trend was reported by Weipert (1990) on gels from rice flour dispersions with different concentrations (10, 14 and 18%) heated for 60 min. The effect of concentration on $(\tan \delta)_I$ was significant ($p < 0.05$) at 25 ºC (Table 2). Except rice flour gel, the value of $(\tan \delta)_I$ decreased with increasing flour concentration. The three types of tef scored lower $(\tan \delta)_I$ values than the two wheat flours types indicating that the tef gels were stronger or with more elastic behavior.
The effect of concentration on \((\tan \delta)\) was not significant \((p < 0.05)\) at 90 °C (Table 2).

Low values of the \(a\) exponent for all tef gels at 25°C mainly at high concentrations, mean that the \(G'\) was not dependent on the frequency. This indicates the structural stability of the gels. Similar results were obtained by Lu et al. (2007) for fermented and non-fermented rice gels. The greater values of the \(b\) exponent, mainly observed at the lowest concentrations and at 90 °C, imply a higher dependency of \(G''\) on the frequency. Except for rice gel, the \(c\) exponent decreased with the increase of gel concentrations and this means that the ratio \(G''/G'\) had a lower dependence on frequency at higher gel concentrations (Acevedo et al., 2013, Ronda et al., 2011). This is coherent with the effect of concentration on \(a\) and \(b\) exponents as \(c\) exponent could be estimated from \((b-a)\).

The high dependence of storage modulus on concentration can be used to get information on the gelation efficiency and the structure of the particle network of the gel (Renkema and van Vliet, 2004). Using power-law function, Clark et al. (1990) approximated the relation between concentration and storage moduli as: \(G \propto C^y\). Based on it power-law was fitted to \(G'_i\) and \(G^o_i\) versus gel concentration and led to the equations: \(G'_i = m \cdot C^n\) and \(G^o_i = p \cdot C^q\), where \(m\) and \(p\) are the \(G'_i\) and \(G^o_i\) moduli values at a gel concentration of 1% and at a frequency of 1 Hz and where \(n\) and \(q\) are indexes that reflect the nature of the association behavior and the network structure (Table 3). The high values of \(R^2\) show the good fitting of the data obtained to the model. Except for rice flour, the values of \(n\) and \(q\) at 25 °C were greater than those at 90 °C which shows the formation of a more ordered gel matrix at 25°C by the formation of hydrogen bonds and amylose crystallization (Acevedo et al., 2013; Avanza et al., 2005). Power-law function between \(G^\prime\) and the concentration were also reported for soybean protein isolates (Renkema and van Vliet, 2004) and amaranth protein isolates (Avanza et al., 2005). However, Biliaderis and Juliano (1993) reported linear relationships between \(G^\prime\) and concentration for potato, wheat, corn and rice starch gels. In this study the \(n\) value for rice flour gels was near 1, indicating an almost linear dependence, similar to that found in starch gels. The rest of flours led to \(n\) values notably higher than rice (Table 3), more similar to that systems richer in proteins. The different variation of \(G^o_i\) with the concentration among tef, wheat and rice flour gels could be mainly due to the relative variations in starches and proteins in these flours.
Table 2. Rheological properties of three tef, wheat (whole and refined) and rice grain flour gels

<table>
<thead>
<tr>
<th>Flour</th>
<th>Temperature (°C)</th>
<th>Concentration (%)</th>
<th>$G'_i$ (Pa)</th>
<th>$a$</th>
<th>$G''_i$ (Pa)</th>
<th>$b$</th>
<th>$(\tan \delta)_i$</th>
<th>$c$</th>
<th>$\tau_{max}$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tef-brown (DZ-01-99)</td>
<td>90</td>
<td>6</td>
<td>18±4</td>
<td>0.11±0.01</td>
<td>4.5±0.7</td>
<td>0.47±0.02</td>
<td>0.24±0.01</td>
<td>0.35±0.03</td>
<td>10±1</td>
</tr>
<tr>
<td></td>
<td></td>
<td>8</td>
<td>78±5</td>
<td>0.11±0.01</td>
<td>18±3</td>
<td>0.40±0.03</td>
<td>0.23±0.03</td>
<td>0.29±0.03</td>
<td>19±3</td>
</tr>
<tr>
<td></td>
<td></td>
<td>10</td>
<td>168±3</td>
<td>0.14±0.01</td>
<td>37±1</td>
<td>0.34±0.01</td>
<td>0.22±0.01</td>
<td>0.20±0.01</td>
<td>37±6</td>
</tr>
<tr>
<td></td>
<td></td>
<td>12</td>
<td>223±48</td>
<td>0.15±0.02</td>
<td>51±8</td>
<td>0.34±0.01</td>
<td>0.24±0.02</td>
<td>0.19±0.01</td>
<td>66±10</td>
</tr>
<tr>
<td></td>
<td></td>
<td>14</td>
<td>413±39</td>
<td>0.13±0.03</td>
<td>81±6</td>
<td>0.29±0.03</td>
<td>0.20±0.01</td>
<td>0.17±0.01</td>
<td>115±14</td>
</tr>
<tr>
<td></td>
<td>25</td>
<td>6</td>
<td>22±3</td>
<td>0.15±0.01</td>
<td>6.3±0.5</td>
<td>0.47±0.01</td>
<td>0.28±0.01</td>
<td>0.31±0.01</td>
<td>10±1</td>
</tr>
<tr>
<td></td>
<td></td>
<td>8</td>
<td>97±5</td>
<td>0.15±0.01</td>
<td>23.6±0.1</td>
<td>0.37±0.01</td>
<td>0.24±0.01</td>
<td>0.23±0.01</td>
<td>117±15</td>
</tr>
<tr>
<td></td>
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<td>10</td>
<td>740±21</td>
<td>0.09±0.01</td>
<td>84±2</td>
<td>0.24±0.01</td>
<td>0.11±0.01</td>
<td>0.15±0.01</td>
<td>482±15</td>
</tr>
<tr>
<td></td>
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<td>12</td>
<td>1157±11</td>
<td>0.08±0.01</td>
<td>122±5</td>
<td>0.21±0.02</td>
<td>0.11±0.01</td>
<td>0.13±0.02</td>
<td>688±20</td>
</tr>
<tr>
<td></td>
<td></td>
<td>14</td>
<td>1724±67</td>
<td>0.07±0.02</td>
<td>172±7</td>
<td>0.22±0.01</td>
<td>0.10±0.01</td>
<td>0.15±0.01</td>
<td>829±30</td>
</tr>
<tr>
<td>Tef-white (DZ-Cr-37)</td>
<td>90</td>
<td>6</td>
<td>33.4±0.3</td>
<td>0.07±0.01</td>
<td>6.6±0.3e</td>
<td>0.32±0.05</td>
<td>0.19±0.20</td>
<td>0.25±0.06</td>
<td>7±3</td>
</tr>
<tr>
<td></td>
<td></td>
<td>8</td>
<td>114±0.9</td>
<td>0.08±0.02</td>
<td>19±1</td>
<td>0.35±0.01</td>
<td>0.32±0.20</td>
<td>0.29±0.04</td>
<td>27±4</td>
</tr>
<tr>
<td></td>
<td></td>
<td>10</td>
<td>229±1</td>
<td>0.10±0.01</td>
<td>34±1</td>
<td>0.31±0.02</td>
<td>0.15±0.01</td>
<td>0.21±0.01</td>
<td>43±5</td>
</tr>
<tr>
<td></td>
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<td>12</td>
<td>353±10</td>
<td>0.12±0.01</td>
<td>56±3</td>
<td>0.29±0.02</td>
<td>0.16±0.01</td>
<td>0.17±0.03</td>
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</tr>
<tr>
<td></td>
<td></td>
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<td>470±26</td>
<td>0.11±0.02</td>
<td>72±9</td>
<td>0.30±0.01</td>
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<td>0.19±0.02</td>
<td>105±10</td>
</tr>
<tr>
<td></td>
<td>25</td>
<td>6</td>
<td>35.2±0.7</td>
<td>0.12±0.01</td>
<td>7.3±0.1e</td>
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<td>0.28±0.01</td>
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</tr>
<tr>
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<td></td>
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<td>144±17</td>
<td>0.12±0.01</td>
<td>24±2</td>
<td>0.33±0.02</td>
<td>0.18±0.01</td>
<td>0.21±0.02</td>
<td>34±5</td>
</tr>
<tr>
<td></td>
<td></td>
<td>10</td>
<td>361±14</td>
<td>0.10±0.01</td>
<td>56±4</td>
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<td>0.17±0.01</td>
<td>223±10</td>
</tr>
<tr>
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<td>645±11</td>
<td>0.08±0.02</td>
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<td>0.16±0.01</td>
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</tr>
<tr>
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<td>14</td>
<td>860±8</td>
<td>0.08±0.02</td>
<td>113±3</td>
<td>0.26±0.02</td>
<td>0.13±0.01</td>
<td>0.17±0.02</td>
<td>570±30</td>
</tr>
<tr>
<td>Tef-white (DZ-Cr-387)</td>
<td>90</td>
<td>6</td>
<td>20.0±0.3</td>
<td>0.13±0.01</td>
<td>5.6±0.1e</td>
<td>0.43±0.01</td>
<td>0.27±0.01</td>
<td>0.30±0.01</td>
<td>7±1</td>
</tr>
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<td>8</td>
<td>57±5</td>
<td>0.13±0.01</td>
<td>15.1±0.4</td>
<td>0.39±0.01</td>
<td>0.26±0.02</td>
<td>0.25±0.05</td>
<td>19±5</td>
</tr>
<tr>
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<td></td>
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<td>112±10</td>
<td>0.16±0.01</td>
<td>28±1</td>
<td>0.37±0.01</td>
<td>0.25±0.01</td>
<td>0.22±0.01</td>
<td>34±4</td>
</tr>
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<td></td>
<td></td>
<td>12</td>
<td>178±15</td>
<td>0.16±0.01</td>
<td>44±3</td>
<td>0.34±0.01</td>
<td>0.24±0.01</td>
<td>0.19±0.01</td>
<td>69±8</td>
</tr>
<tr>
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<td>14</td>
<td>227±8</td>
<td>0.18±0.02</td>
<td>62±6</td>
<td>0.33±0.01</td>
<td>0.27±0.04</td>
<td>0.16±0.03</td>
<td>115±14</td>
</tr>
<tr>
<td></td>
<td>25</td>
<td>6</td>
<td>17±3</td>
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<td>0.46±0.01</td>
<td>0.41±0.01</td>
<td>0.24±0.01</td>
<td>9±1</td>
</tr>
<tr>
<td></td>
<td></td>
<td>8</td>
<td>89±2</td>
<td>0.17±0.01</td>
<td>25±1</td>
<td>0.36±0.01</td>
<td>0.28±0.02</td>
<td>0.20±0.01</td>
<td>108±28</td>
</tr>
<tr>
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<td>481±3</td>
<td>0.10±0.01</td>
<td>64±2</td>
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<td>0.16±0.01</td>
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<td>987±4</td>
<td>0.08±0.01</td>
<td>113±2</td>
<td>0.24±0.01</td>
<td>0.11±0.01</td>
<td>0.16±0.01</td>
<td>570±20</td>
</tr>
<tr>
<td></td>
<td></td>
<td>14</td>
<td>1348±19</td>
<td>0.07±0.02</td>
<td>151±1</td>
<td>0.24±0.01</td>
<td>0.11±0.01</td>
<td>0.14±0.03</td>
<td>688±30</td>
</tr>
</tbody>
</table>

The power law model was fitted to experimental results from frequency sweeps. $G'(\omega) = G'_i a^{-\omega}$, $G''(\omega) = G''_i a^{-\omega}$, $(\tan\delta)_i = (\tan\delta)_i a^{-\omega}$. $\tau_{max}$ was obtained from stress sweeps. Data are the mean ± standard deviation. Values with the same letter in a column for each temperature and flour are not significantly different (p>0.05).
Table 2. Rheological properties of three teff, wheat (whole and refined) and rice grain flour gels (Continued)

<table>
<thead>
<tr>
<th>Flour</th>
<th>Temperature (°C)</th>
<th>Concentration (%)</th>
<th>$G'_i$ (Pa)</th>
<th>$G''_i$ (Pa)</th>
<th>$\tan\delta_i$</th>
<th>$\tau_{\text{max}}$</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Wheat (refined)</strong></td>
<td>90</td>
<td>6</td>
<td>$24\pm3^c$</td>
<td>$170\pm0.01^a$</td>
<td>$841^c$</td>
<td>$0.33\pm0.02^a$</td>
</tr>
<tr>
<td></td>
<td></td>
<td>8</td>
<td>$53\pm10^c$</td>
<td>$190\pm0.04^a$</td>
<td>$2041^d$</td>
<td>$0.35\pm0.03^a$</td>
</tr>
<tr>
<td></td>
<td></td>
<td>10</td>
<td>$111\pm3^b$</td>
<td>$180\pm0.01^a$</td>
<td>$3311^c$</td>
<td>$0.35\pm0.01^a$</td>
</tr>
<tr>
<td></td>
<td></td>
<td>12</td>
<td>$171\pm17^a$</td>
<td>$190\pm0.01^a$</td>
<td>$5211^b$</td>
<td>$0.34\pm0.01^a$</td>
</tr>
<tr>
<td></td>
<td></td>
<td>14</td>
<td>$199\pm4^a$</td>
<td>$220\pm0.1^a$</td>
<td>$682^a$</td>
<td>$0.33\pm0.01^a$</td>
</tr>
<tr>
<td><strong>Wheat (whole)</strong></td>
<td>90</td>
<td>6</td>
<td>$24\pm7^c$</td>
<td>$220\pm0.04^a$</td>
<td>$154^d$</td>
<td>$0.26\pm0.01^c$</td>
</tr>
<tr>
<td></td>
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<td>8</td>
<td>$73\pm10^d$</td>
<td>$110\pm0.01^c$</td>
<td>$287^d$</td>
<td>$0.33\pm0.01^b$</td>
</tr>
<tr>
<td></td>
<td></td>
<td>10</td>
<td>$209\pm18^c$</td>
<td>$200\pm0.01^b$</td>
<td>$713^c$</td>
<td>$0.25\pm0.01^c$</td>
</tr>
<tr>
<td></td>
<td></td>
<td>12</td>
<td>$477\pm10^b$</td>
<td>$15\pm0.01^c$</td>
<td>$120^4^b$</td>
<td>$0.22\pm0.01^d$</td>
</tr>
<tr>
<td></td>
<td></td>
<td>14</td>
<td>$736\pm31^a$</td>
<td>$140\pm0.01^c$</td>
<td>$1614^a$</td>
<td>$0.24\pm0.01^d$</td>
</tr>
<tr>
<td><strong>Rice</strong></td>
<td>90</td>
<td>6</td>
<td>$79\pm10^d$</td>
<td>$210\pm0.01^b$</td>
<td>$498^d$</td>
<td>$0.23\pm0.01^d$</td>
</tr>
<tr>
<td></td>
<td></td>
<td>8</td>
<td>$63\pm3^d$</td>
<td>$190\pm0.01^a$</td>
<td>$2331^c$</td>
<td>$0.35\pm0.01^a$</td>
</tr>
<tr>
<td></td>
<td></td>
<td>10</td>
<td>$183\pm5^c$</td>
<td>$200\pm0.01^a$</td>
<td>$652^c$</td>
<td>$0.30\pm0.01^b$</td>
</tr>
<tr>
<td></td>
<td></td>
<td>12</td>
<td>$335\pm17^b$</td>
<td>$200\pm0.01^a$</td>
<td>$100^4^b$</td>
<td>$0.30\pm0.01^b$</td>
</tr>
<tr>
<td></td>
<td></td>
<td>14</td>
<td>$573\pm29^a$</td>
<td>$170\pm0.01^b$</td>
<td>$1494^a$</td>
<td>$0.28\pm0.01^c$</td>
</tr>
<tr>
<td></td>
<td></td>
<td>25</td>
<td>$60\pm4^d$</td>
<td>$080\pm0.01^a$</td>
<td>$151^c$</td>
<td>$0.25\pm0.01^a$</td>
</tr>
<tr>
<td></td>
<td></td>
<td>8</td>
<td>$154\pm7^c$</td>
<td>$090\pm0.01^a$</td>
<td>$234^c$</td>
<td>$0.29\pm0.01^b$</td>
</tr>
<tr>
<td></td>
<td></td>
<td>10</td>
<td>$215\pm24^b$</td>
<td>$510\pm0.56^b$</td>
<td>$354^b$</td>
<td>$0.29\pm0.01^b$</td>
</tr>
<tr>
<td></td>
<td></td>
<td>12</td>
<td>$256\pm7^a$</td>
<td>$110\pm0.01^a$</td>
<td>$413^b$</td>
<td>$0.30\pm0.01^b$</td>
</tr>
<tr>
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<td></td>
<td>14</td>
<td>$291\pm2^a$</td>
<td>$110\pm0.02^a$</td>
<td>$552^a$</td>
<td>$0.31\pm0.01^b$</td>
</tr>
<tr>
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<td>25</td>
<td>$120\pm10^d$</td>
<td>$100\pm0.01^c$</td>
<td>$192^c$</td>
<td>$0.27\pm0.01^c$</td>
</tr>
<tr>
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<td></td>
<td>8</td>
<td>$212\pm2^d$</td>
<td>$120\pm0.01^c$</td>
<td>$362^c$</td>
<td>$0.28\pm0.01^c$</td>
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<tr>
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<td>$252\pm7^c$</td>
<td>$120\pm0.01^c$</td>
<td>$452^c$</td>
<td>$0.30\pm0.01^b$</td>
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<tr>
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<td>12</td>
<td>$282\pm2^b$</td>
<td>$140\pm0.01^d$</td>
<td>$562^b$</td>
<td>$0.31\pm0.01^b$</td>
</tr>
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</table>
|                   |                  | 14                | $318\pm19^a$ | $150\pm0.02^a$ | $712^c$         | $0.33\pm0.02^a$ | $0.22\pm0.01^a$ | $0.18\pm0.01^a$ | $39330^a$        

The power law model was fitted to experimental results from frequency sweeps. $G'(\omega)=G'_i-\omega^a; G''(\omega)=G''_i-\omega^b; \tan\delta(\omega)=(\tau_{\text{max}}-\omega^c)$. $\tau_{\text{max}}$ was obtained from stress sweeps. Data are the mean ± standard deviation. Values with the same letter in a column for each temperature and flour are not significantly different (p>0.05)
Table 3. Parameters correspond to the fitting of experimental measured of $G'$ and $G''$ to power-law function ($G' = m \cdot C^n$; $G'' = p \cdot C^q$) at 90 °C and 25 °C for three tef, wheat (whole and refined) and rice grain flour gels.

<table>
<thead>
<tr>
<th>Flour</th>
<th>T (°C)</th>
<th>$m$</th>
<th>$n$</th>
<th>$R^2$</th>
<th>$p$</th>
<th>$q$</th>
<th>$R^2$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tef-brown (DZ-01-99)</td>
<td>90</td>
<td>0.038±0.02</td>
<td>3.55±0.36</td>
<td>0.970</td>
<td>0.015±0.008</td>
<td>3.31±0.33</td>
<td>0.970</td>
</tr>
<tr>
<td></td>
<td>25</td>
<td>0.0014±0.001</td>
<td>5.45±0.41</td>
<td>0.960</td>
<td>0.0056±0.003</td>
<td>4.01±0.41</td>
<td>0.969</td>
</tr>
<tr>
<td>Tef-white (DZ-Cr-37)</td>
<td>90</td>
<td>0.149±0.06</td>
<td>3.12±0.19</td>
<td>0.975</td>
<td>0.045±0.01</td>
<td>2.85±0.19</td>
<td>0.987</td>
</tr>
<tr>
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<td>0.044±0.03</td>
<td>3.83±0.20</td>
<td>0.979</td>
<td>0.024±0.01</td>
<td>3.28±0.29</td>
<td>0.977</td>
</tr>
<tr>
<td>Tef-white (DZ-Cr-387)</td>
<td>90</td>
<td>0.124±0.07</td>
<td>2.90±0.22</td>
<td>0.984</td>
<td>0.039±0.02</td>
<td>2.82±0.13</td>
<td>0.993</td>
</tr>
<tr>
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<td>25</td>
<td>0.0013±0.001</td>
<td>5.39±0.32</td>
<td>0.973</td>
<td>0.009±0.002</td>
<td>3.77±0.30</td>
<td>0.981</td>
</tr>
<tr>
<td>Wheat (refined)</td>
<td>90</td>
<td>0.23±0.10</td>
<td>2.62±0.21</td>
<td>0.981</td>
<td>0.088±0.06</td>
<td>2.56±0.15</td>
<td>0.990</td>
</tr>
<tr>
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<td>25</td>
<td>0.0066±0.003</td>
<td>4.45±0.14</td>
<td>0.996</td>
<td>0.021±0.02</td>
<td>3.45±0.23</td>
<td>0.987</td>
</tr>
<tr>
<td>Wheat (whole)</td>
<td>90</td>
<td>0.36±0.22</td>
<td>2.47±0.36</td>
<td>0.938</td>
<td>0.65±0.22</td>
<td>1.73±0.20</td>
<td>0.996</td>
</tr>
<tr>
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<td>25</td>
<td>0.021±0.005</td>
<td>3.89±0.20</td>
<td>0.986</td>
<td>0.028±0.02</td>
<td>3.29±0.29</td>
<td>0.972</td>
</tr>
<tr>
<td>Rice</td>
<td>90</td>
<td>13±2</td>
<td>1.14±0.11</td>
<td>0.992</td>
<td>1.1±0.6</td>
<td>1.47±0.16</td>
<td>0.991</td>
</tr>
<tr>
<td></td>
<td>25</td>
<td>48±11</td>
<td>0.72±0.05</td>
<td>0.997</td>
<td>1.5±0.8</td>
<td>1.18±0.09</td>
<td>0.972</td>
</tr>
</tbody>
</table>

3.3. Texture of flour gels and staling kinetics

Less aged whole wheat gels (< 24 h) and all rice gels (even at higher concentrations as 20%) disintegrated up on taking out undisturbed samples from the RVA canisters. The gel texture measurement was only possible for the three tef varieties and wheat flour.

The evolution of the firmness of the gels from the five types of flours stored between 0 h to 192 h (8 days) is depicted in Fig. 1a. The initial firmness of the DZ-01-99 flour gel (3.6N) was the highest while that of the wheat (1.0N) was the lowest. Although it was not possible to measure the whole wheat gels at times < 24 h, it could be anticipated that firmness values to be lowest of all. Similar order was found in the viscoelastic moduli of these gels at 25°C and high concentrations. Lower initial firmness of wheat flour gels could be because of gluten-starch interactions that hinder starch molecules to reorganize and retrograde (Rao, 2007). The presence of fiber in whole wheat probably obstructed and hindered the starch reorganization needed for its recrystallization (Santos et al., 2008). Variation in amylopectin molecular weight and size among tef varieties (Bultosa et al., 2008) might also explain the higher initial firmness scored by DZ-01-99 flour gel,
which was twice the gels from remaining two tef cultivars. With the exception of whole wheat the firmness of the gels showed significant (p < 0.05) differences with time. The firmness change over long storage periods is mainly related to amyllopectin recrystallization (Jacobson et al., 1997) and could also be affected by the presence of protein, lipids and fiber (Eliasson, 1994; Santos et al., 2008). Except for whole wheat gel, modeling of the firmness kinetics was successfully carried out by fitting the Avrami equation to the acquired data. Avrami model parameters (P₀, Pₓ, k and n) and the half life time are depicted in Table 4. The gel firmness after eight days of storage (Fig. 1a) and the leveling-off value (Pₓ) followed similar trend with the initial gel firmness. The hardening kinetics of the gels can be directly evaluated from k or t₁/₂. The half-life, t₁/₂, showed that gels from refined wheat leveled off much faster than the tef flour gels. The tef gels took around two days (48-58 h) to pass from P₀ to Pₓ/₂ while wheat gels needed only 6 hours. This indicates that the hardness of gel products from wheat could stabilize faster than tef flour gels. The total increase in hardness during storage, which could be analysed from the (Pₓ - P₀) difference, was also higher in tef gels than wheat gels. This could also be related to the absence of gluten in tef gels leading to higher extent of amyllopectin retrogradation (Ziobro et al., 2012).

The springiness of the tef gels dropped significantly (p < 0.05) with storage time while that of refined wheat gel did not change significantly (with averaged value of 0.8) and was always above tef gels (Table 4 and Fig. 1b). Springiness of whole wheat gels increased the first four days and then maintained a constant value of 0.7. The Avrami relation was fitted only to the data acquired for the three tef varieties. The constant of velocity (k) of DZ-01-99 (0.03 hⁿ) was much higher than those of the other two varieties (≈ 10⁻² hⁿ). The half-life followed the same pattern indicating that for the DZ-01-99 (25 h) was much lower than for DZ-Cr-387 (84 h) and DZ-Cr-37 (99 h). This means that the springiness of the brown tef gel leveled-off much faster than that of the both white tef gels.

The two wheat flour gels did not show appreciable (p > 0.05) differences in cohesiveness along the storage period, with averaged values of 0.48 and 0.36 for refined and whole wheat respectively. However, in the tef gels, it decreased significantly (Fig.1c) from 0.4 to less than 0.2. Avrami model was fitted for the three tef flours gels (Table 4 and Fig. 1c). The highest k value was obtained for the DZ-01-99 that also had
the lowest half-life time, 29 h versus 58 h for DZ-Cr-387 and 133 h for DZ-Cr-37. This indicates that the loss of cohesiveness with storage time was slower for the two white tef (DZ-Cr-387 and DZ-Cr-37) gels.

The resilience of tef gels decreased significantly (p < 0.05) with storage time from 0.2-0.3 to 0.08, while wheat flour gels exhibited a constant value (0.31 on average) (Fig. 1d). Resilience is a textural parameter that informs about the instant springiness and how well the product regains its original position. Whole wheat gels had very small resilience, and also lower cohesiveness and springiness than refined wheat gels. Similar textural effects of fibers were previously reported by Collar and Bollain (2005). Wheat bran caused discontinuities in gel and reduced significantly the recovery capacity of gel after a deformation force. The Avrami relation was fitted well only to the data of the three types of tef varieties. Based on the k values recorded the faster decrease of resilience corresponded to brown tef (DZ-01-99) gels. The computed half-life times also confirm the faster kinetics for this gel giving DZ-01-99 (12 h) < DZ-Cr-387 (22 h) < DZ-Cr-37 (29 h).
Figure 1: Evolution of a) Firmness; b) Springiness; c) Cohesiveness; d) Resilience; e) Adhesiveness; f) Gumminess, of tef flour gels stored at 4ºC. Gels made from refined and whole wheat flours were included as references. Each point represents the mean of four replicates. The confidence interval (95% confidence) is included in each point. The solid lines correspond to exponential equations that resulted from fitting the Avrami equation to experimental data (n=28).
Table 4: Staling kinetics of three tef, wheat (whole and refined) and rice grain flour gels at 4°C: Values of Avrami model factors.

<table>
<thead>
<tr>
<th>Texture property</th>
<th>Cereal</th>
<th>$P_c$ ($N$)</th>
<th>$P_{\infty}$ ($N$)</th>
<th>$k$ (h$^{-n}$)</th>
<th>$n$</th>
<th>$R^2$</th>
<th>$t_{1/2}$ (h)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Firmness</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Tef-brown (DZ-01-99)</td>
<td>3.64±0.24</td>
<td>5.81±0.60</td>
<td>0.036±0.050</td>
<td>0.73±0.51</td>
<td>0.93</td>
<td>59</td>
<td></td>
</tr>
<tr>
<td>Tef-white (DZ-Cr-37)</td>
<td>1.92±0.10</td>
<td>4.06±0.38</td>
<td>0.044±0.014</td>
<td>0.70±0.12</td>
<td>0.99</td>
<td>53</td>
<td></td>
</tr>
<tr>
<td>Tef-white (DZ-Cr-387)</td>
<td>1.82±0.90</td>
<td>5.05±0.50</td>
<td>0.075±0.065</td>
<td>0.57±0.37</td>
<td>0.97</td>
<td>48</td>
<td></td>
</tr>
<tr>
<td>Wheat (refined)</td>
<td>1.02±1.14</td>
<td>2.58±0.05</td>
<td>0.19±0.039</td>
<td>0.77±0.57</td>
<td>0.98</td>
<td>6</td>
<td></td>
</tr>
<tr>
<td><strong>Springiness</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Tef-brown (DZ-01-99)</td>
<td>0.80±0.01</td>
<td>0.46±0.01</td>
<td>0.025±0.009</td>
<td>1.02±0.10</td>
<td>0.99</td>
<td>25</td>
<td></td>
</tr>
<tr>
<td>Tef-white (DZ-Cr-37)</td>
<td>0.74±0.01</td>
<td>0.45±0.01</td>
<td>0.6·10$^5$±1·10$^{-5}$</td>
<td>2.54±0.43</td>
<td>0.99</td>
<td>99</td>
<td></td>
</tr>
<tr>
<td>Tef-white (DZ-Cr-387)</td>
<td>0.75±0.03</td>
<td>0.43±0.05</td>
<td>1.2·10$^5$±1·10$^{-5}$</td>
<td>2.43±0.43</td>
<td>0.93</td>
<td>84</td>
<td></td>
</tr>
<tr>
<td>Wheat (refined)</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td></td>
</tr>
<tr>
<td><strong>Cohesiveness</strong></td>
<td></td>
<td></td>
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<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Tef-brown (DZ-01-99)</td>
<td>0.40±0.01</td>
<td>0.16±0.01</td>
<td>0.028±0.010</td>
<td>0.96±0.10</td>
<td>0.97</td>
<td>29</td>
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<tr>
<td>Tef-white (DZ-Cr-37)</td>
<td>0.41±0.02</td>
<td>0.04±0.20</td>
<td>0.016±0.010</td>
<td>0.77±0.20</td>
<td>0.99</td>
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<tr>
<td>Tef-white (DZ-Cr-387)</td>
<td>0.46±0.02</td>
<td>0.13±0.10</td>
<td>0.019±0.021</td>
<td>0.88±0.34</td>
<td>0.92</td>
<td>58</td>
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<td>Wheat (refined)</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
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<td></td>
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<tr>
<td><strong>Resilience</strong></td>
<td></td>
<td></td>
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<td></td>
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<td></td>
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</tr>
<tr>
<td>Tef-brown (DZ-01-99)</td>
<td>0.27±0.01</td>
<td>0.09±0.01</td>
<td>0.096±0.019</td>
<td>0.79±0.07</td>
<td>0.99</td>
<td>12</td>
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</tr>
<tr>
<td>Tef-white (DZ-Cr-37)</td>
<td>0.24±0.02</td>
<td>0.09±0.03</td>
<td>0.032±0.048</td>
<td>0.91±0.42</td>
<td>0.95</td>
<td>29</td>
<td></td>
</tr>
<tr>
<td>Tef-white (DZ-Cr-387)</td>
<td>0.31±0.02</td>
<td>0.09±0.03</td>
<td>0.052±0.056</td>
<td>0.84±0.32</td>
<td>0.96</td>
<td>22</td>
<td></td>
</tr>
<tr>
<td>Wheat (refined)</td>
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<td>-</td>
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<tr>
<td><strong>Gumminess</strong></td>
<td></td>
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<td></td>
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<td></td>
<td></td>
</tr>
<tr>
<td>Tef-brown (DZ-01-99)</td>
<td>1.65±0.10</td>
<td>0.92±0.10</td>
<td>0.066±0.086</td>
<td>0.85±0.41</td>
<td>0.93</td>
<td>16</td>
<td></td>
</tr>
<tr>
<td>Tef-white (DZ-Cr-37)</td>
<td>0.74±0.01</td>
<td>0.45±0.01</td>
<td>5.8·10$^{-5}$±1·10$^{-4}$</td>
<td>2.54±0.43</td>
<td>0.99</td>
<td>99</td>
<td></td>
</tr>
<tr>
<td>Tef-white (DZ-Cr-387)</td>
<td>0.78±0.01</td>
<td>0.42±0.01</td>
<td>2.2·10$^{-5}$±1·10$^{-4}$</td>
<td>1.82±0.22</td>
<td>0.99</td>
<td>84</td>
<td></td>
</tr>
<tr>
<td>Wheat (refined)</td>
<td>0.76±0.40</td>
<td>1.26±0.05</td>
<td>0.13±0.10</td>
<td>0.80±0.70</td>
<td>0.90</td>
<td>9</td>
<td></td>
</tr>
</tbody>
</table>

The data shown are the regression coefficients ± confidence interval

Adhesiveness of the gels from tef flours increased during the earlier periods of storage, and went down later (Fig 1e). Gels from the two wheat flours did not show significant (p > 0.05) variation with time. In general, the tef and the whole wheat gels had markedly higher adhesiveness than refined wheat gels, that showed a very low and constant adhesiveness (0.9 - 0.5 N·s). The adhesiveness data could not be fitted to Avrami model.

Gumminess is often employed to characterize the energy to disintegrate semisolid foods and is nicely correlated to sensory evaluation by a trained panel (Truong et al., 1997).
Though gumminess usually follow similar trend with firmness, the gumminess exhibited by tef gels decreased significantly. Except for the whole wheat the Avrami relation was fitted well to the data (Table 4 and Fig. 1f). DZ-01-99 gel had an initial gumminess 55% higher than DZ-Cr-37 & DZ-Cr-387 which were similar to that of refined wheat gel. However, the gumminess leveling-off values scored by the tef gels were significantly lower than that of refined wheat flour (1.26 N). This could be attributed to the maintenance of cohesiveness by wheat gels during storage. Among the tef flours DZ-01-99 gel had the highest k value indicating that its gumminess leveled-off much quicker than the remaining two tef varieties. Refined wheat gels also seemed to level-off much quicker (k= 0.13) than the tef flour gels. The calculated half-life time was 9 h for wheat, 16 h for brown tef and 84 - 99 h for white tef flours.

3.4. Gel color

The chromatic coordinates (a*- b*) and the Luminosity (L*) of the gels of the different cereal flours and its evolution with storage time at 4ºC are depicted in Figure 3. There were significant differences in the color of gels depending on the type of tef flour. The hue angle (h) of the fresh flour gels varied from the more reddish to the yellow in the order: DZ-01-99 < whole wheat< DZ-Cr-37 < DZ-Cr-387 < refined wheat. The Luminosity (L*) of the gels followed approximately the same order DZ-01-99 (41.7) < DZ-Cr-37 (53.9) < whole wheat (59.1) < DZ-Cr-387 (61.6) < refined wheat (64.5). The high pigment content in DZ-01-99 led to the darkest and the most reddish gel. Though tef flours were also a whole grain flours, the DZ-Cr-387 gels showed very similar hue and lightness to refined wheat gel. Hence, in formulating foods where tef is taken as a component and brighter color is mandatory DZ-Cr-387 could be the best preference among the tef cultivars studied. With regard to the chroma (C*) of the gels the most vivid color (the highest chroma) corresponded to the DZ-01-99 (14.1) and the paler color to the refined wheat (7.0).

With the exception of refined wheat gel, the storage time affected significantly (p < 0.05) L* and C*, but not the h coordinate (Fig. 2). In general, the chroma decreased and the lightness increased with storage. DZ-01-99 and DZ-Cr-387 gel colors were the most affected. Nevertheless, although significant, the calculated ΔE were always <5 indicating that the color differences could not be visibly differentiated (Garcia-Viguera
The stability of gel colors at short-term storage periods seems to be good although the results indicate a certain variation in the gel pigment composition, mainly in DZ-01-99 and DZ-Cr-387 tef gels. Complementary studies should be done to chemically quantify the pigment evolution and to evaluate the changes in longer storage periods.

**Figure 2:** Plot of chromatic coordinates (a*-b*) of tef flour gels stored at 4°C for eight days. Gels made from refined and whole wheat flours were included as references. Each point represents the mean of four replicates. The arrows indicate the evolutions with storage time.

**4. Conclusions**

This research provides important information on color, rheological and textural properties of gels from the flours of three tef cultivars. The minimum concentrations required for the gel formation from the three tef cultivars were 6-8%, similar to that from wheat flour. Elastic and viscous moduli of the gels formed from the suspensions significantly increased (p < 0.05) with the increasing flour concentration according to
the power-law. At same concentration levels gels from the tef cultivars had higher consistency. The gels formed from the tef cultivars were solid-like \((\tan \delta)_{1}<1\) and this indicates that they can form self-supporting gels. Avrami model was successfully fitted to the evolution of the textural properties of the tef gels stored at 4°C. Among tef gels, the one obtained from DZ-01-99 flour had the highest initial firmness and gumminess, and showed also different evolution of textural properties during storage. In general, tef gels had higher initial hardness and closer initial springiness, cohesiveness resilience adhesiveness and gumminess than refined wheat gel. However, evolution of the texture properties of the tef gels varied notably during storage, taking longer time to level off. Hence, getting a stabilized gel product from tef flours will take longer time. This could be considered a drawback of tef flour gels.

The three tef cultivars could be considered suitable food ingredients in the formulation of gel-like foods. Different consistencies, texture properties and color can be obtained depending on the concentration and the tef variety selected. The information reported in this study will help in the formulation of these foods.

**Acknowledgement**

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References


Viscoelastic properties and stickiness of tef grain flour enriched wheat dough matrices

Viscoelastic properties and stickiness of tef grain flour enriched wheat dough matrices

Abstract

Currently, consumers’ preference towards baked goods with additional (functional and nutritional) value is increasing, leading food industries to look at more natural nutrient-dense alternatives like grain tef. Impact of tef grain flour incorporation (three Ethiopian tef varieties at 10, 20, 30 and 40% levels) on dough viscoelastic profiles and stickiness of wheat-based dough matrices were investigated. Oscillatory and creep-recovery tests together with dough stickiness were performed. Incorporation of tef flours affected the structure of the dough matrices visibly by reducing viscoelastic moduli and the maximum stress doughs can tolerate before its structure is broken and increased dough instantaneous and retarded elastic compliances. Effect of dose was not always significant in the parameters measured. Tef flour incorporation up to 30% level led to breads with higher volume than the control associated to optimal consistency and higher deformability of doughs. Higher tef doses increased dough stickiness. This will affect dough handling and shaping/flattening to get continuous strands or thin sheets. On average, the DZ-Cr-37 supplemented doughs exhibited higher elastic and viscous moduli, lower compliances and higher steady state viscosity and led to significantly lower volume breads. Hence, based on dough viscoelastic and stickiness properties, incorporation of DZ-01-99 and DZ-Cr-387 into wheat flour based formulations could be more preferable.

Key words: Tef, dough, oscillatory test, creep-recovery, stickiness
1. Introduction

Obesity, type-2-diabetes, coronary heart disease and colo-rectal cancer are among the rising challenges of western population, due to changes in both life style and eating behavior (WHO, 2005). Currently consumers’ awareness for healthy foods to get a healthy life has changed their preferences considerably regarding cereal products. Accordingly, the interest for breads for special dietary requirements and with increased nutritional value is rising. Hence, nutrient-rich whole grain incorporated baked goods with low glycemic index and/or enriched with dietary fiber are promising ways of producing healthy alternatives.

Tef [Eragrostis tef (Zucc.) Trotter] is a tropical cereal which has gained a rapidly growing global interest due to its nutritional composition and health benefits. Literature indicates it is gluten free, with equivalent protein content to other more common cereals like wheat and relatively richer than other cereals in the essential amino acid lysine (National Research Council, 1996; Dekking et al., 2005). It is composed of complex carbohydrates with slowly digestible starch. It is also known to be a good source of essential fatty acids, fiber, minerals (especially calcium and iron), and some phytochemicals such as polyphenols and phytates (Baye, 2013). In addition, tef grain and derived starch have suitable techno-functional properties like high water absorption capacity, foaming stability and a slow amylose retrogradation, dependent on tef variety type, that could have a positive impact on the quality of cereal based products (Bultosa, 2007; Abebe et al., 2015). These merits of tef make the grain as one of the best alternative ingredients in addressing the aforementioned demand. So far, some studies have been made to produce tef supplemented- and gluten-free western type breads from grain tef flours (Mohammed et al., 2009; Renzetti and Aredndt, 2009; Alaunyte et al., 2011) with encouraging results. However, these studies do not include information of either the tef varieties used or their effects on dough viscoelastic fundamental properties.

The replacement of wheat in bakery products is a major technological challenge, as the wheat protein gluten is essential for structure-formation. The gluten matrix is a major determinant of the important rheological characteristics of dough, such as elasticity, extensibility, resistance to stretch, mixing tolerance, and gas holding ability. Tef is
always consumed in the whole grain form (germ, bran and endosperm) and the composition and types of starch and proteins available are distinct from wheat. Consequently, dilution or removal of wheat gluten during supplementation and/or substitution in the dough system impairs proper dough development capacity during kneading, leavening and baking. Stickiness is a combination of adhesion — the interaction between a material and a surface, and cohesion — the interactions within the material. Therefore, in a dough system it is a result of a combination of surface and rheological properties. Dough stickiness is a major problem in the industry, particularly in large mechanized bakeries, as sticky and poor machinable doughs lead to process disruption and product loss (Armero and Collar, 1997).

Studying the rheological properties of wheat doughs supplemented with tef flours are of paramount importance because it may influence the machinability, elasticity, extensibility, resistance to stretch, mixing tolerance and the gas holding capacity of the dough and eventually the quality of the baked bread. Viscoelastic and stickiness properties of wheat flour dough matrices enriched with known Ethiopian grain tef cultivars have not been explored so far. Hence, the effect of tef variety type and addition level in the flour blend on dough rheological properties and bread volume were studied.

2. Materials and methods

2.1. Material

Three tef varieties DZ-01-99 (brown grain tef), DZ-Cr-37 (white grain tef) and DZ-Cr-387 (Quncho, white grain tef) were obtained from the Debre Zeit Agricultural Research Center of the Ethiopian Institute of Agricultural Research (EIAR). Wheat flour was supplied by Emilio Esteban SA (Valladolid, Spain). Wheat flour alveographic characteristics were (supplier data): Tenacity (P) 129 mm; Extensibility (L) 107 mm, Energy of Deformation (W) 466x10^-4 J; P/L ratio: 1.21. The general purpose bread improver used was Toupan Puratos® supplied by Puratos (Barcelona, Spain) that contained mono- and di-glyceride of fatty acids, ascorbic acid, α-amylase and xylanase. The chemical composition of the wheat and the three tef variety flours are summarized in Table 1.
Table 1: Proximate composition, starch (% on dry basis) and amylose (%) of three grain tef varieties and reference wheat and rice grain flour

<table>
<thead>
<tr>
<th>Flour</th>
<th>Moisture (%)</th>
<th>Proteins (% w/w)</th>
<th>Ash (% w/w)</th>
<th>Fat (% w/w)</th>
<th>Carbohydrates (% w/w)</th>
<th>Starch (% w/w)</th>
<th>Amylose (% starch)</th>
</tr>
</thead>
<tbody>
<tr>
<td>DZ-01-99</td>
<td>10.5±0.1a</td>
<td>8.9±0.3a</td>
<td>2.71±0.19b</td>
<td>2.84±0.08c</td>
<td>85.6±0.6b</td>
<td>75.5±0.1b</td>
<td>21.6±0.3a</td>
</tr>
<tr>
<td>DZ-Cr-37</td>
<td>10.3±0.1a</td>
<td>10.5±0.2b</td>
<td>3.52±0.01c</td>
<td>2.63±0.06b</td>
<td>83.4±0.2a</td>
<td>74.0±0.3a</td>
<td>21.8±0.3a</td>
</tr>
<tr>
<td>DZ-Cr-387</td>
<td>10.4±0.1a</td>
<td>8.9±0.2a</td>
<td>2.63±0.09b</td>
<td>3.24±0.06d</td>
<td>85.3±0.3b</td>
<td>75.5±0.4b</td>
<td>21.1±0.4a</td>
</tr>
<tr>
<td>Wheat</td>
<td>12.2±0.1b</td>
<td>14.5±0.2d</td>
<td>0.66±0.01a</td>
<td>1.47±0.06a</td>
<td>85.1±0.2b</td>
<td>78.8±0.4c</td>
<td>23.2±0.5b</td>
</tr>
</tbody>
</table>

Data are the mean ± standard deviation. Values with a letter in common in the same column are not significantly different (p <0.05)

2.2. Milling

Grain tef varieties were manually cleaned by siftings and winnowing before milling. Disc attrition mill, being used traditionally in cottage tef grain-milling house (Bishoftu, Ethiopia) to mill tef grain for injera making in Ethiopia, was used to mill the tef grain.

2.3. Dough preparation and breadmaking

A straight dough for a ciabatta bread type was performed using the following formula on a 100g flour basis: 1.8% salt, 0.5% bread improver, 2% dry yeast (only added for bread making; not to doughs intended for rheological test) and 85% water. For dough rheological measurements yeast was not added. Each of the three tef varieties (DZ-01-99, DZ-Cr-37 and DZ-Cr-387) was incorporated at 0%, 10%, 20%, 30% and 40% dose level and mixed with the wheat flour for 15 min using Chopin MR2L/MR10L mixer (Chopin technologies, France). The dough was prepared by blending the solid ingredients first in a kitchen-aid professional mixer (KPM5) for 2 min at speed 2. Then the kneading process was made in three phases: at speed 4 for 5 min. by adding water during the first minute, at speed 6 for 1 min. and finally at speed 4 for 8 min. In all preparations the temperature of the water was adjusted in order to get the sum of the room temperature, flour and water temperatures to be 52°C. This enabled to maintain the dough temperature around 26°C. The dough, 300 g, was placed into aluminium pans and was proofed at 28°C and (75 ± 5) % relative humidity for 40 min. Subsequently, baking was carried out in a Salva oven (Lezo, Spain) at 190°C for 40 min. After baking, breads were left for one hour at room temperature before analysis.
2.4. Oscillatory and creep recovery tests

Oscillatory and creep-recovery tests were carried out with a RheoStress 1 rheometer (Thermo Haake, Karlsruhe, Germany) with parallel plate geometry (60 mm diameter) of serrated surface and with 3 mm gap. The excess of dough was removed and vaseline oil was applied to cover the exposed sample surfaces. All measurements were done at 25 °C. Before each assay the dough was allowed to rest for 10 min for relaxation. Frequency sweeps were carried out from 10 to 0.1 Hz in the linear viscoelastic region (LVR). A constant stress value of 1 Pa was chosen for the frequency sweeps of all doughs after establishing this value fell in the LVR of all doughs by means of stress sweeps from 0.1 to 100 Pa at 1 Hz. From the curves, the maximum stress beyond which the dough structure was broken, \( \tau_{\text{max}} \), was established. Frequency sweep data were fitted to the power law model as in previous works (Ronda et al., 2011):

\[
G'(\omega) = G'_1 \cdot \omega^a \\
G''(\omega) = G''_1 \cdot \omega^b \\
\tan\delta(\omega) = \frac{G''(\omega)}{G'(\omega)} = \left(\frac{G''}{G'}\right)_1 \cdot \omega^c = (\tan\delta)_1 \cdot \omega^c
\]

The coefficients \( G'_1, G''_1, \) and \((\tan\delta)_1\), stand for the elastic modulus, viscous modulus and the loss tangent at a frequency of 1 Hz. The \( a, b \) and \( c \) exponents quantify the degree of dependence of these moduli and the loss tangent with the oscillation frequency, \( \omega \) expressed in Hz.

Creep tests were performed by imposing a sudden step shear stress in the LVR and outside the linear viscoelastic region (OLVR). For the creep study in the LVR a constant shear stress of 1 Pa was applied for 120 s while in the recovery phase the stress was suddenly removed and the sample was allowed for 240 s to recover the elastic (instantaneous and retarded) part of the deformation. For the study OLVR a constant shear stress of 50 Pa was applied for 60 s and the sample was allowed to recover for 200 s after removing the load. Each test was performed in triplicate. The data from creep tests were modelled to the 4-parameter Burgers model (Lazaridou et al., 2007) given by:
In the equation, $J_c(t)$ is the creep compliance (strain divided by stress), $J_{0c}$ is the instantaneous compliance, $J_{1c}$ is the retarded elastic compliance or viscoelastic compliances, $\lambda_{Jc}$ is the retardation time and $\mu_0$ gives information about the steady state viscosity. Similar equations were used for the recovery compliance $J_r(t)$. As there is no viscous flow in the recovery phase, equations consist only of parameters describing the elastic response after removal of the shear stress. The data from creep tests were modelled to the 3-parameter Burgers model given by:

$$J_c(t) = J_{0c} + J_{1c} \left( 1 - \exp \left( \frac{-t}{\lambda_{Jc}} \right) \right) + \frac{t}{\mu_0} \quad (4)$$

$J_{max}$ is the maximum creep compliance obtained at the end of the creep step. The steady-state compliance in recovery step, $J_{\text{steady}}$, was also calculated by subtracting the compliance value at the terminal region of curve (where dough recovery reached equilibrium) from the $J_{max}$. The ratio $J_{\text{steady}}/J_{max}$ (elastic recovery) was also calculated and expressed as Recovery (%).

### 2.5. Stickiness

This assay was conducted by following the procedure proposed by Grausgruber et al. (2003) and used by Ronda et al. (2011). A texturometer TA-XT2 from Stable Microsystem (Surrey, UK) provided with a SMS/Chen-Hoseney device where the sample was placed, and a methacrylate 25 mm cylinder (P/25P) as compression cell, were used. The stickiness of the dough was determined at pre-test and test speed of 0.5 mm/s, a post-test speed of 10.0 mm/s and 40 g force. Three parameters were used to define stickiness: the positive maximum force or adhesive force, which is the measure of stickiness, the positive area under the curve or the adhesive energy, which is the work of adhesion, and the distance the sample is extended on probe return, which is an indication of sample cohesion/dough strength. Six replicates were carried out for all doughs.
2.6. Statistical analysis

Experimental data were analyzed using two-way analysis of variance (MANOVA) and then means were compared at p<0.05 using Fisher’s least significant difference (LSD) test. Correlations among the viscoelastic parameters and bread volume were evaluated at p<0.01 and p<0.05 using Pearson’s correlation method. Statistical analysis was done by Statgraphics Centurion XVI program (StatPoint Technologies, Inc. 1982-2010).

3. Results and discussion

3.1. Dynamic oscillatory rheology

The results of the stress and frequency sweeps are presented in Table 2. The values of $\tau_{\text{max}}$, $G'$ and $G''$ exhibited by the control dough in this study were lower than usual for wheat flour doughs (Dobraszczyk and Morgenstern, 2003) due to the higher amount of water used in the ciabatta formulation. Water plays an important role in determining the viscoelastic properties of dough. Both $G'$ and $G''$ decreasing with increasing water content, because water can act as an inert filler causing the dynamic properties to reduce proportionally to moisture content or water can behave as a lubricant enhancing the relaxation phenomena (Masi et al., 1998). The $\tau_{\text{max}}$ of tef enriched doughs, showed significant variation with the control independently on the wheat substitution level, by decreasing more than 41% on average (Table 2). Such lower breakpoint for the tef incorporated doughs might be due to the dilution and breaking of the strong network formed during dough development from the gluten found in wheat. The dose of tef addition and the variety type did not appreciably change the $\tau_{\text{max}}$ score of the doughs.
Table 2. Single effects of tef grain flour incorporation level and tef variety on dynamic parameters of bread doughs.

<table>
<thead>
<tr>
<th>Tef dose (%)</th>
<th>( G'_1 ) (Pa)</th>
<th>a</th>
<th>( G''_1 ) (Pa)</th>
<th>b</th>
<th>((\tan \delta)_{i} )</th>
<th>c</th>
<th>( \tau_{\text{max}} ) (Pa)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>4314±83c</td>
<td>0.20±0.02c</td>
<td>1472±41b</td>
<td>0.260±0.0203c</td>
<td>0.341±0.006e</td>
<td>0.058±0.009c</td>
<td>3.31±0.38c</td>
</tr>
<tr>
<td>10</td>
<td>3293±342a</td>
<td>0.18±0.02b</td>
<td>1001±114a</td>
<td>0.249±0.030c</td>
<td>0.304±0.007d</td>
<td>0.067±0.014d</td>
<td>1.94±0.65b</td>
</tr>
<tr>
<td>20</td>
<td>3405±235a</td>
<td>0.17±0.01b</td>
<td>1039±105a</td>
<td>0.222±0.022b</td>
<td>0.300±0.007c</td>
<td>0.048±0.012b</td>
<td>1.93±0.62b</td>
</tr>
<tr>
<td>30</td>
<td>3560±350a</td>
<td>0.17±0.02ab</td>
<td>1021±98a</td>
<td>0.211±0.017ab</td>
<td>0.287±0.006b</td>
<td>0.037±0.006a</td>
<td>1.36±0.26a</td>
</tr>
<tr>
<td>40</td>
<td>3678±525b</td>
<td>0.16±0.01a</td>
<td>1007±146a</td>
<td>0.192±0.019a</td>
<td>0.273±0.008a</td>
<td>0.031±0.007a</td>
<td>1.29±0.23a</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Tef variety</th>
<th>( G'_1 ) (Pa)</th>
<th>a</th>
<th>( G''_1 ) (Pa)</th>
<th>b</th>
<th>((\tan \delta)_{i} )</th>
<th>c</th>
<th>( \tau_{\text{max}} ) (Pa)</th>
</tr>
</thead>
<tbody>
<tr>
<td>DZ-01-99</td>
<td>3540±446A</td>
<td>0.18±0.02A</td>
<td>1077±210A</td>
<td>0.226±0.032A</td>
<td>0.302±0.023A</td>
<td>0.047±0.015A</td>
<td>1.92±0.76A</td>
</tr>
<tr>
<td>DZ-Cr-37</td>
<td>4019±379B</td>
<td>0.18±0.02A</td>
<td>1224±168B</td>
<td>0.224±0.034A</td>
<td>0.300±0.025A</td>
<td>0.044±0.017A</td>
<td>1.45±0.24A</td>
</tr>
<tr>
<td>DZ-Cr-387</td>
<td>3549±524 A</td>
<td>0.18±0.02A</td>
<td>1088±256A</td>
<td>0.236±0.035A</td>
<td>0.303±0.028A</td>
<td>0.055±0.016B</td>
<td>1.52±0.46A</td>
</tr>
</tbody>
</table>

The power law model was fitted to experimental results from frequency sweeps. \( G'(\omega) = G'_1 \omega^a; G''(\omega) = G''_1 \omega^b; \tan \delta(\omega) = \tan(\delta)_{i} \omega^c \). \( \tau_{\text{max}} \) was obtained from stress sweeps. Data are the mean ± standard deviation. Values with the same letter in a column are not significantly different (p>0.05). Lower case letters are used to compare the effect of tef level and capital letters to compare the effect of variety.
Frequency sweeps showed that for all dough formulations the elastic (or storage) modulus, $G'$, was greater than the viscous (or loss) one, $G''$, in the whole range of frequencies. This led all values of loss tangent, included those at a frequency of 1Hz, $(\tan\delta)_i$, to be lower than 1 suggesting a solid elastic-like behavior of the dough formulations. Both moduli slightly increased with frequency. This variation, which is quantified by $a$ and $b$ exponents from $G'$ and $G''$ fittings to power law (Table 2), decreased significantly with tef addition. The incorporation of tef grain flours also markedly reduced both viscoelastic moduli, $G_1'$ and $G_1''$, leading to values 20% and 30% lower respectively, than the control dough regardless the wheat substitution level. It can be noted that 10% tef addition was enough to exert the gluten dilution and the weakening effect of the gluten network. The additional increase of tef dose did not lead to the concomitant decrease of viscoelastic moduli. Though it is small, a significant increase in $G_1'$ was observed on samples with 40% tef addition with respect to lower doses. This could be explained by the higher water absorption capacity, water holding capacity and swelling volume of tef flour in comparison with wheat flour (Abebe et al., 2015a). The explanation is consistent with the increase in dough consistency that may counteract the gluten dilution effect. Other authors have found higher viscoelastic moduli in rice-wheat composite doughs than in wheat doughs associated to stronger starch–gluten interactions in composite flour (Sivaramakrishnan et al., 2004). Sivaramakrishnan and coworkers (2004) also report that, rice starch granules in the dough can act as filler that reinforces the gluten and produce strong bonds to given higher modulus. The viscous modulus decreased with tef addition in a greater extent than the elastic one (Table 2). Consequently, the loss tangent decreased significantly ($p < 0.05$) with tef addition, from 0.34 (0% tef) to 0.27 (40% tef) implying an increase in the solid like behavior of tef-added doughs that increased with the tef level. This could be attributed to the differences in protein, starch, lipid, non-starch polysacharide types and their contents and profiles (Abebe and Ronda, 2014; Hager et al., 2012; Ronda et al., 2011). The marked variation in the lipid profiles, fiber, and shape and size of starch granules of wheat and tef flours observed by Abebe and Ronda (2014) and Hager et al. (2012) could also be a key factor. The $c$ exponent, as was reported for $a$ and $b$, also decreased constantly with tef addition level, meaning the $G_1''/G_1'$ ratio to have lower dependence on frequency (Ronda et al., 2011) associated to a lower frequency dependence structure (Sivaramakrishnanana et al., 2004) in tef-supplemented doughs.
Significant effect of tef variety type on \( G'_1 \) and \( G''_1 \) was observed. The DZ-Cr-37 tef variety flour exhibited the highest \( G'_1 \) and \( G''_1 \) average moduli, 14% higher than the remaining two tef varieties.

### 3.2. Creep-recovery tests

The results of the analysis of creep curves obtained both in LVR and OLVR are summarized in Table 3. The strong correlation (\( p < 0.001 \)) found for all creep compliance parameters and the equivalents for the recovery phase in the LVR (Ronda et al., 2014) suggested the omission of those data in Table 3 since they do not provide additional information to those of the creep phase. The dough had typical viscoelastic creep-recovery curves combining both viscous and elastic components. Both tef incorporation and variety affected creep-recovery parameters. However, as it was observed in oscillatory tests, the effect on creep results was not proportional to tef addition. The incorporation of 10% tef flour to replace wheat made creep phase instantaneous (\( J_{0c} \)) and retarded (\( J_{1c} \)) elastic compliances to increase significantly (28% and 33% in LVR and 53% and 46% OLVR) with respect to control dough values. The increase of compliances in the recovery phase was 66% and 38% for \( J_{0r} \) and \( J_{1r} \) with respect to the control dough values. This indicates that tef enriched doughs had higher instant and retarded deformations when subjected to a constant stress and higher recoveries when the stress was removed. Higher levels of tef in the flour blend, in general, did not lead to significant increases in the elastic or viscoelastic answers obtained in the LVR and OLVR with respect to that of the 10% tef-supplemented dough. \( J_{0c} \) and \( J_{1c} \) compliances in the LVR tended to decrease again with tef dose attaining at 40% level very similar values to the control dough (+12% and +6% respectively). The steady-state viscosity, \( \eta_0 \), which gave the flowability of the material at the end of the applied load decreased with 10% tef addition being significantly higher than the control dough for the OLVR measurement (-35%). For durum wheat doughs, it was found that the entire elastic compliance curve was shifted to higher values as the strength of the dough (measured by extensigraph) decreased (Edwards et al., 2001), while the steady-state viscosity increased with strength (Edwards et al., 2001). The authors interpreted the differences in creep behavior by differences in strength of the associative network established by non-covalent intermolecular associations within gluten chains. The 10% whole grain tef flour addition represents a supply of insoluble...
fiber that could explain the wheat gluten network disruption. The non-proportional effects of tef substitution levels could be due to differences in tef functional properties with respect to wheat (Abebe et al., 2015) dependent on their different composition and particularly their different protein and starch nature (Sivaramakrishnan et al., 2004). The \( \lambda \) values (Table 3) calculated did not show any significant variation with tef addition. The maximum creep compliances, both in and outside the LVR assays, increased with 10% tef addition although it was much more pronounced (13% versus 30%) in OLVR assays. An additional increase of 30% in the maximum creep compliance in OLVR assays was observed in 40% tef added dough. This can be partly attributed to its higher flowability (lower \( \eta_0 \)) and partly to its higher viscoelastic deformation (higher \( J_{1c} \)). The total elastic compliance (\( J_{0c} + J_{1c} \)) represented 56% of the maximum creep compliance in wheat dough. This ratio did not vary with tef addition in the LVR measurements meanwhile increased significantly in OLVR test, increasing until 64% independently of the dose of tef. In the recovery phase approximately 55% and 65% elastic recovery could be seen for pure and 10% tef-added wheat doughs respectively. This means a lower viscous characteristics of tef added doughs which is coherent with the lower tan \( \delta \) values already reported in the oscillatory tests.

The study effect of tef variety type on creep-recovery properties demonstrated that DZ-Cr-37 behaved differently, as was already commented with respect to oscillatory test results and the differences were more marked in OLVR assays. Accordingly, flour from this variety led to significantly lower average elastic compliances (-23% for \( J_{0c} \) and \( J_{or} \), -30% for \( J_{1c} \) and \( J_{1r} \), 33% for \( J_{max} \) in the creep phase, and -23% for \( J_{steady} \) from recovery phase) and higher average steady-state viscosity (+49%) than DZ-Cr-387 tef flour doughs. The DZ-Cr-387, which is a white tef as variety as DZ-Cr-37, showed the maximum difference with it in all the creep-recovery parameters meanwhile gave similar values to the brown grain tef variety, DZ-01-99. In the LVR the creep compliances of different tef varieties doughs showed maximum average differences of 16 – 19% and non-significant differences among steady-state viscosities. The relatively higher consistency (higher \( G_1' \) and \( \eta_0 \) values) DZ-Cr-37 incorporated dough and its lower deformability versus a stress may explain the lower dough development during proofing and baking, resulting in lower bread volumes. The incorporated tef variety type did not significant on \( \lambda_c \) in the creep phase of the test carried out in the LVR.
However, in OLVR tests impact of tef variety was significant on $\lambda_c$ and DZ-Cr-37 showed the lowest value indicating that the retardation time of the elastic retarded response was smaller than the doughs with the remaining varieties. No significant difference was observed in the retardation time of the recovery phase. Previous works have correlated the retardation times after creep with the bread volume reporting lower bread volumes for doughs with faster recoveries (Van Bockstaele et al., 2011). In this work, differences in retardation times, both in the creep or recovery phases were too small to explain the differences found in bread volume.

Although significant effects were observed among the doughs different in dose or tef variety type on some creep parameters obtained from LVR assays, it can be concluded their effect in OLVR were much more pronounced allowing better dough discrimination. Probably the general thought that higher correlation between dough creep parameters and bread volume are obtained outside the LVR can be partly due to the more marked differences found among samples in the latter test. In any case, very high correlations between all the parameters obtained in and outside LVR were found.
Table 3. Single effects of tef dose and tef grain variety on the creep-recovery parameters of bread doughs

<table>
<thead>
<tr>
<th>Parameter</th>
<th>LVR</th>
<th>Creep phase</th>
<th>Creep phase</th>
<th>OLVR</th>
<th>Recovery phase</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>J_{0c} (10^{-5} Pa)</td>
<td>J_{tc} (10^{-5} Pa)</td>
<td>\lambda_c (s)</td>
<td>\mu_{0c} (10^{-2} Pa·s)</td>
</tr>
<tr>
<td>Tef Dose (%)</td>
<td></td>
<td>J_{0c} (10^{-5} Pa)</td>
<td>J_{tc} (10^{-5} Pa)</td>
<td>\lambda_c (s)</td>
<td>\mu_{0c} (10^{-2} Pa·s)</td>
</tr>
<tr>
<td>0</td>
<td>30±1a</td>
<td>51±7a</td>
<td>15±2a</td>
<td>2.1±0.5b</td>
<td>142±8</td>
</tr>
<tr>
<td>10</td>
<td>38±3c</td>
<td>68±6b</td>
<td>15±1a</td>
<td>1.9±0.6ab</td>
<td>160±15b</td>
</tr>
<tr>
<td>20</td>
<td>35±5bc</td>
<td>50±7a</td>
<td>14±2a</td>
<td>1.7±0.2a</td>
<td>158±20ab</td>
</tr>
<tr>
<td>30</td>
<td>36±4bc</td>
<td>50±6a</td>
<td>15±1a</td>
<td>1.7±0.6ab</td>
<td>158±32b</td>
</tr>
<tr>
<td>40</td>
<td>33±7ab</td>
<td>48±8a</td>
<td>15±2a</td>
<td>1.8±0.4ab</td>
<td>145±27ab</td>
</tr>
</tbody>
</table>

Tef variety

| DZ-01-99 | 35±3B | 52±6AB | 15±1a | 1.8±0.5A | 153±20B | 56±2A | 69±2b | 140±7B | 8.1±0.3B | 0.50±0.03A | 358±14B | 61±6a | 120±4B | 86±3B | 22.6±0.5A | 206±7B | 58±3a |
| DZ-Cr-37 | 32±5A | 49±9A | 17±2A | 2.0±0.5A | 138±18A | 57±2A | 57±2A | 105±7A | 7.7±3.5A | 0.67±0.03B | 261±14A | 63±5a | 101±4A | 67±3A | 23.7±0.5A | 168±7A | 64±3a |
| DZ-Cr-387 | 38±5C | 59±7B | 16±2A | 1.8±0.6A | 160±19B | 57±2A | 75±2B | 150±7B | 8.0±0.4B | 0.45±0.04A | 384±14B | 61±6a | 131±4B | 92±3B | 22.3±0.5A | 223±7B | 58±3a |

LVR: Results obtained in the Linear Viscoelastic Region (at 1Pa); OLVR: Results obtained from creep test carried out Outside the Linear Viscoelastic Region (at 50Pa); J_{max}, J_{0c}, and J_{1c} = maximum, instantaneous and retarded or viscoelastic compliances (respectively), \lambda_c = retardation time and \mu_{0c} = steady state viscosity in the creep phase; J_{steady}, J_{0r}, and J_{1r} = steady-state, instantaneous and retarded compliances (respectively), \lambda_r = retardation time and \mu_{0r} = steady state viscosity in the recovery phase; J_{c,r} Elastic compliance in creep phase = J_{0c}+J_{1c}; Recovery: 100*J_{steady}/J_{max}.

Data are the mean± standard deviation. Values with the same letter in a column are not significantly different (p>0.05). Lower case letters are used to compare the effect of tef level and capital letters the effect of variety.
3.3. Dough stickiness

Results of the stickiness test on the formulated doughs are shown in Table 4. The stickiness (adhesive force) of the tef enriched doughs was lower than the control at lower doses, mainly at 10% and 20%, and conversely the cohesiveness of these doughs were higher (Hoseney and Smewing, 1999). However the adhesive forces recorded tended to rise with tef dose level so that, 40% tef-added doughs showed considerably higher average stickiness (+36%) than the control. The adhesive energy and the distance on return also showed a marked decrease since the smallest tef addition but, in this case, they continued decreasing until the 30% dose, and started to rise at the highest tef content. Tef supplemented doughs did not overpass the control adhesive energy and the distance on return values. Then, the three dough stickiness parameters showed similar tendency with a minimum value versus tef concentration, shifted toward higher concentrations in the case of adhesiveness energy and distance on return.

The study shows that incorporation of tef at higher percentage significantly increases the adhesive force and this may affect the handling and shaping/flattening purposes to get continuous strands or thin sheets of the doughs. In any case, stickiness did not overpass the 100 g value, discarding important dough handling problems (Armero and Collar, 1997; Chen and Hoseney 1995). Slight variations due to tef variety were also observed, in accordance with earlier observations reported on wheat revealing that varieties, growing season, protein concentration, water absorption, milling process and extraction rate may influence dough stickiness (Van Velzen et al., 2003; Yildiz et al., 2012).
Table 4. Single effects of tef dose and tef variety on bread dough stickiness

<table>
<thead>
<tr>
<th>Tef dose (%)</th>
<th>Adhesive Force (N)</th>
<th>Adhesive energy (Positive area) (mN·s)</th>
<th>Distance on return (mm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>0.58±0.05b</td>
<td>113±2d</td>
<td>4.39±0.03e</td>
</tr>
<tr>
<td>10</td>
<td>0.47±0.05a</td>
<td>63±2b</td>
<td>3.56±0.05c</td>
</tr>
<tr>
<td>20</td>
<td>0.50±0.07a</td>
<td>52±2a</td>
<td>3.16±0.05b</td>
</tr>
<tr>
<td>30</td>
<td>0.64±0.08c</td>
<td>51±2a</td>
<td>2.64±0.05a</td>
</tr>
<tr>
<td>40</td>
<td>0.79±0.13d</td>
<td>75±2c</td>
<td>3.87±0.05d</td>
</tr>
</tbody>
</table>

Tef variety

<table>
<thead>
<tr>
<th>Tef variety</th>
<th>Adhesive Force (N)</th>
<th>Adhesive energy (Positive area) (mN·s)</th>
<th>Distance on return (mm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>DZ-01-99</td>
<td>0.59±0.01A</td>
<td>66±2A</td>
<td>3.40±0.90A</td>
</tr>
<tr>
<td>DZ-Cr-37</td>
<td>0.58±0.01AB</td>
<td>76±2A</td>
<td>2.63±0.10A</td>
</tr>
<tr>
<td>DZ-Cr-387</td>
<td>0.61±0.01B</td>
<td>70±2A</td>
<td>2.54±0.04A</td>
</tr>
</tbody>
</table>

Data are the mean ± standard deviation. Values with the same letter in a column are not significantly different (p>0.05). Lower case letters are used to compare the effect of tef level and capital letters the effect of variety.

3.4. Bread volume

Figure 1 represents the bread volume evolution for different doses and tef variety. Both, tef variety type and its content in the formulation, had a significant effect on the specific volume of bread (p < 0.001). Unexpectedly, the substitution of wheat flour by tef flour until the 30% level, led to ciabatta type breads with significantly higher specific volume than the control wheat flour bread. The highest effect on volume was obtained with 10% or 20% additions (+12% on average) depending on the tef variety, but still 30% tef-enriched breads showed a significant (p < 0.05) 5% volume increase with respect to the control breads. The loaves with 40% tef flour showed a small (-2%) although significant, lower volume than the wheat counterpart. Aluyante et al. (2012) showed that replacing wheat flour by tef grain flour up to 10% in straight dough bread making did not affect loaf volume, while larger incorporations had a detrimental effect. Mohammed et al., (2009) obtained declining bread volumes on additions to wheat flour higher than 10-15% tef. The higher amount of admitted tef in the present samples could
be due to bread formulation, the tef varieties used and the type of wheat flour used in the blend. In this case, high grade wheat flour was used while previous authors reported to use all-purpose wheat flour (Mohammed et al., 2009). The very high gluten content of the wheat flour (11.9%) withstood the dilution with tef without losing suitable dough rheological characteristics. The higher gelatinization temperature of tef starch than wheat starch (Bultosa et al., 2002; Whistler and Be Miller, 1997) could also explain the higher volume of tef enriched breads as a higher dough is allowed in the oven before to reach a rigid structure. Tef variety type exerted remarkable effects on bread loaf volume in the order DZ-01-99 > Quncho DZ-Cr-387 > DZ-Cr-37.

Figure 1. Evolution of bread volume with tef dose of three different varieties: DZ-01-99 (■); DZ-Cr-37 (●); DZ-Cr-387 (▲)

3.5. Correlations among rheological properties and bread volume

Pearson correlation analysis showed a significant interdependence among the oscillatory and creep-recovery parameters (Table 5). As reported Ronda et al. (2014) both, the storage and loss moduli, showed strong interdependence. The loss tangent \((\tan \delta)\) was more dependent on loss moduli than the storage modulus. The creep compliances parameters showed strongly significant correlations with recovery phase counterparts \((r\)
In the LVR the viscosity at steady state ($\mu_0$) was only dependent on the maximum creep compliance $J_{\text{max}}$ ($r = -0.40$, $p < 0.01$). However, for measurements outside the LVR, $\mu_0$ strongly decreased ($r > -0.81$, $p < 0.01$) with increasing $J_{\text{max}}$, $J_0$ and $J_1$. In agreement with Ronda et al (2014), the higher maximum stress ($\tau_{\text{max}}$) explaining structural integrity of the doughs, increased in parallel with dynamic moduli, and decreased with instantaneous compliance. Bread volume was negatively correlated with the elastic modulus $G_1'$ ($r = -0.5$, $p < .01$) and positively with elastic compliances, $J_{oc}$ and $J_{fc}$ in the LVR assays ($r = 0.3$, $p < 0.5$). This can be explained by the dilution of the strong gluten network of wheat dough due to tef addition which lowered the dough consistency and increased its deformation capacity versus a constant stress. The condition allowed a higher dough development as a consequence of the gas production during proofing and its further expansion during baking (Villanueva et al., 2015). Bread volume had a highly significant positive correlation with dough elastic recovery after creep (Recovery) ($p < 0.01$; $r = 0.62$) and with the ratio $(J_{oc} + J_{fc})/J_{\text{maxc}}$ in the creep phase ($p < 0.01$; $r = 0.45$) both in OLVR assays. This means that doughs with smaller relative viscous parts led to higher bread volumes. Bread volume showed also a highly negative correlation with dough stickiness (adhesive force) ($r = -0.87$; $p < 0.01$) showing that doughs with the highest level of tef that became stickier gave lower bread volumes. Armero and Collar (1997) recommended for maximized dough cohesiveness and minimized dough stickiness for providing good bread-making performance. Therefore, dough stickiness could be one of the drawbacks of incorporating tef flours at higher percentages.

4. Conclusions

In general, incorporation of tef flours affected the structure of the dough matrices visibly in terms of lower viscoelastic moduli and $\tau_{\text{max}}$ values and larger instantaneous and retarded elastic compliances. Effect of dose level on these parameters was also visible. Tef flour supplemented breads up to 30% level had higher volume than the control ascribed to lower consistency and higher deformability of the doughs. However, at 40% tef dose the bread volume decreased to lower values than wheat bread. Viscoelastic properties do not explain easily this observation, as in general fundamental properties did not change markedly in samples over 10% tef addition. The elastic recovery capacity after creep and stickiness strongly correlated with bread volume. The
present study also show that incorporation of tef at higher percentage (40%) increases dough stickiness and this may affect the handling and shaping/flattening purposes to obtain continuous strands or thin sheets of the doughs. On average, the DZ-Cr-37 supplemented doughs showed higher elastic and viscous moduli, lower compliances, and higher steady state viscosity and in both LVR and OLVR than those supplemented with other tef varieteis. In addition, DZ-Cr-37 supplemented doughs also led to breads with lower volume. However, tef variety type did not appreciably affect dough stickiness. Hence, based on the dough viscoelastic and surface-related handling properties studied, the incorporation of DZ-01-99 and DZ-Cr-387 could be more preferable than DZ-Cr-37.
### Table 5. Correlations between viscoelastic properties and bread volume

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J_{maxI}, J_{est}, and J_{estO} = maximum, instantaneous and retarded compliances (respectively); λ_{ret} = retardation time and μ_{ret} = steady state viscosity in the creep phase obtained from creep test in linear viscoelastic region (at 1Pa); J_{maxCO}, J_{estO} = maximum, instantaneous and retarded compliances (respectively) outside the linear VR; λ_{steady} = Retardation time and μ_{steady} = steady state viscosity in the creep phase obtained from creep test outside the viscoelastic region (at 50Pa) and ι_{steady}, ι_{estO} = steady-state, instantaneous and retarded compliances (respectively) in the recovery phase outside, ι_{estO} = Retardation time in the recovery phase obtained from test outside the viscoelastic region (at 50Pa). J_{est} = Elastic compliance in creep phase = J_{est}+J_{1}; Rec: Recovery = J_{steady}/J_{max}; G1', G1'' and (tan δ) are the elastic and viscous moduli and the loss tangent at 1Hz and a, b, c are the exponents obtained after power law fitting of frequency sweeps data; tan δ = the maximum stress the dough can tolerated in the LVR. F1 = Adhesive force; A = Adhesive energy, and D = Distance on return * = p < 0.05 and ** = p < 0.01, - = not significant (p>0.05).
**Acknowledgements**

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**5. References**


Suitability of tef varieties in mixed wheat flour bread matrices: a physico-chemical and nutritional approach

Suitability of tef varieties in mixed wheat flour bread matrices: a physico-chemical and nutritional approach.

Abstract

Wheat flour replacement from 0 to 40% by single tef flours from three Ethiopian varieties DZ-01-99 (brown grain tef), DZ-Cr-37 (white grain tef) and DZ-Cr-387 (Quncho, white grain tef) had yielded a technologically viable ciabatta type composite bread with acceptable sensory properties with enhanced nutritional value, as compared to 100% refined wheat flour. Incorporation of tef flour from 30% to 40% imparted discreet negative effects in terms of decreased loaf volume and crumb resilience, and increase of crumb hardness in brown tef blended breads. Increment of crumb hardness on aging was in general much lower in tef blended breads compared to wheat bread counterparts, revealing slower firming kinetics, especially for brown tef blended breads. Blended breads with 40% white tef exhibited similar extent and variable rate of retrogradation kinetics along storage, while brown tef-blended breads retrograded slower but in higher extent than control wheat flour breads. Breads that contains 40% tef grain flour were found to contain five folds (DZ-01-99, DZ-Cr-387) to 10 folds (DZ-Cr-37) Fe, three fold Mn, twice Cu, Zn and Mg, and 1.5 times Ca, K, and P contents as compared to the contents found in 100% refined wheat grain flour breads. In addition, suitable dietary trends for lower rapid digestible starch and starch digestion rate index were met from tef grain flour fortified breads.

Key words: Composite wheat breads, grain tef, nutritional profile, physical properties, sensory
1. Introduction

With the constant search for diversity and innovation in foods, an alternative niche market for nutrient-dense fermented baked goods has emerged to satisfy the interest of health conscious people diet, which became the dietary needs of a significant part of the world human population. Tef (*Eragrostis tef*) is a nutritious cereal wheat type gluten-free grain indigenous to Ethiopia, rich in carbohydrates and fibre, microelements and phytochemicals (Baye, 2014) that contains superior amounts of iron, calcium and zinc than wheat, barley and sorghum (Abebe et al., 2007). The high nutritional profile makes tef a good candidate for designing innovative functional foods for health promotion and disease prevention.

In general, replacement of wheat by non-gluten forming cereals is a major technological challenge in breadmaking, as the wheat protein gluten is essential for structure-formation. Dilution of wheat gluten during supplementation and/or substitution at higher amounts in the dough system impairs proper dough development capacity during kneading, leavening and baking. Tef has been incorporated into breadmaking systems encompassing detrimental effects on bread physical and sensory quality when tef flour levels reached 20% (Mohammed et al., 2009) and 30% (Ben-Fayed et al., 2008; Alaunyte et al., 2012). Tef breads deserved significantly lower sensory scores, as only 10% and 5% tef breads had comparable acceptability scores to wheat bread in Ben-Fayed et al. (2008) and Mohammed et al. (2009) studies, respectively. More recently, a combination of enzymes has been successfully used to improve the quality of tef-enriched breads in terms of loaf volume and crumb firmness during storage in both straight dough and sourdough breadmaking processes (Alaunyte et al., 2012).

Since major challenge to include high levels of tef grain flours into breadmaking matrices relates the production of bread with good volume, textural and sensory attributes, changing the bread formulation and process conditions might be necessary. In addition, exploring the suitability of different tef varieties for bread formulation could be useful since most physicochemical, functional and nutritional properties of cereal-based goods are variety dependent. Therefore, in this study the impact of three Ethiopian grain tef varieties at different incorporation levels is evaluated for the physical, sensory and nutritional performance in making *ciabatta* type bread.
2. Experimental

2.1. Materials

Three tef varieties DZ-01-99 (brown grain tef), DZ-Cr-37 (white grain tef) and DZ-Cr-387 (Quncho, white grain tef) were obtained from the Debre Zeit Agricultural Research Center of the Ethiopian Institute of Agricultural Research (EIAR). Tef grain was manually cleaned by siftings and winnowing before milling. Disc attrition mill, being used traditionally in cottage tef grain-milling house (Bishoftu, Ethiopia) to mill tef grain for injera making in Ethiopia, was used to mill the tef grain. Tef flours (per 100 g, dry basis) from the different varieties (DZ-01-99, DZ-Cr-37, DZ-Cr-387) accounted for 8.9, 10.5, 8.9% protein, 2.8, 2.6, 3.2% fat, and 86, 83, 85% total carbohydrate, respectively as reported earlier (Abebe et al., 2015) were used. Wheat flour of extra-strength (14.5 % protein, 1.47% fat, and 85% total carbohydrate, Energy of Deformation (W) 466x10⁴J, P/L ratio 1.21) was supplied by Emilio Esteban SA (Valladolid, Spain). A general purpose bread improver Toupan Puratos® (Puratos, Barcelona, Spain) containing mono- and di-glyceride of fatty acids, ascorbic acid, α-amylase and xylanase was used.

2.2. Dough preparation and breadmaking

A straight dough process for a ciabatta bread type was performed using the following formula on a 100g flour (tef + wheat) basis: 1.8% salt, 0.5% bread improver, 2% dry yeast and 85% water. Tef flours were incorporated at 0%, 10%, 20%, 30% and 40% of wheat flour replacement and mixed for 15 min. using a Chopin MR2L/MR10L mixer (Chopin Technologies, France). Dough (300 g) was placed into aluminium pans and proofed at 28ºC and (75 ± 5) % relative moisture for 40 min. Subsequently, baking was carried out in a Salva oven (Lezo, Spain) at 190ºC for 40 min, and resulting breads were left for one hour at room temperature before analysis.

2.3. Bread physical characteristics

Bread volume was determined in duplicate using a volume analyser BVM-L370 TexVol Instruments (Viken, Sweden). Bread mechanical properties -firmness (N), cohesiveness, springiness, resilience and chewiness- were determined in fresh and 7 days stored breads using a TA-XT2 texture analyzer (Stable Microsystems, Surrey, UK) fitted with the “Texture Expert” software. A 25-mm diameter cylindrical aluminium probe was
used in a Texture Profile Analysis (TPA) with double compression test to penetrate to
50% of the sample depth at a test speed of 2 mm/s and with a 30 s delay between first
and second compressions. Analysis were carried out at (20 ± 2) ºC on two slices of 20
mm thickness taken from the centre of the loaf of two breads (2x2) per sample, taking
the average of the 4 measurements. Crumb and crust moisture contents were determined
by drying the samples in an oven for 24 hours at 105 ºC. Color was measured using a
Minolta spectrophotometer CN-508i (Minolta, Co.LTD, Tokyo, Japan). Results were
expressed in the CIE L* a* b* color space and were obtained using the D65 standard
illuminant, and the 2° standard observer. Color determinations were made 4x5 times on
each bread loaf (two breads per formula): crumb and crust color was checked at four
different points per loaf, and five measurements per point were made.

2.4. Mineral determination

Mineral content (Ca, Cr, Cu, Fe, K, Mg, Mn, Na, P, Zn) of flours and breads were
determined using a Radial Simultaneous inductively coupled plasma optical emission
spectrometry (ICP-OES) Varian 725-ES spectrophotometer (Agilent Technologies,
Santa Clara, CA, US). Aliquots of flours and freeze-dried breads (0.5 g) were placed in
Teflon cups, diluted with 6 mL of 65% HNO₃ and 2 mL of 30% H₂O₂, heated for 6 min
up to 200ºC and hold for 15 min at 200ºC for mineralisation in a microwave digester
(MLS 1200 mega, Milestone, Shelton, CN, US) and finally diluted to 25 mL.

2.5. Starch digestibility

In vitro starch digestibility of breads was measured according to the modified method
by Englyst et al. (2000), as previously applied by Ronda et al. (2012). The hydrolyzed
glucose at 20 min (G₂₀) and 120 min (G₁₂₀) and the total glucose (TG) were determined
by the glucose oxidase/peroxidase colorimetric method. The free sugar glucose (FGS)
content was also determined through a separate test following the procedure proposed
by Englyst et al. (2000). From the above results, rapidly digested starch (RDS) = 0.9 *
(G₂₀−FGS), slowly digestible starch (SDS) = 0.9 * (G₁₂₀−G₂₀), resistant starch (RS) =
0.9 * (TG − G₁₂₀), total starch (TS) = 0.9 * (TG − FGS) and rapidly available glucose
(RAG) = G₂₀ were calculated. Starch digestibility rate index (SDRI) was computed from
the percentage of RDS in TS in the flours.
2.6. Amylopectin retrogradation

A Mettler Toledo Differential Scanning Calorimeter DSC 822e (Schwerzenbch, Switzerland) equipped with a ceramic sensor (FSR5) of high sensitivity, liquid nitrogen cooling system and nitrogen purge gas was used. Bread crumb samples (20-25 mg) taken from the center of the bread loaf were hermetically sealed in aluminum pans (40 µL) and stored in the refrigerator at 4°C from 0 to 9 days. Starch retrogradation was analyzed from DSC endotherms obtained for crumb samples during temperature scanning from 0°C to 105°C at a heating rate of 5°C/min. Each measurement was performed at least in duplicate. The melting enthalpy was expressed in J/g of solids. Crystallization data using melting enthalpies after storage were fitted to the Avrami equation:

\[
\frac{H_\infty - H_t}{H_\infty - H_0} = e^{-k t^n}
\]

where \( t \) is time, \( k \) is a rate constant, and \( n \) is the Avrami exponent describing the type of crystal growth, \( H_\infty \) is the levelling-off value of melting enthalpy, \( H_t \) is the melting enthalpy at time \( t \), and \( H_0 \) is the melting enthalpy at initial time. The values of the constants \( k \) and \( n \) were used to calculate the half-life, \( t_{1/2} \) (Ronda and Roos, 2011) according to:

\[
t_{1/2} = \left( -\ln 0.5 \right)^{\frac{1}{n}}.
\]

2.7. Extraction and determination of polyphenols

*Extractable (soluble) phenols* from bread samples were extracted by concentrated hydrochloric acid:methanol:water (1:80:10, v/v) mixture at room temperature for 5 h, as reported by Milella et al. (2011). *Hydrolyzable (insoluble) phenolics* extraction was conducted with methanol and concentrated sulfuric acid (10:1, v/v) at 85°C for 20 h according to the procedure of Hartzfeld et al. (2002). Total phenolic content was calculated as the sum of extractable and hydrolyzable polyphenolic fractions as suggested by Perez-Jiménez and Saura-Calixto (2005).

*Bioaccessible phenol determination* was carried out by conducting an “in vitro” digestive enzymatic mild extraction that mimics the conditions in the gastrointestinal
tract according to Angioloni and Collar (2011a). Polyphenols content were determined according to the Folin-Ciocalteau procedure as described by Singleton et al. (1999). Results were expressed as gallic acid (GA) equivalents.

For the detection of flavonoids, 1 g of ground freeze-dried bread was extracted in 10 ml of 40% (v/v) ethanol for 30 min at room temperature according to Collar et al (2014a). The results expressed as mg of catechin equivalents (CE) per g of dry matter (dm).

2.8. Anti-radical activity

The stable 2,2-diphenyl-1-picrylhydrazyl (DPPH•) radical was used to measure the radical scavenging capacity of bread samples according to the DPPH• method adapted by Collar et al. (2014b). Plots of μmol DPPH vs time (min) were drawn, and calculations were made to know the antiradical activity (AR). AR= \([([DPPH]_{\text{INITIAL}} - [DPPH]_{\text{PLATEAU}}) \times 100]/ [DPPH]_{\text{INITIAL}}\).

2.9. Sensory analysis

Hedonic sensory tests were conducted by 60 untrained panellists. Breads were evaluated on the basis of acceptability of their visual appearance, odour intensity, texture, taste intensity, persistency of taste and overall acceptability by a hedonic 9-point scale where 9 means very much liked and 1 very much disliked. Two types of control breads were presented simultaneously with the samples and were evaluated in random order by panellists. Control 1 was bread made from 100% refined wheat flour and control 2, from 85% refined wheat flour and 15% wheat bran.

2.10. Statistical analysis

Experimental data were analysed using single and multivariate analysis of variance, and then means were then compared at p<0.05 using Fisher’s least significant difference (LSD) test. Statistical analysis was performed by using the Statgraphics Centurion XVI program (StatPoint Technologies, Inc. 1982-2010).
3. Results and Discussion

3.1. Physicochemical pattern and sensory performance of tef-wheat blended breads

The effect of wheat flour replacement from 0% up to 40% by tef flours with brown grain (DZ-01-99) and white grain (DZ-Cr-37, DZ-Cr-387) varieties on physicochemical analysis (Table 1), sensory acceptability (Table 2), staling kinetics (Table 3) and images of the tef-wheat blended breads (Fig. 1) are discussed below.

Physical characteristics data (Table 1), showed that replacing wheat flour with tef up to the level of 30% in straight dough breads did not affect either loaf volume (DZ-Cr-37, DZ-Cr-387) or crumb hardness and cohesiveness, and even provided 10% higher volume blended breads when a brown tef variety was used (DZ-01-99). Further incorporation of tef grain flour from 30% to 40% had negative effects in terms of decreasing 3.4 % loaf volume regardless the tef variety used, 17% increase of crumb hardness in brown grain tef flours (DZ-01-99) blended breads, and 15 % decrease in the crumb resilience, irrespective of grain tef variety. Increments of crumb hardness at 7 days of storage were in general much lower in tef blended breads compared to wheat bread counterparts, revealing slower firming kinetics, especially for brown grain tef (DZ-01-99) blended breads. A dramatic deleterious effect of tef flour incorporation up to 20-30% has been previously reported for mixed breads quality (Alaunyte et al., 2012; Ben-Fayed et al., 2008; Mohammed et al., 2009), particularly regarding reduced bread volume, harder texture and compact crumb structure. The wheat flour type used in our study (extra-strength), capable of standing the gluten dilution, instead of a common bread making flour may explain these differences. Close examination of bread crumb grain (Fig. 1) by visual inspection revealed changes in cell features depending on both the tef variety and the tef addition. Slice area decreased significantly in 40% tef breads, particularly for the brown grain tef variety DZ-01-99, in good accordance with data for specific volume (Table 1). Breads with 40% tef exhibited a more open and coarse crumb structure with less and larger cells, and thicker cell walls, particularly pronounced for the brown grain tef variety DZ-01-99 compared to their respective wheat counterparts and to breads with 10% tef.

Slice brightness (L*) significantly decreased gradually in both crumb and crust with increased levels of brown tef flour from 0% up to 40% (Table 1). L* values ranged
from 63.8 to 42.9 (crumb) and from 58.5 to 49.1 (crust) in brown grain tef variety DZ-01-99. In white grain tef breads, a slight decrease in crumb brightness was only observed from 30% to 40% of tef addition ranging from -19% (DZ-Cr-37) to -14% (DZ-Cr-387). Earlier reports (Alaunyte et al., 2012; Ben-Fayed et al., 2008) found similar decreases of slice brightness with grain tef flour addition, attributed to bran particles in wholegrain flours causing a darker crumb color (Fig. 1). The crumb hue (h), associated to the original color of ingredients, decreased from -14% to -29% with brown tef (DZ-01-99) addition, denoting a significantly loss of the pure yellow hue of the control bread (h=88 degrees) to evolve toward reddish (Figure 1). White grain tef varieties slightly decreased the crumb hue, although the variety DZ-Cr-387 led to the closer color of wheat bread. The crust hue, more affected by Maillard reactions, only varied slightly with tef addition. The crumb Chroma ($C^*$) increased with tef addition, reaching the maximum at 30% addition, and denoting more vivid colors than control breads. In the crust, only brown tef incorporated breads had visibly decreased $C^*$.

Sensory evaluation of blended breads (Table 2) revealed that increased tef grain flour levels from 0% to 30%, provided in general no dramatic decrease in sensory ratings, particularly for breads blended with white grain tef flours of DZ-Cr-37 and DZ-Cr-387 DZ-Cr-37 and DZ-Cr-387 which their scores were being similar or discreetly lower than the control bread processed from refined wheat grain flours. Blended breads with brown grain tef flour (DZ-01-99) at 30% produced poor ratings on visual appearance and overall acceptability as compared the whole wheat control bread. Increased tef addition from 30 to 40% encompassed significant lower scores on odour and overall acceptability of DZ-Cr-37 white breads, and similar ratings for brown DZ-01-99 and white DZ-Cr-387 blended breads. Previous results by Ben-Fayed et al. (2008), Mohammed et al. (2009), and Alaunyte et al. (2012) reported bread processed by substitution of grain tef flour from 10 to 5% (basic formulation), and 30% (with enzyme addition) respectively were acceptable as that of control bread. In this work even the 40% grain tef flours blended breads were rated >5/10 and judged good.

The DSC thermal analysis data for 40% grain tef flour-blended breads generated from storage after 9 days were defined according to the tef variety used, and the kinetics of amylpectin recrystallization on aging were modelled using the Avrami equation. Results on the model factors $H_0$, $H_\infty$, $n$, and $k$ for the enthalpy of amylpectin
retrogradation are compiled in Table 3. Compared to the control wheat breads, white grain tef flour-blended breads (DZ-Cr-37, DZ-Cr-387) exhibited similar extent ($H_\infty$: 5.63, 5.97 J/g vs 5.79 J/g) and variable rate ($n$: 0.69, 0.83 vs 0.85) of retrogradation kinetics with storage. Whereas the brown grain tef flour-blended breads (DZ-01-99) retrograded slower ($k$: 0.31 vs 0.49; $n$: 0.66 vs 0.85; $t_{1/2}$: 3.52 vs 1.51) but in higher extent ($H_\infty$: 8.19 J/g vs 5.79 J/g) than control wheat breads.
<table>
<thead>
<tr>
<th>Variety/Dose</th>
<th>Specific volume (mL/g)</th>
<th>Hardness (N)</th>
<th>Cohesiveness</th>
<th>Resilience</th>
<th>∆Firmness 7days (N)</th>
<th>Crumb L* h C*</th>
<th>Crust L* h C*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>3.21±0.09b</td>
<td>2.42±0.19de</td>
<td>0.69±0.01f</td>
<td>0.54±0.02f</td>
<td>5.95±0.26f</td>
<td>63.8±2.8g</td>
<td>87.50±0.94i</td>
</tr>
<tr>
<td>DZ-01-99</td>
<td>3.57±0.03ef</td>
<td>2.08±0.23abc</td>
<td>0.69±0.01ef</td>
<td>0.53±0.01ef</td>
<td>2.99±0.30ab</td>
<td>59.5±1.3f</td>
<td>75.43±0.47d</td>
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<tr>
<td>10</td>
<td>3.8±0.06ef</td>
<td>1.74±0.39a</td>
<td>0.72±0.04g</td>
<td>0.55±0.04f</td>
<td>2.82±0.53a</td>
<td>52.4±2.4cd</td>
<td>69.18±0.52c</td>
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<tr>
<td>20</td>
<td>3.54±0.07de</td>
<td>2.31±0.28cde</td>
<td>0.66±0.01abcd</td>
<td>0.48±0.01abcd</td>
<td>3.73±0.15abc</td>
<td>47.9±1.0b</td>
<td>65.61±0.66b</td>
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<td>40</td>
<td>3.09±0.01a</td>
<td>2.83±0.20f</td>
<td>0.65±0.02abc</td>
<td>0.47±0.03abcd</td>
<td>3.78±0.53abc</td>
<td>42.9±3.4a</td>
<td>61.94±0.82a</td>
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<td>DZ-Cr-37</td>
<td>3.49±0.02cde</td>
<td>2.37±0.17cde</td>
<td>0.68±0.01def</td>
<td>0.50±0.01de</td>
<td>5.29±0.28def</td>
<td>60.2±3.1f</td>
<td>85.82±0.57h</td>
</tr>
<tr>
<td>10</td>
<td>3.42±0.01c</td>
<td>2.46±0.08cdef</td>
<td>0.67±0.02cde</td>
<td>0.50±0.02cd</td>
<td>4.11±0.89bc</td>
<td>60.8±2.7f</td>
<td>83.61±0.99g</td>
</tr>
<tr>
<td>20</td>
<td>3.20±0.01b</td>
<td>2.21±0.32bcd</td>
<td>0.67±0.02bcde</td>
<td>0.49±0.03cde</td>
<td>5.49±0.55f</td>
<td>60.7±2.2f</td>
<td>82.57±1.28f</td>
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<tr>
<td>30</td>
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<td>2.60±0.37ef</td>
<td>0.65±0.02ab</td>
<td>0.45±0.02a</td>
<td>6.04±0.53f</td>
<td>49.3±8.2bc</td>
<td>79.71±1.33e</td>
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<td>DZ-Cr-387</td>
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<td>2.16±0.22bcd</td>
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<td>0.54±0.02f</td>
<td>4.27±0.72cd</td>
<td>58.7±5.3ef</td>
<td>87.49±1.04i</td>
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<td>3.64±0.06f</td>
<td>1.87±0.16ab</td>
<td>0.67±0.01bcde</td>
<td>0.50±0.01cd</td>
<td>3.30±0.90abc</td>
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<td>2.20±0.20bcd</td>
<td>0.68±0.01def</td>
<td>0.506±0.02de</td>
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<td>62.2±2.8f</td>
<td>85.94±0.72h</td>
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<td>3.09±0.01a</td>
<td>2.35±0.12cde</td>
<td>0.64±0.01a</td>
<td>0.456±0.01ab</td>
<td>3.90±0.53abcd</td>
<td>53.6±3.5d</td>
<td>83.10±1.57f</td>
</tr>
</tbody>
</table>

Control: 100% wheat bread, ∆Firmness 7 day: Firmness increase over 7 storage days.

(*) Mean values ± standard deviation. Values with the same letters in a column are not significantly different (p > 0.05).
Table 2: Sensory properties of composite breads processed by substitution with 10, 20, 30 and 40% three grain tef flour varieties to refined wheat grain flours and two type control breads

<table>
<thead>
<tr>
<th>Variety/ Dose (%)</th>
<th>Appearance</th>
<th>Odour</th>
<th>Texture</th>
<th>Taste</th>
<th>Persistency</th>
<th>Overall acceptance</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control -1</td>
<td>7.1 e</td>
<td>6.5 cd</td>
<td>6.9 de</td>
<td>6.4 cd</td>
<td>6.2 ab</td>
<td>6.9 e</td>
</tr>
<tr>
<td>Control- 2</td>
<td>6.4 cd</td>
<td>6.3 abcd</td>
<td>5.6 a</td>
<td>6.2 bcd</td>
<td>6.3 b</td>
<td>6.2 bcd</td>
</tr>
<tr>
<td>DZ-01-99</td>
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<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>10</td>
<td>6.0abc</td>
<td>6.6 cd</td>
<td>6.9 de</td>
<td>6.2 bcd</td>
<td>6.0 ab</td>
<td>6.5 cde</td>
</tr>
<tr>
<td>20</td>
<td>5.6 a</td>
<td>5.8 a</td>
<td>6.8 cde</td>
<td>6.5 cd</td>
<td>6.0 ab</td>
<td>6.3 cde</td>
</tr>
<tr>
<td>30</td>
<td>5.7 ab</td>
<td>6.2 abcd</td>
<td>5.8 ab</td>
<td>5.8 abc</td>
<td>5.7 ab</td>
<td>5.7 ab</td>
</tr>
<tr>
<td>40</td>
<td>5.5 a</td>
<td>6.6 cd</td>
<td>6.1 abc</td>
<td>5.4 a</td>
<td>5.7 ab</td>
<td>6.0 abc</td>
</tr>
<tr>
<td>DZ-Cr-37</td>
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<tr>
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<td>6.5 bcd</td>
<td>7.0 de</td>
<td>6.2 bcd</td>
<td>6.0 ab</td>
<td>6.7 de</td>
</tr>
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<td>6.2 bcd</td>
<td>6.1 ab</td>
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<td>5.9 ab</td>
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<td>DZ-Cr-387</td>
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<td>6.0 abc</td>
<td>7.2 e</td>
<td>6.6 d</td>
<td>6.2 ab</td>
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</tr>
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<td>6.1 abc</td>
<td>6.7 cde</td>
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<td>6.4 cde</td>
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<td>6.1 abc</td>
<td>6.6 cde</td>
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<td>6.1 abc</td>
</tr>
<tr>
<td>40</td>
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<td>6.1 abc</td>
<td>5.5 a</td>
<td>5.9 abcd</td>
<td>5.7 ab</td>
<td>5.9 abc</td>
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<tr>
<td>SD</td>
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<td>0.25</td>
<td>0.27</td>
<td>0.27</td>
<td>0.28</td>
<td>0.24</td>
</tr>
</tbody>
</table>

Control 1 = 100% refined wheat bread and control 2 = wheat bread processed from 85% wheat and 15% wheat bran. Values with the same letters in a column are not significantly different (p > 0.05).
Figure 1: Effect of tef addition on the external appearance and internal structures of bread. A) Control 1: 100% refined wheat bread; B) Control 2: whole wheat bread; C) 10% DZ-01-99 (brown grain tef flour) addition to refined wheat bread D) 40% DZ-01-99 (brown grain tef flour) addition to refined wheat bread E) 10% DZ-Cr-38 (white grain tef flour) addition to refined wheat bread F) 50% DZ-Cr-37 (white grain tef flour) addition to refined wheat bread; G) 10% DZ-Cr-387 (white grain tef flour) addition to refined wheat bread F) 40% DZ-Cr-378 (white grain tef flour) addition to refined wheat bread flour.
3.2. Nutritional features/profile of tef-wheat blended breads

3.2.1. Mineral elements

Potassium, P, Mg, and Ca are the most abundant minerals in wheat flour (Piironen et al. 2009; De Brier et al., 2015), while tef grain flours have a higher Fe, Ca, Zn and Cu content than other common cereals including wheat (Hager et al., 2013). The mineral contents are dependent on the genetic and environmental factors (Baye, 2014). Incorporation of 40% (Table 4) in the bread resulted into significantly higher amounts of micro-elements compared to the refined wheat, in agreement with previous studies (Alaunyte et al., 2012). This is especially true for Ca, Cu, Fe, K, Mg, Mn and P, regardless the tef variety used for blending (Table 4). Breads that contain 40% tef grain flour were found to contain five folds (DZ-01-99, DZ-Cr-387) to 10 folds (DZ-Cr-37) Fe, three fold Mn, twice Cu, Zn and Mg, and 1.5 times Ca, K, and P contents as compared to the contents found in 100% refined wheat grain flour breads. The most noticeable difference in contribution between wheat and tef breads was the dietary iron, which would be notably higher if tef breads were incorporated as a part of the diet, particularly using DZ-Cr-37 variety. If bioavailability of iron is assumed 100%, based on Recommended Dietary Allowances (RDA) for adequate intake (AI) of Fe (female = 18 mg/day  and male = 8 mg/day), daily consumption of 170-180 g of tef-wheat (40-60%) breads depending on tef grain variety will satisfy 60% and 135% (DZ-01-99), 141% and 318% (DZ-Cr-37) and 54% and 123% (DZ-Cr-387), for female and male adults, respectively. Similarly, if copper bioavailability is assumed 100%, copper requirements (0.9 mg/day) can be met by 43% (DZ-01-99, DZ-Cr-37) and 48% (DZ-Cr-387) by consumption of 170-180 g blended breads. However, daily consumption of 170-180 g of wheat bread can only delivers 13% (female) and 29% (male) of the required amount of iron and less than 28% of copper daily requirements.

3.2.2. Starch digestibility

Rate of starch hydrolysis and the subsequent nutritionally relevant starch fractions obtained from tef-wheat (40:60) blended breads are presented in Table 4. Significant differences (p < 0.05) in free sugar glucose (FSG) contents (% d. b.) of tef enriched breads were observed (1.30-1.58%), in accordance with similar observed in the grain tef flours (1.48-1.86%) of the different varieties (Abebe et al., 2015). Starch fractions
(RDS, SDS and RS), rapidly available glucose (RAG) and starch digestion rate index (SDRI) did not show dependence on tef variety. Amounts of digestible starch (RDS +SDS) of mixed breads were significantly lower than values found for the reference wheat bread (71.1-72.5% vs 77.1%). This is probably because of the relatively lower starch contents (74.0-75.5% vs 78.8%) and higher dietary fiber and ash contents in the respective tef flours (Abebe et al., 2015) as compared to wheat flours (Collar and Angioloni, 2014). Results are in accordance with the superior total starch content (TS) found in wheat breads (75.6%) compared to tef-enriched breads (71.4-72.3%) (Table 3). Suitable dietary trends for lower RDS and SDRI, and higher SDS contents (statistically non significant) in tef-enriched breads (67.5-68.2%, 94.2-94.7%, 3.5-4.3%) compared to wheat breads (74.3%, 98.3%, 2.8%) were found. In addition to the interference by dietary fibre, a stronger and denser mixed protein network may be formed hindering the starch availability to enzyme attack (Hager et al., 2013), which may contribute to the reduced rate of enzymatic starch hydrolysis.
Table 3. Starch fractions, FSG, RAG and SDRI expressed in % referred to dry matter and values of Avrami model factors for crumb amylopectin recrystallization in terms of melting enthalpy (H) of tef-wheat (40:60) blended breads

<table>
<thead>
<tr>
<th>Tef variety</th>
<th>FSG* (%)</th>
<th>RAG* (%)</th>
<th>RDS* (%)</th>
<th>SDS* (%)</th>
<th>RS* (%)</th>
<th>TS* (%)</th>
<th>SDRI* (%)</th>
<th>H_o (J/g solids)</th>
<th>H_∞ (J/g solids)</th>
<th>k</th>
<th>n</th>
<th>t_{1/2}</th>
<th>R^2</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control (0%)</td>
<td>0.11 ± 0.01a</td>
<td>82.7 ± 3b</td>
<td>74.3 ± 2b</td>
<td>2.8 ± 3a</td>
<td>-1.4 ± 1.9a</td>
<td>75.6 ± 0.6b</td>
<td>98.3 ± 0.8b</td>
<td>1.22±0.40b</td>
<td>5.79±0.83a</td>
<td>0.49±0.17a</td>
<td>0.85±0.40b</td>
<td>1.51±0.4a</td>
<td>0.975</td>
</tr>
<tr>
<td>DZ-01-99</td>
<td>1.58 ± 0.05c</td>
<td>76.7 ± 3a</td>
<td>67.6 ± 3a</td>
<td>3.5 ± 2a</td>
<td>0.3 ± 0.7a</td>
<td>71.4 ± 0.7a</td>
<td>94.7 ± 0.9a</td>
<td>1.10±0.34b</td>
<td>8.19±2.9b</td>
<td>0.31±0.21a</td>
<td>0.66±0.33a</td>
<td>3.52±0.5b</td>
<td>0.981</td>
</tr>
<tr>
<td>DZ-Cr-37</td>
<td>1.30 ± 0.03b</td>
<td>76.2 ± 4a</td>
<td>67.5 ± 2a</td>
<td>3.6 ± 2a</td>
<td>0.8 ± 1.8a</td>
<td>71.4 ± 1.7a</td>
<td>94.6 ± 2.3a</td>
<td>0.63±0.24a</td>
<td>5.63±0.10a</td>
<td>0.78±0.11b</td>
<td>0.69±0.10a</td>
<td>0.84±0.4a</td>
<td>0.999</td>
</tr>
<tr>
<td>DZ-Cr-387</td>
<td>1.47 ± 0.04c</td>
<td>77.2 ± 5a</td>
<td>68.2 ± 4a</td>
<td>4.3 ± 1a</td>
<td>-0.1 ± 1.0a</td>
<td>72.3 ± 1.0a</td>
<td>94.2 ± 1.3a</td>
<td>0.26±0.22a</td>
<td>5.97±0.48a</td>
<td>0.60±0.19b</td>
<td>0.83±0.22b</td>
<td>1.19±0.4b</td>
<td>0.999</td>
</tr>
</tbody>
</table>

*All results are expressed as the mean of six replicates ± standard deviation. FSG: Free glucose and sucrose; RDS = rapidly digestible starch, SDS = slowly digestible starch, RS = resistant starch, TS = total starch, RAG = rapidly available glucose, and SDRI = starch digestion rate index. Values with a letter in common in the same column are not significantly different (p>0.05).
Table 4. Moisture (%) and micro-element contents (mg/100g) of 40% tef- 60% wheat blended breads.

<table>
<thead>
<tr>
<th>Moisture (%)</th>
<th>Ca</th>
<th>Cu</th>
<th>Fe</th>
<th>Cr</th>
<th>K</th>
<th>Mg</th>
<th>Mn</th>
<th>P</th>
<th>Zn</th>
</tr>
</thead>
<tbody>
<tr>
<td>Breads</td>
<td></td>
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<tr>
<td>Control (0%)</td>
<td>43.4±0.7 b</td>
<td>151±1 a</td>
<td>&lt;0.25 a</td>
<td>2.3±0.2 a</td>
<td>&lt;0.25</td>
<td>201±5 a</td>
<td>50±1 a</td>
<td>0.86±0.02 a</td>
<td>208±1 a</td>
</tr>
<tr>
<td>DZ-01-99</td>
<td>42.5±0.6 ab</td>
<td>191±14 b</td>
<td>0.40±0.03 b</td>
<td>10.8±0.2 b</td>
<td>&lt;0.25</td>
<td>347±35 b</td>
<td>105±7 b</td>
<td>3.02±0.11 c</td>
<td>319±25 b</td>
</tr>
<tr>
<td>DZ-Cr-37</td>
<td>41.5±0.7 a</td>
<td>209±2 b</td>
<td>0.39±0.01 b</td>
<td>25.4±1.5 c</td>
<td>&lt;0.25</td>
<td>319±2 b</td>
<td>102±4 b</td>
<td>2.98±0.26 c</td>
<td>295±2 b</td>
</tr>
<tr>
<td>DZ-Cr-387</td>
<td>42.8±0.7 ab</td>
<td>208±6 b</td>
<td>0.43±0.02 b</td>
<td>9.8±0.3 b</td>
<td>&lt;0.25</td>
<td>358±31 b</td>
<td>101±2 b</td>
<td>2.47±0.04 b</td>
<td>313±13 b</td>
</tr>
<tr>
<td>Flours</td>
<td></td>
<td></td>
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</tr>
<tr>
<td>DZ-01-99</td>
<td>10.5±0.1 A</td>
<td>129±2 A</td>
<td>0.63±0.01 A</td>
<td>17.4±2.1 A</td>
<td>&lt;0.25</td>
<td>475±1 B</td>
<td>172±1 B</td>
<td>6.07±0.05 B</td>
<td>455±2 C</td>
</tr>
<tr>
<td>DZ-Cr-37</td>
<td>10.3±0.1 B</td>
<td>138±2 B</td>
<td>0.68±0.01 B</td>
<td>77.8±1.5 C</td>
<td>&lt;0.25</td>
<td>375±5 A</td>
<td>156±2 A</td>
<td>6.66±0.07 C</td>
<td>357±2 A</td>
</tr>
<tr>
<td>DZ-Cr-387</td>
<td>10.4±0.1 B</td>
<td>137±1 B</td>
<td>0.65±0.01 A</td>
<td>22.9±0.5 B</td>
<td>&lt;0.25</td>
<td>467±2 B</td>
<td>171±1 B</td>
<td>4.51±0.02 A</td>
<td>409±2 B</td>
</tr>
</tbody>
</table>

Mean values (two replicates) ± standard deviation. Values with in a columns followed by the same letter are not significantly different from each other (p > 0.05). Lower case letters are used to compare bread contents and capital letters to compare flour ones.
Table 5. Polyphenols fractions and subfractions and anti-radical activity of tef-wheat blended breads

<table>
<thead>
<tr>
<th>Variety/ Dose (%)</th>
<th>Extractable polyphenols</th>
<th>Hydrolyzable polyphenols</th>
<th>Total polyphenols</th>
<th>Hydrolyzable/ Extractable</th>
<th>Flavonoids</th>
<th>Bioaccessible polyphenols</th>
<th>Bioaccessible polyphenols</th>
<th>Anti-radical activity DPPH*</th>
<th>remaining μmolDPPH at steady state</th>
</tr>
</thead>
<tbody>
<tr>
<td>Breads</td>
<td></td>
<td></td>
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<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control 0%</td>
<td>308 ± 40 a</td>
<td>1958 ± 40 a</td>
<td>2265</td>
<td>6.4</td>
<td>97 ± 3 a</td>
<td>1747 ± 25 b</td>
<td>77</td>
<td>0.1802 ± 0.0061 a</td>
<td>28.9</td>
</tr>
<tr>
<td>DZ-01-99</td>
<td>401 ± 34 bc</td>
<td>2299 ± 7 b</td>
<td>2700</td>
<td>5.7</td>
<td>115 ± 4 a</td>
<td>1628 ± 42 a</td>
<td>60</td>
<td>0.1710 ± 0.0054 a</td>
<td>32.6</td>
</tr>
<tr>
<td>10</td>
<td>391 ± 2 b</td>
<td>2352 ± 48 b</td>
<td>2744</td>
<td>6.0</td>
<td>121 ± 0 b</td>
<td>1603 ± 47 a</td>
<td>58</td>
<td>0.1616 ± 0.0037 a</td>
<td>36.3</td>
</tr>
<tr>
<td>20</td>
<td>459 ± 51 cd</td>
<td>2320 ± 32 b</td>
<td>2780</td>
<td>5.1</td>
<td>132 ± 3 c</td>
<td>1611 ± 53 a</td>
<td>58</td>
<td>0.1646 ± 0.0034 a</td>
<td>35.1</td>
</tr>
<tr>
<td>30</td>
<td>496 ± 6 d</td>
<td>2397 ± 55 b</td>
<td>2893</td>
<td>4.8</td>
<td>155 ± 6 d</td>
<td>1591 ± 49 a</td>
<td>55</td>
<td>0.1729 ± 0.0090 a</td>
<td>31.8</td>
</tr>
<tr>
<td>DZ-Cr-37</td>
<td>408 ± 21 b</td>
<td>2505 ± 72 d</td>
<td>2913</td>
<td>6.1</td>
<td>95 ± 1 a</td>
<td>1810 ± 59 b</td>
<td>62</td>
<td>0.1907 ± 0.0084 a</td>
<td>24.8</td>
</tr>
<tr>
<td>10</td>
<td>417 ± 6 b</td>
<td>2479 ± 11 d</td>
<td>2896</td>
<td>5.9</td>
<td>102 ± 4 a</td>
<td>1658 ± 51 a</td>
<td>57</td>
<td>0.1926 ± 0.0027 a</td>
<td>24.1</td>
</tr>
<tr>
<td>20</td>
<td>456 ± 25 c</td>
<td>2399 ± 67 c</td>
<td>2855</td>
<td>5.3</td>
<td>92 ± 6 a</td>
<td>1647 ± 51 a</td>
<td>58</td>
<td>0.1847 ± 0.0112 a</td>
<td>27.2</td>
</tr>
<tr>
<td>30</td>
<td>508 ± 9.70 d</td>
<td>2235 ± 16 b</td>
<td>2743</td>
<td>4.4</td>
<td>103 ± 16 a</td>
<td>1608 ± 60 a</td>
<td>59</td>
<td>0.1824 ± 0.0104 a</td>
<td>28.1</td>
</tr>
<tr>
<td>DZ-Cr-387</td>
<td>445 ± 25 b</td>
<td>2036 ± 61 b</td>
<td>2481</td>
<td>4.6</td>
<td>83 ± 33 a</td>
<td>1862 ± 29 d</td>
<td>75</td>
<td>0.1816 ± 0.0048 a</td>
<td>28.4</td>
</tr>
<tr>
<td>10</td>
<td>413 ± 49 b</td>
<td>2037 ± 27 b</td>
<td>2450</td>
<td>4.9</td>
<td>91 ± 9 a</td>
<td>1790 ± 12 c</td>
<td>73</td>
<td>0.1715 ± 0.0044 a</td>
<td>32.4</td>
</tr>
<tr>
<td>20</td>
<td>539 ± 33 c</td>
<td>1984 ± 32 ab</td>
<td>2523</td>
<td>3.7</td>
<td>102 ± 4 a</td>
<td>1703 ± 18 ab</td>
<td>67</td>
<td>0.1705 ± 0.0112 a</td>
<td>32.8</td>
</tr>
<tr>
<td>30</td>
<td>585 ± 6 d</td>
<td>1942 ± 35 a</td>
<td>2527</td>
<td>4.3</td>
<td>87 ± 2 a</td>
<td>1612 ± 97 a</td>
<td>64</td>
<td>0.1741 ± 0.0062 a</td>
<td>31.4</td>
</tr>
<tr>
<td>Flours</td>
<td></td>
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<td></td>
<td></td>
</tr>
<tr>
<td>DZ-01-99</td>
<td>907 ± 177 b</td>
<td>1971 ± 155 a</td>
<td>2879</td>
<td>2.2</td>
<td>266 ± 5 b</td>
<td>1406 ± 13 b</td>
<td>49</td>
<td>0.1724 ± 0.0057 a</td>
<td>32.0</td>
</tr>
<tr>
<td>DZ-Cr-37</td>
<td>685 ± 56 a</td>
<td>1972 ± 78 a</td>
<td>2657</td>
<td>2.9</td>
<td>117 ± 22 a</td>
<td>1249 ± 44 a</td>
<td>47</td>
<td>0.1852 ± 0.0033 b</td>
<td>27.0</td>
</tr>
<tr>
<td>DZ-Cr-387</td>
<td>670 ± 23 a</td>
<td>1840 ± 147 a</td>
<td>2510</td>
<td>2.7</td>
<td>108 ± 4 a</td>
<td>1496 ± 108 b</td>
<td>60</td>
<td>0.1849 ± 0.0044 b</td>
<td>27.1</td>
</tr>
</tbody>
</table>

(*) Corresponding to 2.5 mg flour or freeze-dried bread that consumed DPPH when 0.247 μmol of the free radical are initially available to react. The plateau was decided at 90 min of reaction. (*) Mean values ± standard deviation. Values (mean of two replicates) within a column with the same letters are not significantly different (p > 0.05).
3.2.3. Polyphenol fractions and anti-radical activity of tef-wheat blended breads

The profile of phenolic fractions and subfractions and anti-radical activity of 100% wheat bread used as a control, tef enriched breads and from three teff grain flour varieties are given Table 5. Contents of extractable, hydrolyzable and total polyphenols of tef-enriched breads were higher than those of wheat flour bread counterparts (0% tef), regardless the tef variety and the percent of wheat flour replacement used. When the tef blended wheat breads are compared to that of control breads values (mg GA/100 g sample, d.b.) the extractable polyphenols ranged from 391 (DZ-01-99, 20%) to 585 (DZ-Cr-387, 40%) vs 308 (0% tef) and the hydrolyzable polyphenols varied from 1942 (DZ-Cr-387, 40%) to 2505 (DZ-Cr-37, 10%) vs 1958 (0% tef). The estimated total polyphenol content ranged from 2481 (DZ-Cr-387, 10%) to 2912 (DZ-Cr-37, 10%) vs 2265 (0% tef). The results show the content of extractable polyphenols increased with an increase in the dosages of grain tef flours from 10 to 40% leading to a concomitant decrease of hydrolyzable polyphenol contents except for DZ-01-99 tef enriched samples. Compared to wheat flour breads counterparts, the larger increase in extractable polyphenols corresponded to 40%-DZ-Cr-387 breads (+90%), followed by 40%-DZ-Cr-37 (+65%) and 40%-DZ-01-99 (+61%) breads, while the larger decrease in hydrolyzable polyphenols with dose (from 10 to 40%) was observed for DZ-Cr-37 breads (-11%). As a result of the translocation of insoluble polyphenols to accumulation of soluble components in tef-enriched breads, total polyphenol content changed little with dose for each tef variety: 2913-2743 mg/100g (DZ-Cr-37), 2481-2527 mg/100g (DZ-Cr-387), and 2700-2893 mg/100g (DZ-01-99) (Table 5). Values for extractable polyphenols of tef-enriched samples changed little according to the tef variety, covering similar ranges: 408-508 mg/100g (DZ-Cr-37), 445-585 mg/100g (DZ-Cr-387), and 401-496 mg/100g (DZ-01-99), while content of hydrolyzable polyphenols followed the decreasing order: DZ-Cr-37 (2505-2235 mg/100g) > DZ-01-99 (2299-2397 mg/100g) >DZ-Cr-387 (2036-1942 mg/100g).

The result shows the contents of non-extractable (hydrolyzable) phenolics were significantly higher than the soluble phenolic fraction (from 3.7-fold in 30%-DZ-Cr-387 sample to 6.1-fold in sample containing 10% DZ-Cr-37 tef flour) vs 6.4-fold in the control wheat flour sample (Table 5). The average ratio between hydrolyzable and extractable phenolic content in the present samples was very similar to the one obtained.
by Saura-Calixto et al. (2007) for cereal grain products. Amounts of phenolic fraction and subfractions were substantially higher than expected from sum of the respective values of the flours. This fact can be ascribed to the breadmaking process, mainly through the mixing and baking stages that encompass mechanical and thermal input, respectively. Both breadmaking steps may favour either depolymerization/unfolding and linkage breaking of insoluble, bound forms and further release, or may increase the accessibility of soluble free compounds and soluble conjugates. The content of flavonoids (mg CE/100 g sample, d.b.) was significantly higher in bread samples enriched with brown tef DZ-01-99 (115-155 mg/100g) compared to control wheat flour sample (97 mg), values being higher with tef flour dose. The tef grain flours dose with white tef varieties (DZ-Cr-37, DZ-Cr-387) within the range 10 to 40% had insignificant effect (p > 0.05) on the total flavonoids contents of the bread (80-100 mg/100g) opposite to dosing effect of brown grain tef variety. This is in agreement with the high flavonoid contents in brown grain tef flour variety (DZ-01-99) of 266 mg/100g as compared to white grain tef varieties (DZ-Cr-37 = 117 mg/100g and DZ-Cr-387 = 108 mg/100g).

The bioaccessible polyphenol content (mg GA/100 g sample, d.b.) of the blended breads decreased with an increase of tef grain flours doses from 10 to 40%, ranging from 1810 to 1608 mg/100g (DZ-Cr-37), from 1862 to 1612 mg/100g (DZ-Cr-387) and from 1628 to 1591 mg/100g (DZ-01-99). The bioaccessible polyphenols contents of the control wheat flour breads (1747 mg/100g) were found to be higher than the breads processed by enriching with different grain tef varieties (1249 mg/100g for DZ-Cr-37, 1496 mg/100g for DZ-Cr-387, 1406 mg/100g for DZ-01-99) (Table 5). Accumulation of bioaccessible polyphenols from flour to bread is in line with previous results observed on multigrain blended breads (Angioloni and Collar, 2011b; Collar et al., 2014b). Mechanical input during mixing and thermal treatment during baking may induce depolymerization of the constituents, mainly fibre, and hence may favour bread accessibility to enzyme attack and the subsequent release of fibre-associated polyphenols. In addition, Maillard reactions during bread baking can result in the synthesis of substances with antioxidant properties (Vogrincic et al., 2010). Nevertheless, replacement of wheat flour by increasing amounts of tef flour resulted in either a decline in the absolute level of bioaccessible polyphenols (DZ-Cr-37, DZ-Cr-
387) or a reduction in the percentage of bioaccessible compounds with respect to total polyphenol content (DZ-Cr-37, DZ-01-99). This fact possibly attributed to a physical/sterical interference by tef grain flour constituents, particularly dietary fibre that may hinder the accessibility of pepsin and pancreatin to achieve gastric and intestinal digestion. It has been stated that other compounds of proven resistance to the action of digestive enzymes, such as resistant starch, resistant protein, Maillard reaction compounds and other associated compounds, may reduce the bread phenol bioaccessibility (Saura-Calixto et al., 2000).

Anti-radical activity was determined by the extent of the reduction of the stable DPPH• radical, and results expressed as the remaining unreacted DPPH• amount when 0.247 µmol of the free radical are initially available to react with enzyme extracts from 2.5 mg flour or freeze-dried bread. Anti-radical activity for flours and for breads ranged from 24 to 36.3% (Table 5). It should be noticed the superior anti-radical activity of brown DZ-01-99 flour (32%) compared to white tef flours DZ-Cr-37 and DZ-Cr-387 that showed 27% in good accordance with the higher flavonoid content that are known to be good radical scavengers due to the presence of polyhydroxyl groups in the molecule. This resulted in a concomitant higher anti-radical activity in DZ-01-99 tef-blended breads (32-36%) regardless the dose of wheat flour replacement, compared to control wheat flour breads (29%) and white tef-blended breads (24-32%) (Table 5). For white tef-blended samples, irrespective of the dose of addition, incorporation of tef flour into formulations did not induced/contributed to enhanced anti-radical activity of breads. The observation, can be ascribed, to the changes occurring over breadmaking steps in terms of oxidation of phenolic compounds by coupled reaction due to substantial incorporation of oxygen in the dough during mixing (Eyoum et al., 2003), and to losses or degradation of phenolic compounds during baking (Angioloni and Collar, 2011b) as a result of the susceptibility of phenolic acids and flavonoids to heat.

4. Conclusions

Wheat flour replacement from 0% up to 40% by single tef flours from three Ethiopian varieties DZ-01-99 (brown grain tef), DZ-Cr-37 (white grain tef) and DZ-Cr-387 (Quncho, white grain tef) yielded technologically viable and sensory acceptable ciabatta type blended breads with enhanced nutritional value, as compared to the 100% refined
wheat flour breads. Addition of tef grain flours up to 30% had insignificant effect on either loaf volume (DZ-Cr-37, DZ-Cr-387) or crumb hardness and cohesiveness, and provided even 10% higher volume when brown grain tef flour (DZ-01-99) was used as compared to the control bread. Further incorporation of tef flour from 30% to 40% imparted discreet negative effects in terms of decreasing loaf volume and crumb resilience regardless the tef variety used, and increase of crumb hardness in brown tef blended breads. Increment of crumb hardness on aging was in general much lower in tef blended breads as compared to wheat bread counterparts, revealing slower firming kinetics, especially for brown grain tef flour blended breads. Blended breads with 40% white grain tef flour exhibited similar extent and variable rate of retrogradation kinetics along storage, while brown tef-blended breads retrograded slower although in higher extent than control wheat flour breads. If the bio-availability can be assumed 100%, a daily intake of 170-180 g of tef-wheat (40-60%) blended breads can provide from 60 to 135% (DZ-01-99), from 141 to 318% (DZ-Cr3-7) and from 54 to 123% (DZ-Cr-387) of the amount of iron recommended for adults, depending on the tef flour variety and the gender, while copper requirements can be met from 43% (DZ-01-99, DZ-Cr-37) to 48% (DZ-Cr-387). In addition, suitable dietary trends for lower rapid digestible starch and starch digestion rate index can be fulfilled. The content of flavonoids and the anti-radical activity were significantly higher in bread samples enriched with brown grain tef flour compared to control wheat flour sample, values for flavonoids being higher with tef flour dose.

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