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BIOCHEMICAL AND TECHNOLOGICAL CHARACTERIZATION OF C4 CYCLE GLUTEN-FREE CEREALS: *ERAGROSTIS TEF* AND *SORGHUM BICOLOR*

Elena Galassi

Coordinatore
Prof. Giulio Iannello

Tutor
Dott.ssa Laura Gazza

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to my beloved parents
especially to my father
so that he is proud of me from up above

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CHAPTER 1: INTRODUCTION

1.1 BACKGROUND: TWO INDIGENOUS AFRICAN CEREALS

There are more than 50,000 known edible plants in the world, yet two-thirds of global plant-derived food is provided by only three major cereals: maize, wheat and rice. The dominance of this triad, now considered truly global food commodities, has led to a decline in the number of crop species contributing to global food supplies. The dependence on only a few crop species limits our capability to deal with challenges posed by the adverse effects of climate change and the consequences of dietary imbalance. Emerging evidence suggests that climate change will cause shifts in crop production and yield loss due to more unpredictable and hostile weather patterns. One solution to this problem is through the wider use of underutilized (also called orphan or minor) crops to diversify agricultural systems and food sources. In addition to being highly nutritious, underutilized crops are resilient in natural and agricultural conditions, making them a suitable surrogate to the major crops (Cheng et al., 2017).

Two such crops are C4 plants (Fig.1A-B) as teff [*Eragrostis tef* (Zucc.) Trotter], a warm-season annual cereal with the tiniest grain in the world, native to Ethiopia, and sorghum (*Sorghum bicolor* L.) an important staple food in parts of Asia and Africa, where usually is consumed as grain or as flour in recipes including fermented and unfermented porridges, bakery products and alcoholic beverages.

Both teff and sorghum plants are efficient in water usage and tolerates drought as well as waterlogged and saliferous soils, this capability makes them better able to grow on marginal lands that do not support maize and other cereal crops. Sorghum and teff, despite being considered minor cereals and having an absolutely not comparable productivity with popular cereals, can take in the short term a prominent role in cereal production also in industrialized countries, where until now are not used for human food.

The phylogenetic tree constructed on partial sequences of the waxy gene, exposed the evolutionary relationships between teff and sorghum (Fig. 2). Cannarozzi et al., (2014) observed that the closest cultivated species to teff was finger millet (*Eleusine coracana*), and the closest subfamily to teff was Panicoideae including sorghum (*Sorghum bicolor*), maize, and pearl millet (*Pennisetum glaucum*).

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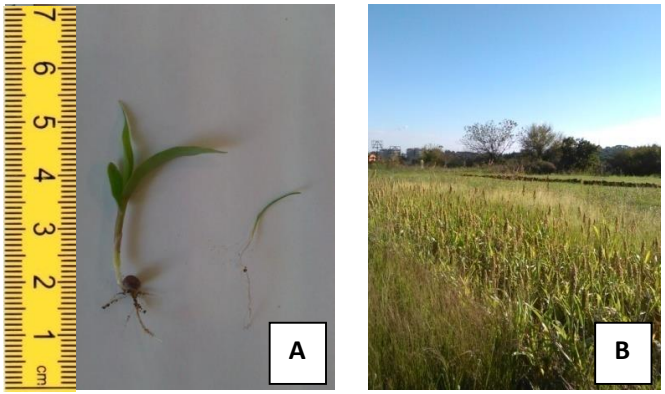

Figure 1. *Sorghum and Teff shoots (A) and plants (B) at Azienda Sperimentale Inviolatella (CREA-IT, Rome).*

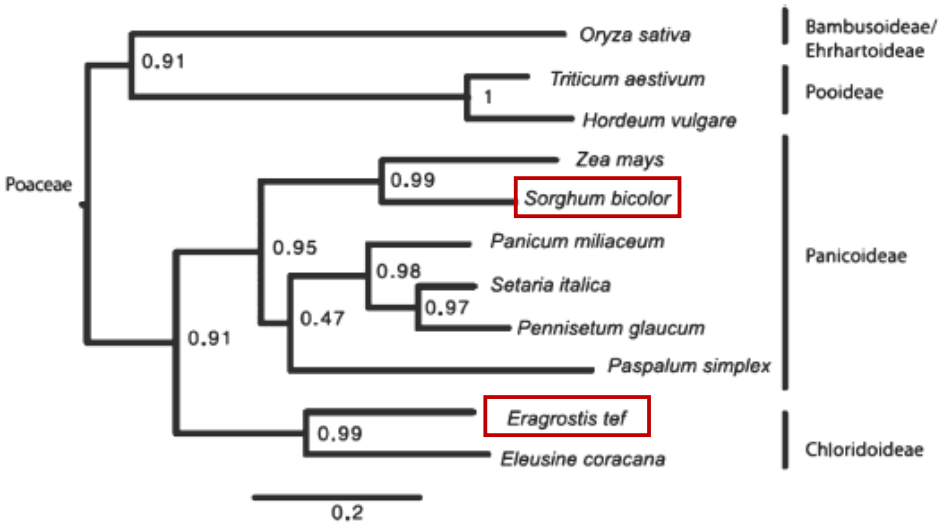

Figure 2. *Phylogenetic tree for five subfamilies from the family Poaceae. The tree was constructed on the basis of partial sequences of the waxy gene (NCBI database: http://www.ncbi.nlm.nih.gov/) from barley (Hordeum vulgare, X07931), wheat (Triticum aestivum, KF861808), finger millet (Eleusine coracana, AY509652), foxtail millet (Setaria italica, AB089143), maize (Zea mays, EU041692), Paspalum simplex (AF318770), pearl millet (Pennisetum glaucum, AF488414), proso millet (Panicum miliaceum, GU199268), rice (Oryza sativa, FJ235770.1), sorghum (Sorghum bicolor, EF089839), and teff (Eragrostis tef , AY136939). Numbers at nodes represent posterior probabilities. Image from Cannarozzi et al. (2014).*

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1.2 SORGHUM (Sorghum bicolor (L.) MOENCH)

Sorghum (*Sorghum bicolor* L.) is a cereal of the Poaceae family, native to Africa where was domesticated between 3,000 and 5,000 years ago (U.S. Grains Council, 2004) and then widespread to other continents.

Sorghum is the fifth most widely grown cereal crop worldwide, after wheat, rice, corn, and barley, with a world area of over 44 million hectares and a production exceeding 63 million tons in year 2016/2017(https://ipad.fas.usda.gov/) (Fig.3).

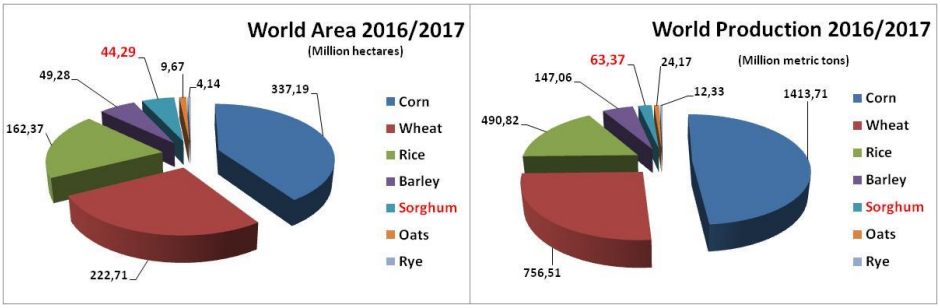

Figure 3. *Pie chart of area (on the left) and production (right) of major cereal crops cultivated in the world in year 2016/2017(Foreign Agricultural Service/USDA, December 2018 report).*

This cereal, due to its peculiar agronomic traits such as drought tolerance and adaptation to tropical and subtropical conditions, is a major human food source in Africa and Asia where agricultural and environmental conditions are unfavorable for other crops (Bean et al., 2011; Goodall et al., 2012). In these developing Countries sorghum usually is consumed as grain or as flour, representing thus the dietary staple and the major source of energy and nutrients for more than 300 million people (Althwab et al., 2015). However, in Western Countries, where 40% of sorghum production worldwide occurred (https://ipad.fas.usda.gov/) (Fig.4), this cereal is mainly used as animal feed and for ethanol production (Wang et al., 2009). Furthermore, it is noteworthy that despite the decrease expected for the subsequent years (projections of 2017/2018 and 2018/2019), both in terms of cultivated area and production for African and American continents, there is a prediction of an increase of 8% for the next 2 years (2017/2018 and 2018/2019) for the sorghum production in the European Union (28 States) (https://ipad.fas.usda.gov/).

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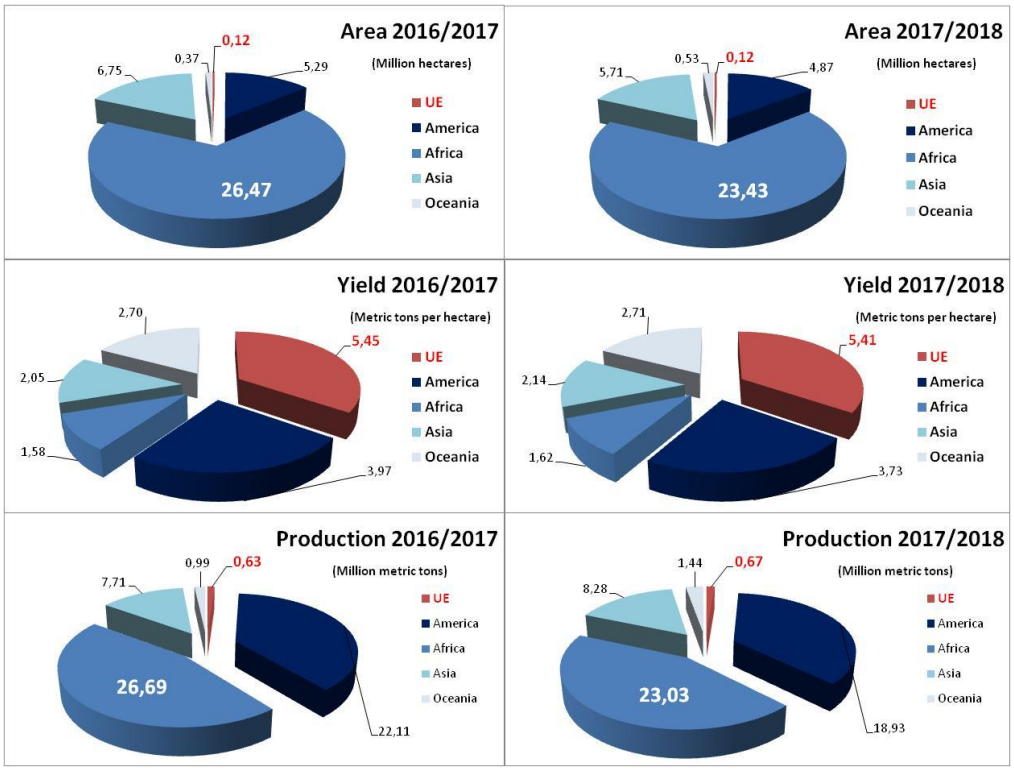

Figure 4. *Pie chart of area yield and production of sorghum crops in 2016/2017 year and preliminary data for 2017/2018 year (Foreign Agricultural Service/USDA, December 2018 report).*

The interest in sorghum for human foods has recently increased because it is considered safe for people with celiac disease (Pontieri et al., 2013). In this sense, due to its high nutritional potential and to a growing demand for gluten-free foods and beverages from people with coeliac disease and other intolerances (Taylor et al., 2006), several studies on sorghum for human consumption have been conducted in developed Countries (Ciacci, et al., 2007; Dykes, et al.,2005), and several sorghum products have been made, including bread, pasta and biscuits (Khan et al., 2013; Stefoska- Needham et al., 2016; Yousif et al., 2012).

In recent years, public and private sector sorghum breeding programs have released many improved sorghum varieties that are adapted to semi-arid and tropic environments including cultivars that meet specific food and industrial requirements; among these, sorghum hybrids with white grain from a tan-color plant (often called "food-grade" sorghum) for production of gluten-free foods (Tuinstra, 2008). Furthermore, epidemiological studies suggest that the consumption of whole cereal grains, including sorghum, lowers the mortality from cardiovascular disease, which is

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probably linked to their antioxidant properties (Kushi et al., 1999; Awika & Rooney, 2004).

1.2.1 Agronomic and physical characteristics of sorghum

Sorghum (*Sorghum bicolor* (L.) Moench) (Fig.5) is a tropical C4 cereal grass, belonging to the Poaceae family (Tab.1), related to sugar cane and maize. Like maize, which in many respects it resembles, it is diploid with 2n = 2x = 20 chromosomes, with a relatively small genome of 740 Mb (Paterson et al., 2009), only one-third the size of maize.

Figure 5. *Panicles of sorghum hybrids at Azienda Sperimentale Inviolatella (CREA-IT, Rome).*

The genus *Sorghum* (Tab.1) is spread across the world but the important species for cultivation is *Sorghum bicolor*.

Table 1. *Botanical classification of the Sorghum bicolor.*

Class	Liliopsida (Monocotyledones)
Order	Poales
Family	Poaceae
Sub-Family	Panicoideae
Species	*Sorghum vulgare*
Synonymous	*Sorghum bicolor (L.) Moench.*

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The species comprises an extremely variable group of cultivated and wild types genotypes, categorized into five separate races and nine intermediate races. Around the world, there are over 7,000 varieties of sorghum (Kangama & Rumei, 2005).
Sorghum can usefully be categorized according to end use:

- Broom sorghum (*Sorghum vulgare* var. *technicum*): the inflorescences, deprived of the grain, and dried, are used for brooms and brushes;
- Sweet sorghum (*Sorghum vulgare* var. *saccharatum*): they are very tall plants, with big buds and wide leaves, juicy and sugary stems due to the presence in the marrow of considerable quantities of sucrose (15-20%). This sucrose is always accompanied by significant amounts of invert sugar that inhibits crystallization, therefore the sugar sorghum is not suitable for sugar production, but it is used for the syrups or alcohol industries. The best varieties have been selected in the United States, and is also grown in Italy;
- Forage sorghum (*Sorghum vulgare* var. *saccharatum* and *Sudan grass*): the plant in its milky or waxy phases lends itself well to feeding livestock. The plant has slender culms and considerable biomass with high sugar content (ensiling);
- Grain sorghum: come from different hybrids, low plants (1-1.5 m), limited tillering, naked kernels, without tannins. They are grown for their grain that is widely used for human food in developing Countries or for feeding livestock in developed Countries;
- Grassy sorghum: tall stems for grazing silage and hay.

As for the other cereals, the edible part in the sorghum is represented by the seeds. The kernel is a naked caryopsis, typically 2-5 mm in length and 2-3 mm thick at the widest point (Taylor & Belton, 2002). The kernel weight varies from 15 to 40 mg, and present an extreme variability of the morphological characteristics, both in the form (round, apiculata), and in the color (white, orange, rosy, red, brown) (Figs. 5 and 7). Each panicle brings to maturation from 1,500 to 2,500 kernels.
The sorghum grain has three distinct anatomical structures called pericarp, endosperm and germ (Fig.6). Some varieties have a fourth structure called testa, located between the pericarp and the endosperm (Earp et al., 2004). Testa is an inner integument, which

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separates the pericarp from the aleurone layer, it is generally very thin and almost invisible in low tannin sorghum varieties (Taylor & Belton, 2002), whereas it is darkly pigmented and generally thicker in the high tannin sorghums (Serna-Salvidar & Rooney, 1995).

The aleurone layer consists of a single layer of cells, which contains proteins and oil. The main endosperm contains protein bodies and starch granules that are tightly packed in a continuous matrix (Taylor & Belton, 2002).

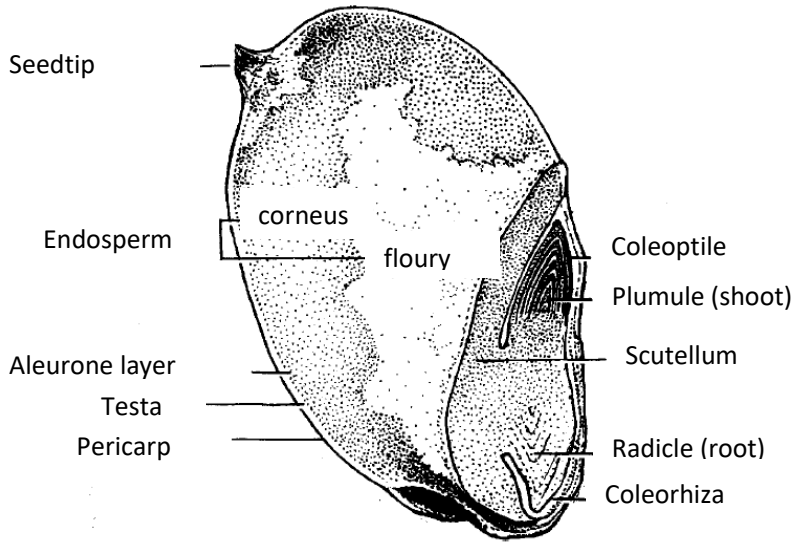

Figure 6. *Section of sorghum kernel.* Manual of good practice for the integrated production of sorghum (Guiducci M., 2000*).*

Generally, the pericarp and the testa are composed of non-starch polysaccharides, phenolic compounds (3-deoxyanthocyanidins, tannins and phenolic acids), and carotenoids. The starch, proteins, B-complex vitamins, and minerals are located in the endosperm (storage tissue); moreover, the germ (embryo) is composed of lipids, fat-soluble vitamins, B-complex vitamins, and minerals (Earp et al., 2004; Slavin 2004; Waniska & Rooney, 2000). However, the proportion and chemical composition of sorghum anatomical structures depend on the variety and growing conditions (Waniska & Rooney, 2000).

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Figure 7. *Sorghum plants with two different colors: on the left hybrids with tannins (red panicles), on the right, without tannins (white panicles).*

The sorghum appears to be very similar to maize, but, in order to germinate, the sorghum plant needs soil temperature of at least 14 °C, whereas for the corn 12 °C are sufficient. Sorghum is also well suited to heavy clayey with a mediocre structure; it tolerates a wide range of acidity (from pH 5.5 to 8.5) and high salinity. Sorghum maturation goes through the same phases described for other cereals: milky, waxy and physiological maturation. The biological cycle of sorghum is very similar to maize. The periods of achievement of the different stages of development are very variable in relation to the time of sowing, the environment and the precocity of the variety; the complete cycle in Italy ranges from 90 days in the early varieties to over 150 days in the late varieties (Fig.8).

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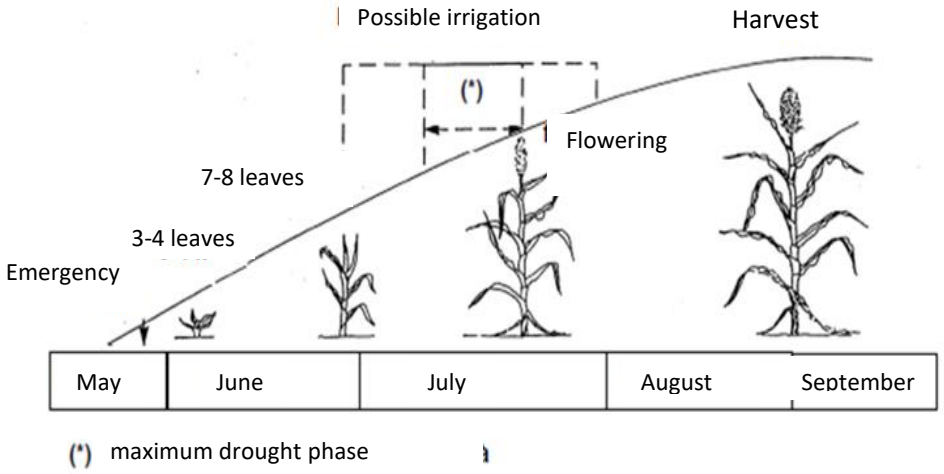

Figure 8. *Vegetative cycle of sorghum from 'Manual of good practice for the integrated production of sorghum' (Guiducci M., 2000).*

Once the physiological maturation has been reached, the grain is rapidly dried, and in the early varieties cultivated in Italy, the desiccation is complete, so at harvest the moisture of the grain is almost lower than the standard value of 14% (Guiducci, 2000). The sorghum harvest occurs using the same combine harvesters of wheat.

The average productions are variable as sorghum is a dry summer crop. However, with the refinement of cultivation techniques (especially about sowing and the choice of hybrids) and the concentration of the crop in the most suitable areas, sorghum can provide permanently yields of 5-6 t ha^{-1}without irrigation subsidy, with tips of 8-9 t ha^{-1} in favorable environments or with limited irrigation aid. Making a comparison with the dry maize crop, of which sorghum should be the substitute, it is interesting to highlight that in favorable environments the sorghum is similar to corn in production, whereas in drought years it surpasses it a lot, because sorghum plant is able to enter into vegetative stasis, slowing down the vital processes and then recovering them with limited damage as soon as the most favorable water conditions have been restored (in corn water stress stops the growth irreparably).

Thus, this cereal represents a valid alternative to maize especially in areas where poor irrigation, reduced summer rainfall and infestations of *Fusarium* make it unproductive. The precise reasons of sorghum drought tolerance are not known, but this ability is due to physiological and morphological causes. Sorghum often has deeper penetrating and more extensive roots. Apparently, it conserves moisture by reducing transpiration

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when stressed by leaf rolling and closing stomata; higher than normal levels of epicuticular wax appear to be of importance in this respect (Jordon & SuHivan, 1980). Sorghum appears to have a higher capacity for osmotic adjustment to water stress (the accumulation of sugars and amino acids in cells to hold water and maintain turgor pressure) than maize (Nguyen et al., 1997).

Among the abiotic adversities, which can damage sorghum, there are excessive rainfall during germination and low temperatures at the beginning of the vegetative phase, which can accentuate the attacks of aphids on young seedlings. In fact, the seed in germination is very sensitive to excess water and low temperatures that cause severe thinning and scalar births, often compromising the uniformity of the crop. Furthermore, the plant lodging due to bad weather events causes damage to the crop as the interruption of apical dominance can lead to the emergence of adventitious shoots and affects the harvest, however this phenomenon does not occur in low and robust varieties.

Different varieties of sorghum, despite having optimal components for feeding, are characterized by the presence of tannins. Although tannins play a role of considerable importance before harvesting, as they give the grain characteristics of repellency to the attack of birds and resistance to mold, currently the market requires grains of sorghum free of tannins, focusing on white or rosy grain types.

The sorghum grain to be marketable in the European Union must have a low tannin content, which presence lowers the digestibility of proteins. Therefore, the hybrids that had been selected for high tannin content, in order to make them resistant to the predation of the birds (BR: Bird Resistant hybrids), at present are dismissing. All sorghum hybrids currently on the market, in fact, are free of tannins, regardless of the color of the grain. Moreover, as sorghum is a safe cereal for celiac patients (Ciacci et al., 2007), its flour is an attractive alternative to wheat flour for the celiac market because of its neutral flavor and the use of hybrids with a white pericarp. Indeed, these white grain sorghum lines produce a flour similar for the color to wheat flour (De Mesa et al., 2010).

The sorghum crop, as for the maize, must include hybrid varieties, for their superior qualities of uniformity and vigor, and therefore of productivity. Sorghum hybrids, which experience has shown to be the most suitable and reliable in Italy, are those with

a medium-early cycle corresponding to 105-110 conventional days (Bonciarelli & Bonciarelli, 1994). In the last 10 years the agricultural area destined to cereal crops decreased from a total surface of about 4 million hectares in 2008 to just over 3 million in 2017, almost 25% in less (Istat 2017) (Fig.9).The decrease concerned the main crops such as wheat, barley, maize and oats, almost unaltered for rice, whereas a slight increase is highlighted for sorghum, from 38600 to 40900 ha (+16.7%) and in other cereals (Fig.9).

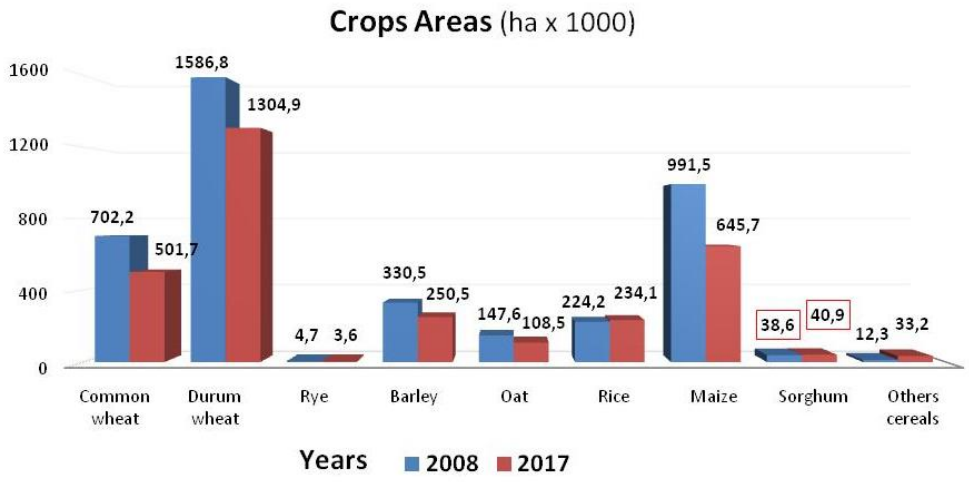

Figure 9. *Histograms of major cereal crops areas in Italy in 2008 (blue) and 2017 years (red) (Istat 2017).*

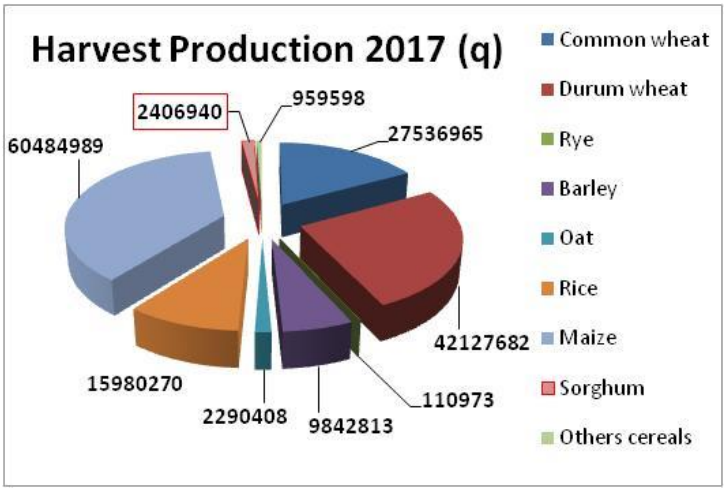

Figure 10. *Pie chart of major cereal harvest production in Italy in 2017 year (Istat 2017.)*

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However, the sorghum crop remains somewhat marginal in Italy, in 2017, its production at harvest was equal to just over 2.4 million quintals, representing about 1.5% of total cereal production (Fig.10), three quarters of this come from Emilia Romagna (Istat 2017).

1.2.2 Chemical and nutritional composition of sorghum

The chemical composition of whole sorghum is very interesting from a nutritional point of view for its contents in fibers, proteins, lipids and polysaccharides; moreover, energy value of 100g of sorghum grains varies between 296 and 356 kcal (Martino et al., 2012). In this crop, starch and proteins are more slowly digested than that of other cereals. Furthermore, most sorghum varieties are rich in phenolic compounds, especially 3-deoxyanthocyanidins and tannins, and they are a source of some minerals and vitamins.

1.2.2.1 Carbohydrates

The content and composition of starch are influenced by the genetic characteristics and growing conditions of the grain (Hill et al., 2012). The starch content of sorghum resides mainly in the endosperm and is distributed between the floury endosperm in the middle of the seed and the glassy endosperm in the outer regions. The granules have a large range of sizes (from 30 to 2-3 µm), with typical values about 10-16 µm range, often misshapen due to the compressive effects of contact with the protein bodies and as a result may take on complex shapes (Taylor & Belton, 2002). It is characteristic of all starches that they swell when exposed to hot water and, if the temperature increases sufficiently, they will gel. Typical gelling temperatures for sorghum starch cover the range 68-78°C for onset to completion of gelation; this is similar but slightly higher than the range for normal maize starch (Becker & Hanners, 1991).

In some varieties, starch ranges between 32 and 72.5 g/100g and is composed mainly of amylopectin (81.0-96.5%) and amylose (3.5-19.0%) (Shegro et al., 2012; Udachan et al., 2012). The proportion of these two components affects the rheological proprieties as gelatinization, retrogradation and gelling, and consequently the digestibility of sorghum starch (Sang et al., 2008; Singh et al., 2010a). Moreover,

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among cereals, sorghum has the lowest starch digestibility due to the strong association between its granules and proteins and tannins (Barros et al., 2012; Mkandawire et al., 2013).

The non-starch polysaccharides of sorghum (6 to 15 %) include insoluble fibers (75-90%), mainly arabinoxylans, and soluble fibers (10-25%) (Martino et al., 2012; Taylor & Emmambux, 2010). The total sugars content present in the sorghum varies according to the different stages of plant development and the main sugars found in the mature seed are sucrose, glucose and fructose. Raffinose, glucose and fructose are present in greater quantities in the varieties of sugary sorghum.

1.2.2.2 Proteins

Sorghum grain has protein content varying from 7% to 15%, with an average of 10% (Bean et al., 2011). Sorghum proteins can be broadly classified into prolamin and non-prolamin proteins. Kafirins, the major storage proteins, are classified as prolamins, and as such, they contain high levels of proline and glutamine and are soluble in nonpolar solvents such as aqueous alcohols. Kafirins account for 77% to 82% of the protein in the endosperm, whereas non-prolamin proteins (albumins, globulins, and glutelins) make up about 30% of the proteins (Belton et al., 2006).

Since maize and sorghum are closely related genetically, the large volume of research on maize prolamins, called zein, has served as a framework for studying kafirins. Shull and others (1991) utilized procedures developed for maize to characterize the proteins of sorghum based on solubility, molecular weight, and structure. Thus, kafirins are classified in α (66-84%), β (8-13 %), γ (9-21%) and δ (low levels). The α-kafirins are divided into 2 groups of polypeptides with molecular weights (MW) of 23 and 27 kDa and they are rich in nonpolar amino acids and are found primarily as monomers and oligomers. These proteins do not crosslink extensively and form mainly intramolecular disulfide bonds. The β-kafirins have a MW of approximately 18 kDa, they are rich in the sulfur-containing amino acids methionine and cysteine, and they are found in monomeric and polymeric forms. The γ-kafirins have a MW of approximately 28kDa and they are rich in the amino acids proline, cysteine, and histidine. These subunits are found as oligomers and polymers. Both β- and γ-kafirins form intermolecular and intramolecular disulfide bonds and they are highly crosslinked. The δ-kafirins have a

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MW of about 13 kDa and they are rich in methionine (Belton et al., 2006; Bean et al., 2011). The kafirins are stored in the endoplasmic reticulum in spherical protein bodies, between 0.4 to 2 μm in diameter (Taylor et al., 1984), where the α-kafirins compose the core and the β- and γ-kafirins decorate the periphery of these protein bodies, which are embedded in a glutelin protein matrix and surrounded by starch granules. It is thought that the organizational structure of the protein bodies has a major impact on protein digestibility of sorghum food and feed products (Hicks et al., 2001). Overall, the prolamins of sorghum, rich in glutamic acid and non-polar amino acids (proline, leucine and alanine), are almost totally free of essential amino acids such as lysine. Taylor and Belton (2002) reported an indicative table of the amino acid composition of the different kafirine forms compared to the homologous corn proteins (Tab.2). In recent years, the importance of kafirins as a potential source of protein for gluten-free products has been recognized (De Mesa-Stonestreet et al., 2010). Yet, the low digestibility of kafirins (Duodu et al., 2003; Afify et al.,2012b) and the poor functionality due to their encapsulation in protein bodies (Hamaker & Bugusu, 2003) hinder their use in food systems.

Table 2. *Comparison of amino acid content (% moles) in kafirins and zeins. (Taylor & Belton, 2002).*

Amino acid	α-Zein	α-Kafirin	β-Zein	β-Kafirin	γ-Zein	γ-Kafirin
Asn	5.3	6	2.5[a]	3.3[a]	0	0
Asp	0	0.4			0	0
Thr	2.8	4	2.5	4.6	4.4	4.7
Ser	6.9	6	5	4.6	3.9	5.2
Gln	20.7	24.6	18.1[b]	17.79[b]	14.7	11.9
Glu	0.8	0.4			1	1
Pro	8.9	7.7	8.8	9.7	25	23.3
Gly	0.8	1.6	8.8	6.8	6.4	8.8
Ala	13.8	14.9	13.8	13.4	4.9	5.7
Cys	0.4	0.4	4.4	4.9	7.4	7.8
Val	6.9	4.4	1.9	5.2	7.4	6.2
Met	2	0.8	11.3	5.7	0.5	1
Ile	4.5	5.6	0.6	2.3	2	2.6
Leu	17.1	15.3	10	12	9.3	8.3
Tyr	2.8	2.8	8.8	3	2	2.1
Phe	3.3	2.4	0	1.9	1	1.6
His	1.2	1.2	0	0.9	7.8	7.8
Lys	0	0	0	0.5	0	0
Arg	1.6	0.8	3.1	2.7	2.5	2.1
Trp	0	0.4	[c]	[c]	0	0

[a] Asn + Asn expressed as Asn.
[b] Gln + Glu expressed as Gln.
[c] Not given.

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Thus, several approaches to achieve efficient food-grade extraction methods to isolate functional kafirins have been performed (Espinosa-Ramírez et al., 2017).

Moreover, the digestibility of sorghum proteins, especially after cooked, is lower than cereals like wheat and maize (Moraes et al., 2012), indeed the kafirins are resistant to peptidase due to the formation of intramolecular disulfide bonds (Belton, et al., 2006), making them less available for the body to use.

In addition, Duodu et al. (2003) reviewed also exogenous factors for sorghum protein digestibility, i.e. interactions of proteins with non-protein components such as polyphenols, starch, non-starch polysaccharides, phytates and lipids, and cooking enhanced these interactions reducing protein digestibility, hence the poor digestibility appears to be multi-factorial.

For celiac patients who typically suffer from malnutrition due to poor nutrient absorption, it is even more important for nutrients to be more readily available. Thus, a challenge exists to make sorghum proteins more digestible.

The studies on the highly digestible mutant sorghum cultivar P851171 showed that the invaginations with a greater surface area for enzymatic digestion gave highly digestible α-kafirins, more homogenously dispersed throughout the interior of the protein body rather than simply localized in the central portion; and the poorly digestible γ-kafirins were concentrated at the base of the invaginations of the protein body rather than at the protein body periphery encapsulating α-kafirins like in normal sorghum cultivars (Oria et al., 2000).

Starch affects sorghum protein digestibility differently. Although the bulk of the literature suggests that sorghum proteins inhibit starch gelatinization and its digestion (Duodu et al., 2002; Ezeogu et al., 2008), the presence of starch mutually reduces sorghum protein digestibility (Duodu et al., 2003; Wong et al., 2009).

Oom and others (2008) studied the rheological properties of kafirins in a viscoelastic dough system. Their study showed that although the extensional viscosity of isolated kafirin dough immediately after mixing was similar to those found in gluten-based dough, it became rapidly stiff over time, probably due to disulfide crosslinking of kafirin monomers. When kafirin was mixed with starch and water, however, no dough could be formed. The researchers inferred that kafirin's inability to form composite viscoelastic doughs could be a result of its extremely hydrophobic nature.

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Unlike the major prolamins of wheat (glutenins and gliadins), rye (secalins), and barley (hordeines), the kafirins do not trigger an allergic response or an autoimmune reaction in humans (De Mesa-Stonestreet et al., 2010). In addition to the qualitative evidence based on the type of proteins found in sorghum, there is genetic evidence that it has characteristics that do not allow the expression of toxic peptides related to gliadin (Pontieri et al., 2013). Thus, sorghum is an effectively safe cereal for consumption by people with celiac disease.

1.2.2.3 Lipids

The lipids of sorghum like those of other cereals are mainly located in the germ although there are smaller amounts present in the endosperm (Taylor & Belton, 2002). Therefore, most of the lipid content is removed as a result of the decortication process (Smith et al., 2000).

In sorghum the variation of total lipid content (range from 2 up to 6.6% of dry seed) (Taylor & Belton, 2002) and their composition, mainly composed of unsaturated fatty acids (83-88%), depends on cultivar type, location and also on the methods of extraction (Osagie et al., 1987).

The three major classes of lipids may be classified as non-polar lipids, glycolipids and phospholipids. In most of the varieties of sorghum the polyunsaturated fatty acids (PUFA) are higher than monounsaturated fatty acids (MUFA) (Afify et al., 2012a). The principal fatty acids of sorghum are linoleic (46-51%), oleic (32-42%), palmitic (12-16 %), and linolenic acids (1.4-2.8%) (Cardoso et al., 2017).

Lee et al. (2011) reported that the total lipid content in the whole grain of sorghum is comparable or superior to that of other common cereals, such as maize (4.7%), wheat (1.7%), barley (1.2%) and rice (0.6%).

As for other cereals, the triacylglycerols, which constitute the energy reserve for germination, represent the group of most abundant lipids in sorghum seeds (10-25%), and are represented by both oils and waxes. Linoleic acid, oleic acid and palmitic acid are reported as the main fatty acids present in the sorghum seed, but their relative percentages vary, probably due to intrinsic and extrinsic factors (Lee et al., 2011).

The data on the sterols, on the other hand, are inferior, even if the attention to these compounds is increasing given the relationship between phytosterols and some

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beneficial effects on health, such as the lowering of cholesterol levels and possible anti-tumor properties. According to the evidence reported for other cereals, even in sorghum the sterols exist in free and in esterified form, but the total content of these compounds is in any case lower than that of maize (Lee et al., 2011). The main sterols in sorghum is β-sitosterol (54%), followed by the stigma sterol (23%). It is also reported in the literature that sorghum contains from 0.03 to 0.08mg per 100g of α-tocopherol. The waxes are on the outer side of the caryopsis in an amount equal to 0.2% (Smith et al, 2000).

1.2.2.4 Minerals and vitamins

The mineral and vitamin compositions of sorghum are similar to that of maize.
Sorghum is a source of minerals (phosphorus, potassium, and zinc) whose content varies according to the place of cultivation (Martino et al., 2012; Shegro et al., 2012; Silva et al., 2012), while calcium and sodium are low (Smith et al., 2000).
The bioavailability of most minerals of sorghum is still little known and information on the content of vitamins is scarce; some B-complex vitamins (thiamine, riboflavin and pyridoxine), except vitamin B12 (Smith et al, 2000), and fat soluble vitamins (D, E, and K) are present in this cereal (Cardoso et al., 2014; Martino et al., 2012; Ochanda, et al., 2010).
Smith et al. (2000), reported that mature kernels do not contain vitamin C, and the decortication process reduces the content of fat-soluble vitamins, which are mainly found in the germ, whereas only the caryopsis with the yellow endosperm have pro-vitamin A activity. Niacin, on the other hand, is present in free form and in a bound form and alkaline treatment promotes bioavailability.

1.2.3 Bioactive Compounds in sorghum

Recently, interest in bioactive compounds has increased exponentially since it is understood how they can beneficially influence human health. Although they are not nutritious in the classical sense, they are defined as substances capable of modulating numerous biological activities and important functions of the organism. Bioactive components (or phytochemicals) include polyphenols, carotenoids, fiber and resistant starch.

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Baye (2014) underlines how, among dietary factors, phytochemicals, such as polyphenols and phytates, constitute major mineral absorption inhibitors and hence were, for a long time, referred to as anti-nutritional factors. However, in recent years, the recognition of their health promoting effects including anti-diabetic, anti-cancer and antioxidative properties made the term anti-nutritional factor obsolete.

1.2.3.1 Phenolic Compounds

Phenolic compounds are the most widely distributed secondary metabolites, ubiquitously present in the plant kingdom; they help in the natural defense of plants against pests and diseases. Located in the vacuole, they are found in free form or linked to carbohydrates (glucose, galactose, rhamnose, mannose, rutinose etc.).

The phenolic compounds are the main bioactive compounds of sorghum with a variety of genetically dependent types and levels including phenolic acids, flavonoids and condensed tannins.

Among cereals, sorghum has the highest content of phenolic compounds reaching up to 6% (w/w) in some varieties (Awika & Rooney, 2004). While all sorghums contain phenolic compounds, its genotype and the environment in which it is grown influence the amount present in any cultivar.

As reported by Dykes & Rooney (2006) all sorghums contain phenolic acids and most contain flavonoids, but only varieties with a pigmented testa have condensed tannins. The types and quantities of phenols present in the grain are genetically controlled. Sorghum genetics relevant to tannins and phenols has been reviewed by Rooney et al. (1982). The pericarp color of the sorghum kernel is controlled by the *R* and *Y* genes. A pericarp is white when *Y* is homozygous recessive (*rryy* or *R_yy*), whereas a yellow pericarp has recessive *R* and dominant *Y* genes (*rrY_*). When both *R* and *Y* genes are dominant, the pericarp is red. The intensifier gene *I* affects the intensity of the pericarp color and is most apparent in red sorghums (Dykes & Rooney, 2006). Pericarp color is not a reliable indicator of tannins in sorghums (Boren & Waniska, 1992). It is erroneously believed that all sorghums with a red/brown pericarp contain tannins. Sorghums with white, yellow, red, or brown color pericarp may or may not have tannins depending upon the presence of a pigmented testa, which is controlled by the *B1* and *B2* genes. Sorghums with a pigmented testa must have both dominant genes

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(*B1_B2_*). The spreader gene S controls the presence of brown pigments, possibly tannins, in the epicarp and endocarp when a pigmented testa is present (Blakeley et al., 1979). The levels of condensed tannins are highest in sorghums containing dominant *B1_B2_SS* genes; these sorghums have high bird and mold resistance.

Sorghum varieties are divided into three groups based upon their genetics and chemical analyses (Rooney et al., 1982). Type I sorghums (*b1b1B2_*, *B1_b2b2*, *b1b1b2b2*) do not have a pigmented testa, and contain low levels of phenols and no tannins. Types II and III both have a pigmented testa and contain tannins. The tannins in Type II sorghums (*B1_B2_ss*) are extracted with acidified methanol (1% HCl methanol) while the tannins in Type III sorghums (*B1_B2_S_*) are extracted with either methanol or acidified methanol when using the vanillin/HCl assay.

1.2.3.1.1 Phenolic acids

All sorghums contain phenolic acids (PA), that, like other phenols, are thought to help in plant defense against pests and pathogens (Dyckes & Rooney, 2006).

Phenolic acids consist of two classes: hydroxybenzoic, directly derived from benzoic acid, including gallic, p-hydroxybenzoic, vanillic, syringic, and protocatechuic acids, and hydroxycinnamic acids, a C6–C3 structure including coumaric, caffeic, ferulic, and sinapic acids.

Hahn et al. (1984) identified free and bound phenolic acids in sorghum. Free phenolic acids were found in the outer layers of the kernel (pericarp, testa, and aleurone), whereas the bound phenolic acids, the most frequent form, were associated with the cell walls with ferulic acid being dominant (24–47%).

The content of phenolic acids in some sorghum varieties ranged between 135.5 and 480 µg/g (Afify, et al., 2012c; Chiremba, et al., 2012), with major amounts of the protocatechuic (150 to 178 µg/g) and ferulic (120.5 to 173.5 µg/g) acids and small amounts of the p-coumaric (42 to 72 µg/g), syringic (15.5 to 17.5 µg/g), vanillic (15 to 23 µg/g), gallic (15 to 21.5 µg/g), caffeic (13.5 to 21 µg/g), cinnamic (10 to 15.0 µg/g), and phydroxybenzoic (6 to 16.5 µg/g) acids (Afify, et al., 2012c; Svensson, et al., 2010).

The PA show good antioxidant activity *in vitro* and thus may contribute significantly to the health benefits associated with whole grain consumption, especially in white

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sorghum varieties which normally show very low levels of flavonoid content (Awika & Rooney, 2004).

1.2.3.1.2 Flavonoids and sorghum anthocyanins

Most flavonoids of the sorghum are located in the outer layers of the grain. Thus, differences in the color and thickness of the pericarp and presence of the testa influence the concentration and profile of flavonoids (Awika et al., 2005; Dykes et al., 2009). Three classes of flavonoids are in large quantities in sorghum: anthocyanins, flavones and flavanones.

Many sorghum flavonoids have been isolated and identified, among these the anthocyanins are the major class studied in sorghum.

Anthocyanins have been extensively detected in fruits and vegetables due to their antioxidant properties and potential as natural food colors. In general, this class of compounds contributes the blues, purples and reds in plants. The six common anthocyanidins are cyanidin, delphinidin, malvidin, pelargonidin, petunidin, and peonidin.

Unlike these common anthocyanins, sorghum anthocyanins are unique since they do not contain the hydroxyl group in the 3-position of the C-ring (Fig. 11) and thus are called 3-deoxyanthocyanins, corresponding up to 79% of the flavonoids' content (Dykes & Rooney, 2006; Shih et al., 2007).

Therefore, sorghum 3-deoxyanthocyanidins have a differentiated structure due to the absence of the hydroxyl group and this unique feature increases their stability at high pH compared to the common anthocyanins, which render these compounds as potential natural food colorants (Awika & Rooney, 2004; Dykes et al., 2009).

The most common sorghum3-deoxyanthocyanidins are the yellow apigeninidin, and the orange luteolinidin (Awika et al., 2004a,b). Moreover, sorghums with a black pericarp present the highest levels of 3-deoxyanthocyanins; indeed it was detected that varieties with pericarp and black testa have 3 to 4 times more total 3-deoxyanthocyanidins than red and brown varieties, which are concentrated in the bran (Awika et al., 2004a,b; Dykes et al., 2005).

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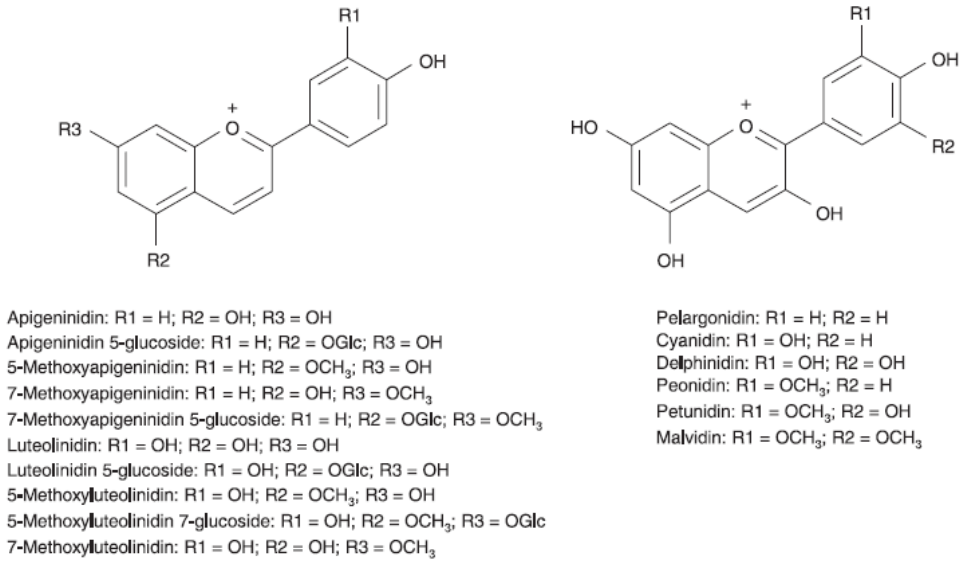
Finally, the 3-deoxyanthocyanidins are also phytoalexins since they are produced as a response to mold invasion or other stresses in sorghum (Lo et al., 1999; Seitz, 2004; Waniska & Rooney, 2000).



Apigeninidin: R1 = H; R2 = OH; R3 = OH
Apigeninidin 5-glucoside: R1 = H; R2 = OGlc; R3 = OH
5-Methoxyapigeninidin: R1 = H; R2 = OCH_3; R3 = OH
7-Methoxyapigeninidin: R1 = H; R2 = OH; R3 = OCH_3
7-Methoxyapigeninidin 5-glucoside: R1 = H; R2 = OGlc; R3 = OCH_3
Luteolinidin: R1 = OH; R2 = OH; R3 = OH
Luteolinidin 5-glucoside: R1 = OH; R2 = OGlc; R3 = OH
5-Methoxyluteolinidin: R1 = OH; R2 = OCH_3; R3 = OH
5-Methoxyluteolinidin 7-glucoside: R1 = OH; R2 = OCH_3; R3 = OGlc
7-Methoxyluteolinidin: R1 = OH; R2 = OH; R3 = OCH_3

Pelargonidin: R1 = H; R2 = H
Cyanidin: R1 = OH; R2 = H
Delphinidin: R1 = OH; R2 = OH
Peonidin: R1 = OCH_3; R2 = H
Petunidin: R1 = OCH_3; R2 = OH
Malvidin: R1 = OCH_3; R2 = OCH_3

Figure 11. *Structures of the 3-deoxyanthocyanidins and their derivatives reported in sorghum compared to the six common anthocyanidins found in fruits, vegetables and some cereals.*

1.2.3.1.3 Tannins (proanthocyanidins)

Sorghum contains polyphenolic compounds called condensed tannins (proanthocyanidins), originally classified as antinutritional factors because they bind to dietary proteins, digestive enzymes, minerals such as iron and B vitamins like thiamin and vitamin B6, but in the last years re-evaluated for possible beneficial effects on human health (Parr & Bolwell, 2000; Awika et al. 2004a).

The tannins in high tannin sorghum are agronomically advantageous as they protect the crop against birds, pre-harvest germination, weathering effects and likely fungi and bacteria (Serna-Saldivar & Rooney, 1995).

They are present in sorghums having a pigmented testa, occur primarily in the pigmented inner integument (testa) and to a limited extent in the pericarp, and are

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absent in white and colored sorghums without a pigmented testa (Anglani, 1994; Taylor & Belton, 2002).

Tannins of sorghum are almost exclusively of the ‘‘condensed’’ type. They are mainly polymerized products of flavan-3-ols and/or flavan-3,4-diols. Glycosylated and non-glycosylated polymers of flavan-4-ols with various substitution patterns have also been reported in sorghum (Gujer et al., 1986).

Tannins are the most important phytochemical components of sorghum since they possess properties that produce obvious and significant effects in animals and have also been associated with various positive and negative impacts on human health. Even though tannins are commonly associated with sorghums, more than 99% of sorghum currently produced in the US is tannin-free. Decades of breeding efforts to eliminate tannins from sorghum were motivated mostly by the reduced feed value of the tannin sorghums. Tannins bind to and reduce digestibility of various food/feed nutrients, thus negatively affecting productivity of livestock. However, in many other parts of the world where pests and diseases are common, tannin sorghums are still grown in significant quantities since they are more tolerant of such conditions than the non-tannin varieties (Awika & Rooney, 2004).

As already described above, based on extractable tannin content, sorghums have been classified as type I (no significant levels of tannins extracted by 1% acidified methanol), e.g., TX2911 (red pericarp), type II (tannins extractable in 1% acidified methanol and not methanol alone), e.g. Early Hegari and, type III (tannins extractable in both acidified methanol and methanol alone), e.g. Early Sumac variety (Cummings & Axtel, 1973; Price et al., 1978). However, this classification does not account for the varying levels of other major phenolic constituents, especially anthocyanins. Another broad way to classify sorghum is based on both appearance and total extractable phenols: white sorghums (also called food-type) with no detectable tannins or anthocyanins and very low total extractable phenol levels; red sorghums which have no tannins but have a red pericarp with significant levels of extractable phenols; black sorghums with a black pericarp and very high levels of anthocyanins and the brown sorghums which have a pigmented testa and contain significant levels of tannins, with varying degrees of pericarp pigmentation.

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1.2.3.2 Carotenoids

Carotenoids are pigments naturally present in plants, liposoluble, yellow or orange. They have a terpene structure and are classified into two large structural groups: carotenes, which have a hydrocarbon molecular structure (beta carotene and lycopene), and xanthophylls, such as lutein, zeaxanthin, luteoxanthine and neoxanthine. Several studies have linked rich diets in carotenoids to a reduced risk of developing various chronic and degenerative diseases, including cancer and cardiovascular disorders (Kean et al., 2007).

Yellow endosperm sorghums contain carotenoids, including β-carotene which is considered the most important precursor of vitamin A, since one molecule can potentially be transformed into two molecules of retinol. Vitamin A deficiency affects approximately 250 million people in semiarid regions of Africa and Asia, where sorghum (*S. bicolor* Moench) is a major staple crop, even though β-carotene content would not be sufficient to cover daily requirement of vitamin A.

Several studies have been carried out on carotenes, Reddy et al. (2005) reported that carotene in raw sorghum ranged from 0.6 to 1.1 mg/kg, also, Salas Fernandez et al. (2008) found that yellow endosperm sorghum had β-carotene ranged from 0.2 to 3.2 mg/kg, and Afify et al. (2012c) found similar values (0.5-1.2 mg/kg) in their white sorghum varieties. Furthermore, Thaddi & Nallamilli in a 2014 study compared the carotenoid content in sorghum varieties with and without pigmented head, and report that pigmented genotypes had higher carotenoid values than those without.

1.2.3.3 Fiber

Sorghum is a rich source of fiber. Fiber is a plant material that is resistant to digestive enzymes of the gastro-intestinal tract. The main components of the fiber are cellulose, hemicellulose, lignin and pectin, which are located in the pericarp and in the endosperm cell wall (Smith et al., 2000), with a structural role and a protective function.

Fibers can be divided into two main categories: soluble fibers and insoluble fibers. Sorghum contains high levels of insoluble fiber (86% of the total fiber) and low levels of soluble fiber, with low levels of β-glucans, the main components of the latter.

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Sorghum contains from 6.5 to 8% of insoluble fiber and from 1.1 to 1.23% of soluble fiber.

The main components of sorghum fiber are cellulose and pentosans. The cellulose levels reported are between 1.2 and 5.2% (Ragaee et al., 2006). The pentosane content of integral sorghum ranges from 2.5 to 5.5%, the variation of which depends on environmental factors and on the variety (Ragaee et al., 2006).

Thus, the fiber content in the sorghum products mainly depends on the degree of removal of the pericarp during milling (Smith et al., 2000).

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1.3 TEFF [ERAGROSTIS TEF/(ZUCC.) TROTTER (1918)]

Teff [*Eragrostis tef* (Zucc.) Trotter] is an ancient tropical cereal that has its center of origin and diversity in the Northern Ethiopian highlands from where it is believed to have been domesticated (Seyfu, 1997; Demissie, 2001). Teff is a minor cereal crop worldwide, whereas in Ethiopia constitutes the staple food for more than half of the Ethiopian population, mainly used to make injera, a traditional fermented Ethiopian pancake. In this Country, teff crop occupies an estimated annual acreage of more than 3 million ha of the 8 million hectares used to cultivate cereals, annually (Central Statistical Agency, CSA, 2005–2013); approximately 3.8 million tons of the crop is produced per year, reaching 4.2 million tons in 2015 in Ethiopia (Ethiopian Agricultural Transformation Agency, 2016), and most is used for domestic consumption. Nutritionally, teff contains higher amounts of the essential aminoacids, its lysine content is higher than that of all other cereals except rice and oats, and its mineral content is substantial (Seyfu, 1997). Teff holds a central place in Ethiopia's cultural dishes and fetches the highest market price among cereals. While teff is Ethiopia's key cereal in terms of cultivation, contribution to food security, nutrition and culture, teff has seen little improvement in terms of breeding, for example for the development of lodging-resistant, high-yielding varieties. The crop is versatile in that it is adapted to diverse agro-ecological conditions due in part to its resilience to abiotic stresses such as drought and water logging (Assefa et al., 2011). Teff cultivation in Ethiopia is almost exclusively rain-fed, and it is grown on different soil types including poorly drained vertisols that are marginal for most other crops.

Time series data of the Central Statistical Agency (2005–2013) show that the productivity of the crop has increased by 30% in a single decade from about 1.0 t ha^{-1} in 2005 to about 1.3 t ha^{-1} in 2013. This increase in productivity can be attributed to progress made unconventional breeding and to adoption of improved varieties and production packages. Teff seeds remain viable for several years provided that direct contact with moisture and sunshine is avoided, furthermore in comparison with other common cereals, teff grain is less prone to attacks by weevils and other storage pests. Thus, it can be safely stored under traditional storage conditions with no chemical protection (Gebremariam et al., 2014).

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Teff flour is a wholemeal because of the very small grain size, hence the flour is rich in fiber due to the incorporate of the bran components, it is also a source of bioactive compounds such as polyphenols (Shumoy & Raes, 2016). As a result of the unique chemical composition and the whole grain form, a range of health benefits have been associated with teff (Zhu, 2018).

Moreover, very recently teff has gained prominence as a food crop in other parts of the world, this interest is mainly associated with its gluten-free property and its nutrient composition, comparable or better than those of other common cereals (Cheng et al., 2017).

Beyond its uses as a staple food, teff plant residues such as its straw are commonly used as fodder for livestock or construction materials to reinforce houses built from mud or plaster (Stallknecht et al., 1993). The straw can be treated for biomethane production and may be used as an adsorbent of Cr(VI) in contaminated waste waters (Chufo et al., 2015; Tadesse et al., 2015). Therefore, comprehensive and green utilization of teff without generating much waste is possible.

1.3.1 Agronomic and physical characteristics of teff

Teff is also commonly written as tef, in this thesis teff is used to follow the nomenclature of Encyclopedia of Food Grains (Bultosa, 2016).

Teff belongs to the grass family Poaceae, sub-family Chloridoideae, used synonymously for Eragrostoidae of teff (Costanza et al., 1979), tribe Eragrostidae, sub-tribe Eragrostae, and genus *Eragrostis* (Tab.3).

Table 3. *Botanical classification of Eragrostis tef.*

Class	Liliopsida (Monocotyledones)
Order	Poales
Family	Poaceae
Sub-Family	Chloridoideae (Eragrostoidae)
Genus	*Eragrostis*
Species	*Eragrostis tef* (Zucc.) Trotter

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The genus *Eragrostis* comprises about 350 species (Watson & Dallawitz, 1992). Although the crop species have had several synonyms previously used by several authors, its presently most accepted binomial nomenclature is *E. tef* (Zucc.) Trotter. In cultivation as a cereal, teff is the only species in the genus *Eragrostis* and, together with finger millet (*Eleusine crocana* L.), they constitute the sole two species in the sub-family Chloridoideae cultivated for human consumption of the grains (Assefa et al., 2011).

As already mentioned, Ethiopia is the centre of both the origin and diversification of teff, and its domestication is predicted to have occurred between 4000 and 1000 BC (Vavilov, 1951). Teff is an allotetraploid (2n=4x=40) autogamous small cereal crop widely cultivated in the Horn of Africa (Eritrea and Ethiopia), originating from two diploid progenitors (Jones et al., 1978). At present, the exact diploid progenitors of teff are still unknown, although the majority of studies on morphological, anatomical, cytological and biochemical characters suggest that teff is closely related to *Eragrostis pilosa*, a wild allotetraploid characterized by its early maturity and seed shattering. *E. pilosa* is also the only species known to be cross-compatible with modern teff varieties (Costanza et al., 1979). This evolutionary relationship was further supported by the DNA sequence data of the nuclear waxy gene and plastid locus *rps16* reported by Ingram and Doyle (2003). Hinged on partial sequences of the waxy gene, a phylogenetic tree has been constructed to reveal the evolutionary relationships between teff and other grasses (Fig.2). The closest cultivated species to teff is finger millet (*Eleusine coracana*), and the closest subfamily to teff is Panicoideae including maize, sorghum (*Sorghum bicolor*) and pearl millet (*Pennisetum glaucum*) (Cannarozzi et al., 2014).

Teff is the first sequenced member of its subfamily. The teff genome is a medium-sized tetraploid genome of approximately 0.7Gb, i.e. relatively small compared to other polyploid crops such as maize (diploid, 2.4 Gb) and wheat (hexaploid, 16.8Gb) (Cannarozzi et al., 2014).

Like sorghum and maize, teff is a C4 plant which utilizes CO_2 very efficiently during photosynthesis. It has a fibrous root system with mostly erect stems, although some cultivars are bending or elbowing types. The sheaths of teff are smooth, glabrous, open and distinctly shorter than the internodes. Its ligule is very short and ciliated while its

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lamina is slender, narrow and nearly linear with elongated acute tips. It has a panicle type of inflorescence showing different forms, from loose to compact, the latter appearing like a spike (Figs. 12A and 12B). The plant height varies from 31 to 155 cm and the panicle length from 14 to 65 cm (Dawit & Hirut, 1995). The number of spikelets per panicle ranged from 190 to 1400, and its spikelets have 2-12 florets. Each floret has a lemma, palea, three stamens, an ovary and mostly two, in exceptional cases three, feathery stigmas (Seyfu, 1997).

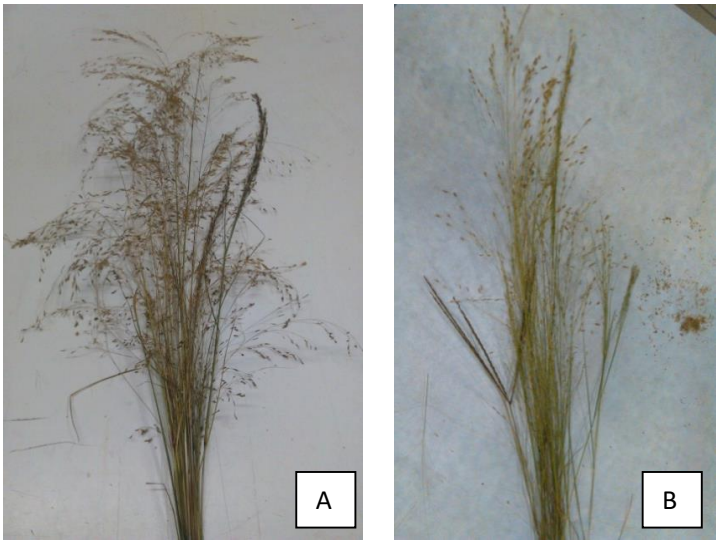

Figure 12. *Panicles of teff, white(A) and brown(B) grain, grown at CREA-IT, Rome.*

The numbers of days to heading and maturity ranged from 25 to 60 and 60 to 120 respectively (Assefa et al., 2015). Fertilization was found to occur in the basal floret of a spikelet when that floret was at the base of the flag leaf blade. The maturation of flowers is basipetal on the panicle and on each branch, while acropetal on the spikelet basis. The flowers of teff are hermaphroditic with both the stamens and pistils being found in the same floret. Florets in each spikelet consist of three anthers, two stigmas and two lodicules that assist in flower opening. Teff is a self-pollinated chasmogamous plant. The degree of outcrossing in teff is very low, 0.2-l.0% (Seyfu, 1997). The word teff is thought to have been derived from the Amharic word *teffa* which means "lost" due to small size of the grain and how easily it is lost if dropped. Indeed, teff is possibly the smallest cereal, the grain is oval-shaped with size 0.9–1.7 mm (length) and 0.6–1.0

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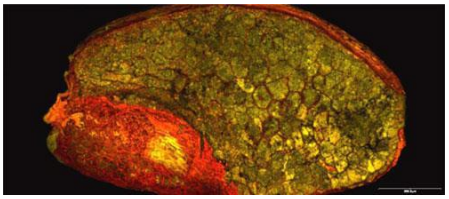
mm (diameter) (Bedane et al., 2015). The average thousand kernel weight (TKW) of 12 teff varieties tested by Bultosa (2007) was 0.264 g (Tab.4)

Table 4. *Teff grain characteristics. Bultosa 2007 (data); Helbing 2009 in Gebremariam et al. 2014 (photo).*

Average of 13 teff varieties	**Scanning electron microscope (SEM) image of a longitudinal section of a teff grain with germ and endosperm**
Length (mm) =1.17 Width (mm) = 0.61 Percent of sample that passes through sieves of different mesh size: 710 microns – 1.1 600 microns – 52.7 300 microns – 45.3 250 microns – 0.1	

The minuteness of teff grains has nutritional and technological implications. For instance, as teff grains are difficult to decorticate, the cereal is consumed as a wholegrain, improving nutrient intake for consumers. The color of teff can vary from white (ivory) to dark brown (black) depending on the variety (Figs.12 and 13).

Figure 13. *Plants of teff (white and brown grain) at Azienda Sperimentale Montelibretti (CREA-IT, Rome).*

Teff remains the main cereal crop in Ethiopia, favored by millions of local smallholder farmers (Seyfu, 1997; CSA, 2014). As with many C4 crop plants, teff can be grown in

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a wide range of agroecological zones in Ethiopia, including arid and semi-arid areas prone to drought and heat where maize, wheat and rice do not thrive. Its adaptability to cold climates allows it to grow at altitudinal ranges between 800 and 3200m above sea level (a.s.l) (Tefera, 2011). Beyond Ethiopia, countries such as Eritrea, USA, the Netherlands and Israel produce small areas of teff as a grain crop (Spaenij-Dekking et al., 2005a). On the other hand, South Africa, India, Pakistan, Australia, Uganda, Kenya and Mozambique grow teff as a forage or pasture crop (Assefa et al., 2010).

Teff shows considerable phenotypic variation, with wide adaptation across a range of agro-ecologies (Assefa et al., 2015). However, teff yields are low, with a mean national yield of 1.47 t ha^{-1} (CSA, 2014). The low yield of teff is attributed to its susceptibility to lodging, frequent moisture stress, and poor agronomic management with few inputs. Teff yield could be enhanced through selective breeding using locally adapted, farmers-preferred, genetically complementary lines.

Abraha et al. (2016) reported the use of SSR markers to determine the genetic relationships within 60 teff genotypes thereby, indicating their usefulness in genetic studies and teff breeding programs. Marker-based identification and selection of the distinct genotypes could be helpful for the development of improved teff varieties from an agronomic point of view.

1.3.2 Chemical and nutritional composition of teff

The chemical composition of cereals varies widely and depends on the environmental conditions, soil, variety and fertilizer. The importance of teff is mainly due to the fact it has attractive nutritional profile and has no gluten found in other common cereals such as wheat, barley and rye.

1.3.2.1 Carbohydrates

Starch is the major component of teff grain and may amount up to over 70% of the dry weight (Emmambux & Taylor, 2013) and the starch content of teff is higher than that of most other cereals. The amylose content of 12 teff varieties tested ranged from 20 to 26%, comparable to other grains, such as sorghum (Bultosa, 2007). Nonetheless, in comparison to wheat, the *in vitro* starch digestibility of teff was found to be significantly lower (Wolter et al., 2013). In line with this, the predicted glycemic index

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(GI) of teff (74) was significantly lower than that of white wheat (100) but comparable to that of sorghum (72) and oats (71) (Wolter et al., 2013). This somewhat lower GI for teff than expected may be explained by its amylose content, lower starch damage, and the possible formation of amylose-lipid complexes that can hinder enzymatic access and thus starch digestibility (Singh et al., 2010a; Wolter et al., 2013). In addition, the high (68-80 °C) gelatinization temperature of teff (Bultosa, 2007; Wolter et al., 2013) can hinder gelatinization and thus decrease susceptibility to enzymatic attack by α-amylase (Fardet et al., 2006).This makes it to be a potential gluten free cereal that replaces wheat and other cereals in their applications as sources of food energy and, moreover, suitable for Type2 diabetic patients owing to its richness in starches which digest slowly, conferring a low glycemic index (GI).

1.3.2.2 Proteins

The average protein level of teff is in the range of 8 to 11%, comparable to that of barley, wheat, maize and pearl millet, and higher than that of rye, rice and sorghum (Baye, 2014; Gebremariam et al., 2014). Teff's fractional protein composition suggests that glutelins (45%) and albumins (37%) are the major protein storages, while prolamins are a minor constituent (~ 12 percent) (Bekele et al., 1995; Tatham et al., 1996). In contrast, in more recent studies Adebowale et al. (2011) reported that prolamins are the major protein storages in teff. The different methods of extraction between these studies may explain the contradictory findings.

As observed by Tatham et al. (1996), the major prolamins of teff are less complex than those of wheat, in terms of their apparent molecular size differences, and resemble more to the α-prolamins of the Panicoideae (maize, sorghum and Coix), although they are classified in a separate sub-family of the Poaceae, the Chloridoideae. Recently, Adebowale et al. (2011) detected the fractionation of teff proteins in SDS-PAGE under non-reducing and reducing conditions, presenting broad bands of teff prolamins at approximately 20.3 and 22.8 kDa. Other bands were at approximately 36.1, 50.2, 66.2 and 90.0 kDa, respectively under non-reducing conditions, but were absent under reducing conditions, indicating that these polypeptides are disulphide bonded. The presence of broad monomeric prolamin bands in teff under non-reducing conditions indicates that teff prolamin is less polymerized than sorghum prolamin. Furthermore,

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the 2-D electrophoresis showed teff protein containing more polypeptides than maize or sorghum (Adebowale et al., 2011). By examining the amino acid profile, the higher contents of glutamine, alanine, leucine and proline and the relatively lower content of lysine further suggests that prolamins are the major storage proteins (Adebowale et al., 2011). Teff's amino acid composition is well balanced. A relatively high concentration of lysine, which is often lacking in most cereals, is found in teff. Similarly, compared to other cereals, higher contents of isoleucine, leucine, valine, tyrosine, threonine, methionine, phenylalanine, arginine, alanine, and histidine are found in teff (Tab.5).

Table 5. *Amino acid content of teff (g/16 gN)*

Lysine	3.68†
Isoleucine	4.07*
Leucine	8.53†
Valine	5.46†
Phenylalanine	5.69†
Tyrosine	3.84†
Tryptophan	1.30*
Threonine	4.32†
Histidine	3.21†
Arginine	5.15†
Methionine	4.06†
Cystine	2.50*
Asparagine + Aspartic Acid	6.4¤
Serine	4.1¤
Glutamine + Glutamic Acid	21.8¤
Proline	8.2¤
Glycine	3.1¤
Alanine	10.1¤

¤ *Bultosa and Taylor 2004; *Jansen et al. 1962, †Seyfu 1997*

Another important feature of teff is that it has no gluten (Hopman et al., 2008). Spaenij-Dekking et al. (2005b) investigated the presence or absence of gluten in pepsin and trypsin digests of 14 teff varieties. The digests were analyzed for the presence of T-cell stimulatory epitopes. In contrast to known gluten containing cereals, no T-cell stimulatory epitopes were detected in the protein digests of all the teff varieties assayed, thus confirming the absence of gluten in teff. Bergamo et al. (2011), reported

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others immunological analysis of alcohol-soluble fraction of teff protein, in which no immune cross-reactivity was showed toward the wheat gliadin, confirming its safety in the diet of celiac disease patients. This makes teff a valuable ingredient for functional foods destined for consumers with coeliac disease and gluten intolerance, and the presence of all nine essential amino acids needed by humans, making it a suitable alternative to wheat and rice (Cheng, 2017).

1.3.2.3 Lipids

The crude fat content of teff is higher than that of wheat and rice, but lower than maize and sorghum (Baye, 2014). Bultosa (2007) found that the crude fat content of teff is in the range of 3.0–2.0 % with mean of 2.3 % which is similar to the review report (3–2 %) of previous findings (Bultosa & Taylor, 2004). In following studies, the lipid content of teff (3.7%) was found higher than a range of other Ethiopian staple grains such as maize and wheat (Forsido et al., 2013). Another comparative study showed that the lipid content of teff (4.4%) was higher than that of wheat (3.6%), rice (0.9%), sorghum (3.5%), and maize (2.5%) flours, and was lower than that of oat (6.7%) and quinoa (8.6%) (Hager et al., 2012a).

Rice, wheat and maize contain negligible amount of linoleic acid (LA) and only traces of α-linoleic acid (ALA). Furthermore, these widespread cereals are consumed after decortication and further refining which reduces their amount of crude fat and n-6 and n-3 poly-unsaturated fatty acids. By maintaining whole grains, as in the case of teff, this provides a better source of fatty acids than refined ones. Most of the free fatty acids were unsaturated (84%), which was similar to that of maize, sorghum and quinoa. Hager et al. in 2012 reported that the major ones were linoleic acid (50%) and oleic acid (29%), which were also similar to those of sorghum and maize. A previous study showed that the content of oleic acid (32%) was higher than that of linoleic acid (24%) (El-Alfy et al., 2012). This may be due to the differences in the analytical method and teff genetics. Furthermore, the above mentioned studies mostly employed just one teff variety. It would be expected that a wide range of lipid contents exists from a large sample collection.

Although a clear consensus has not been reached on the optimal ratio between LA and ALA fatty acids, the Codex standards for infants formula recommends a LA: ALA

ratio in the range of 5 to 15 (Koletzko et al., 2005). In this regard, the LA: ALA ratio of 7:1 for teff can be considered favorable and is comparable to legumes that are good sources of fatty acids, such as soybean.

1.3.2.4 Minerals and vitamins

In general, compared to the other cereals, teff is rich in minerals such as calcium, zinc, magnesium, iron, phosphorous and copper (Gebremariam et al., 2014).
As summarized by Baye (2014), the difference in mineral content between and within teff varieties is wide ranging. Red teff has a higher iron and calcium content than mixed or white teff (Abebe et al., 2007). On the other hand, white teff has a higher copper content than red and mixed teff. Seyfu (1997) analyzed 12 genotypes of teff grown in different agro-ecologic settings and 5 varieties grown in a greenhouse in Great Britain and reported that genetic and environmental factors affect the iron content of teff. This may partly explain the high variability in the mineral content reported in different studies. Notwithstanding the differences described above, teff has a higher iron, calcium and copper content than other common cereals (Mengesha, 1966). The zinc content of teff is also higher than that of sorghum and wheat. However, the very high mineral (i.e. iron) content of teff has been contested and in many instances attributed to soil contamination (Seyfu, 1997; Abebe et al., 2007). Mengesha (1966) found, by washing the grain, that this significantly decreased the iron content as well as the variability between replicates. Despite this decrease, the variability between replicates for teff remained relatively high suggesting that soil contamination in teff is relatively high compared to other cereals. The mineral contamination of teff is probably due to its small size and suggests increased contact with soil over a larger area (Baye et al., 2014). The contamination of cereal grains in Ethiopia, particularly in teff, has often been associated with traditional methods of threshing grain under the hooves of cattle (Bezwoda et al., 1979). More recently, Ambaw (2013) compared the iron content of the same teff variety after laboratory (manually) and traditional threshing. Traditional threshing led to 30 to 38 percent increase in iron content mainly due to soil contamination. The iron content of the laboratory threshed teff was 16 mg/100g, which was still higher than what is found in many cereals. This suggests that although the intrinsic iron content of teff may not be as high as previously thought, teff is still a

better source of iron than other cereals like wheat, barley, sorghum, and maize. This feature of teff as major source of dietary iron explains the relative lack of anemia among Ethiopians (Adish et al.,1999).

In contrast to iron, Baye et al. (2014) showed that under the same conditions, the values reported for calcium and zinc are consistent and are less affected by washing. This suggests that soil contamination contributes little to the content of these minerals in teff. However, teff contains an excellent concentration of calcium (0.16 %) and magnesium (0.18%), the level of these minerals in teff are higher than other cereals, except sorghum which has magnesium concentration (0.18 %) comparable to that of teff (Gebremarian et al., 2014).

Zhu in 2018 highlighted from USDA Food Composition Databases (2017) that vitamins of teff (1 variety, uncooked) include niacin (4 mg/100 g), vitamin B6 (0.5 mg/100 g), thiamin (0.4 mg/100 g), riboflavin (0.3 mg/100 g), vitamin K (phylloquinone) (1.9 mg/100 g), vitamin A (9 IU), and a-tocopherol(0.08 mg/100 g) (wet basis).

1.3.3 Bioactive Compounds in Teff

1.3.3.1 Phenolic compounds

The other most important health-promoting aspect of teff as food is that like other millets it is generally assumed to contain substantial amounts of phenolics (Dykes & Rooney, 2007). Phenolics are notable for their antioxidant activity, which appears to be beneficial in terms of prevention of cardiovascular disease and cancer (Awika & Rooney, 2004). They also act as natural antioxidants for the food industry. At the same time, they might inhibit digestive enzymes and reduce food digestibility (Qiang et al., 2006).

In a study conducted on flour of seven teff varieties Shumoy & Raes (2016) detected the total phenolic content (TPC) ranged from 263 to 448 mg gallic acid equivalents (GAE)/100 g, of which the bound phenolic content (226-376 mg GAE/100 g) contributed to more than 84% of TPC. In the same varieties the contents of free and bound flavonoids showed values from 36 to 64 and from 113 to 258 mg CE (catechin equivalent)/100 g, respectively.

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Comparative analysis on the total phenolic contents showed brown and white whole teff grains (1 variety each) were in the range of 600–700 mg CE/100 g, with values lower than that of red sorghum and cowpea (Salawu et al., 2014). Forsido et al. (2013) reported TPC for teff flour as 123.6 GAE/100 g, which was much higher than some Ethiopian staple cereals such as maize and wheat. Moreover, another study reported brown teff values of TPC higher than white teff and total phenolic contents of 5 teff varieties ranged from 1.41 to 2.19 mg GAE/g (gallic acid equivalent) with higher values for the free phenolics than the bound phenolics (Kotaskova et al., 2016).
Therefore, there is great diversity in the polyphenol contents of teff grains.

1.3.3.1.1 Phenolic acids and Flavonoids

Bound phenolic compounds (PCs) in cereals are cross-linked to cell wall structural components of cellulose, hemicellulose, proteins, pectins and lignins which can survive the upper gastrointestinal digestion, and finally, reach the colon where they can be fermented by different microflora to exert their health benefits (Acosta-Estrada et al., 2014), while soluble PCs, which are readily absorbable in the stomach and the small intestine, could exert their beneficial health effect throughout the body (Liu, 2007).
The major bound phenolic acids and flavonoids identified in teff include protocatechuic acid, vanillic acid, syringic acid, p-coumaric acid, sinapic acid, ferulic acid, rosmarinic acid, catechin and naringenin, whereas catechin, ferulic and rosmarinic acids are the major polyphenols in the soluble fraction of teff (Kotaskova et al., 2016; Shumoy & Raes, 2016).
Research findings revealed that ferulic acid (286 µg/g) is the major phenolic compound in teff. Some other phenolic compounds such as protocatechuic (25.5 µg/g), gentisic (15 µg/g), vanillic (55 µg/g), syringic (15 µg/g), coumaric (37 µg/g), and cinnamic (46 µg/g) acids are also present in teff in considerable amounts (McDonough & Rooney, 2000).
Comparative studies showed that trans-p-coumaric, protocatechuic, ferulic, and gallic acids were the major free phenolics in brown teff, and rutin, ferulic and protocatechuic acids the major ones in white teff, whereas for bound phenolics in brown teff the

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majors were the ferulic and gallic acids, quercetin, and catechin, and the majority in white teff where ferulic acid, rutin, catechin, and quercetin (Kotaskova et al., 2016). Therefore, the polyphenol composition is much dependent on teff variety. Small amounts of quercetin and luteolin are bound to the cell wall material of teff, which was revealed by hydrolysis of the covalent bonds in alkaline conditions (Salawu et al., 2014). Naringenin, naringenin-40-methoxy-7-O-a-lrhamnoside, and eriodictyol-30,7-dimethoxy-40-O-b-d-glucoside were also identified in teff (El-Alfy et al., 2012).
Moreover, fermentation and cooking processes are known to enhance the release of bound PCs and increase the content of soluble PCs (Acosta-Estrada et al., 2014). Indeed, Shumoy et al. (2017) showed that after 72 h of fermentation of dough for *Injera*, from 4 teff varieties of brown and white color, the majority of the phenolic compounds increased in the range of 42–1805% in soluble and decreased by 2–100% in bound extracts in both varieties. FRAP values of the soluble and bound phenolic extracts of *injera* increased by 54–138% and 30–40%, respectively. Total ABTS values, but not DPPH, improved with fermentation. Finally, brown seed colored varieties showed superior total phenolic and antioxidant contents compared to the white varieties. Furthermore, Alaunyte et al. (2012) showed that by supplementing wheat bread with 30 percent teff flour, it was possible to significantly increase the total antioxidant capacity from 1.4 to 2.4 mM trolox equivalent antioxidant capacity (TEAC) per 100g.

1.3.3.2 Fiber

The crude fiber, total and soluble dietary fiber content of teff is several folds higher than in most other gluten containing and gluten-free cereals. There may be several reasons for this, as whole grains have higher fiber content than decorticated ones and small grains have a relatively high proportion of bran, which is high in fiber (Bultosa, 2007). Studies revealed that high fiber diets prevent many human diseases, colon cancer, coronary heart disease and diabetes (Anderson et al., 2009).
Bultosa et al. (2007) compared in 13 teff varieties the crude fiber ranged and detected values ranging from 3.8 to 2.6% with mean 3.3%, and apparently the crude fiber contents observed in these are almost similar with the earlier report of 3.5–2.0% with typical value 3.0% (Bultosa and Taylor, 2004). Moreover, the brown teff varieties

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presented the higher crude fiber contents (3.75% on average). Consumption of dietary fiber provides many health benefits.

Other studies reported the dietary fiber content of 8.0 g/100 g for teff, high value when compared to some fruits, nuts, pulses and cereals such as corn and rice (Saturni et al., 2010). Similar data were shown by Baye et al. (2014) with a value of 9.8% for the dietary fiber content of whole grain teff (one variety).

Another study showed that the total and soluble dietary fiber contents of teff flour (one variety) were 4.5 and 0.85%, respectively (Hager et al., 2012b), which appeared to be much lower than that reported above. The acid (4.3%) and neutral (6.1%) detergent fiber contents of teff were higher than those of a range of other Ethiopian staple foods such as maize, wheat, and cassava (Forsido et al., 2013).

It should be noted that many of above mentioned studies only employed a single genotype of each cereal, and a diversity in the fiber content would be expected from many samples. The analytical methods may also contribute to the large differences in the fiber content among different studies. Zhu (2018) observed in its review that the chemical composition and properties of teff bran and cell wall material, which is a major part of dietary fibers, remain to be studied

1.4 CELIAC DISEASE AND GLUTEN-FREE PRODUCTS

1.4.1. General aspects of Celiac Disease

Celiac disease (CD) is a chronic inflammatory disease of the small intestine that affects genetically susceptible individuals. This disease is unique in that the critical etiologic factor has been identified as the ingestion of proteins found in grains of wheat, barley, rye and triticale (Kagnoff, 2005). While the first description of a patient affected by a disorder related to gluten ingestion has been ascribed to Areteus of Cappadocia, who in the 2nd century A.C. reported a case of chronic diarrhea and malabsorption, its association with the ingestion of wheat flour dates back to about 70 years ago (Dicke, 1950). However, from the mid-1950s onward, an explosion in the understanding of CD has occurred, including the recognition of dermatitis herpetiformis, an intensely itchy skin eruption, known as 'CD of the skin', which rises after ingestion of proteins contained in wheat, rye, or barley kernels and subsides on a gluten-free diet (Marietta et al., 2008).

CD is characterized by the formation of autoantibodies and the destruction of the mucosal lining of the small intestine, which results in nutrients malabsorption. Typical symptoms associated with CD are abdominal pain, diarrhea, and constipation. Long-term complications of this disease include anemia, osteoporosis, miscarriage, liver diseases, cancers of the intestine, and depression or anxiety (NIDDK, 2009). CD is predominantly seen in Caucasians (one in 100 individuals) (Maki et al., 2003),the prevalence of CD is estimated from 0.5% to 1% of the worldwide population, may be higher in Northern Europe or in specific "at risk" groups as first-degree relatives of patients with CD, patients with type-1 diabetes mellitus, Hashimoto's thyroiditis, genetic disorders (Down's syndrome and Turner's syndrome) and IgA deficiency (Elli et al., 2017). Nevertheless, in many individuals, the disease becomes evident only during adulthood, and is sometimes triggered after surgery, pregnancy, childbirth, viral infection, or severe emotional stress. For this reason, even though 1% of the U.S. population is thought to be afflicted with CD, about 97% of these cases are undiagnosed. Additionally, because gluten is found not only in foods but also in medicines, vitamins, beauty products, stamps, and envelope adhesives, celiac have to be exceedingly judicious.

As previously mentioned the celiac disease is an immune-mediated disease that is triggered by the ingestion of wheat gluten or similar proteins found in barley and rye. Gluten is one of the earliest protein fractions described by chemists, a first description by Beccari was in 1728, and it is defined as the "cohesive, visco-elastic proteinaceous material" that remains when wheat dough is washed to remove starch granules and water-soluble constituents (Lamacchia et al., 2014). Gluten is formed by storage proteins necessary for plant germination, a complex mixture of proteins called prolamins, comprising glutenins and gliadins, which contain a relatively high concentration of glutamine and proline aminoacid residues within their primary structures, settling on several gluten-derived domains with high resistance to degradation by human gastrointestinal proteases (Sollid, 2002). Indeed, none of the major human gastrointestinal proteases, such as pepsin, trypsin, chymotrypsin and brush-border membrane enzymes of the small intestine contain the necessary proteolytic capabilities to effectively cleave certain immunogenic gluten peptides, because of a lack of post-proline cleavage-site specificity (Picariello et al., 2013; Shan et al., 2002).CD is strongly associated with particular human leukocyte antigen (HLA) genotypes, as only individuals carrying the DQA1*0501 and DQB1*0201 (DQ2),or DQA1*0301 and DQB1*0302 (DQ8) alleles develop the disease. Regarding the pathogenic mechanisms as summarized by Gianfrani et al. (2012) the immune response against these cereal-derived proteins is mediated by the innate and adaptive immune branches. The adaptive response starts when the gluten peptides are presented by the human leukocyte antigen (HLA)-DQ2/8 molecules of specialized antigen-presenting cells to CD4+ T cells or by HLA class I molecules to CD8+ T cells. More specifically, several gluten peptides were identified to activate proinflammatory T cells that release interferon-γ, which is a dominant cytokine with a key role in tissue damage. It has been shown that gluten also activates a stress-like immune response mediated by lymphokine-activated killer cells of the innate immune system and a marked proliferation of cryptenterocytes. IL-15 expressed by enterocytes is a major mediator of this innate immune response so that it is most likely that the toxicity of cereal prolamins for CD patients is due to the presence of both peptides that are able to activate T cell responses and sequences that induce a stress-innate proliferative response of mucosal epithelial cells.

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Furthermore, in the past few years, forms of gluten sensitivity other than celiac disease have been gaining the attention of physicians. Some patients have reported the appearance of intestinal (bloating, diarrhea) and extra-intestinal (headache, fatigue/irritability, foggy mind) symptoms shortly after the ingestion of gluten in the absence of any serologic celiac disease marker or intestinal mucosal damage but with a variable presence of antigliadin antibodies (AGA) and the disappearance of such symptoms on a gluten-free diet (GFD). This condition has been defined as nonceliac gluten sensitivity (Bucci et al., 2013).

In March 2015the Italian Association of Hospital Gastroenterologists and Endoscopists (AIGO) commissioned an experts' panel, composed of nutritionists, gastroenterologists, allergologists and biologists, to prepare a position statement on the nomenclature and diagnosis definition of gluten-related disorders (GRD). The panel identified celiac disease, wheat allergy and non-celiac gluten sensitivity as the gluten related disorders of gastroenterological interest, defining that celiac disease has an autoimmune nature, wheat allergy is IgE-mediated while the pathogenesis of non-celiac gluten sensitivity is still unknown as is the case of non-IgE mediated allergy. Therefore, diagnosis should start with the serological screening for celiac disease and wheat allergy. In case of normal values, the response to a gluten-free diet should be evaluated and a confirmatory blind food challenge carried out. Consequently gluten-related disorders are clinically heterogeneous, and patients should be carefully managed and specific protocols applied for a correct differential diagnosis in gastroenterological setting (Elli et al., 2017).

To date, a strictly gluten-free diet represents the only medical treatment for celiac disease patients. However, compliance to the gluten free diet is difficult and affects the quality of life of patients because, besides economic and social factors, it involves the consumption of poorly palatable and scarce technological-aptitude bakery products. This is the reason why alternative approaches to the GFD are actively sought, which include the search for and the development of new cereals with less amount of prolamins or poor structured gluten with no or low immunogenic content (Comino et al., 2013; Spaenij-Dekking et al., 2005b; Iacomino et al. 2016).

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1.4.2. Gluten-free foods: market and challenge

The Italian celiac association website reported the annual review to the Italian Parliament on celiac disease for 2016 by the Directorate General for Hygiene and Security of Food and Nutrition for the Italian Ministry of Health. This report refers that from the epidemiological mapping carried out in 2016, 198,427 celiacs are diagnosed in Italy, of which 2/3 belong to the female population. The comparison with the first Annual Report, drawn up in 2007, shows that the diagnoses of celiac disease have increased from 64,398 to 198,427, and this has been done mainly thanks to the sensibilization of doctors and health professionals. It has been calculated, however, that in the Italian population the theoretical number of celiacs in 2016 is more than 600,000 against the almost 200,000 diagnosed. The geographical distribution of celiacs in 2016 in Italy shows that the regions with the highest number of celiac residents are in Lombardy with 37.907 celiac residents (19 %), followed by Lazio with 19.325 (9.7 %) celiac and Campania with 18.720 celiac (9.4 %).

Furthermore, in these last years the increased awareness and diagnosis of CD and gluten sensitivity have spurred the demand for gluten-free products. The term 'gluten-free' refers to products with a gluten residue of not more than 20 ppm based on gluten-free ingredients at source, or with one or more ingredients purified from gluten (European Regulation n.41/2009) (Evangelisti & Restani, 2011) (Fig.14). The European directive 2003/89 requires that the presence of cereals containing gluten and derived products be included as ingredients in the label of the food product.

Figure 14. *The bared-ear of wheat appears on the packaging of foods and beverages free of toxic prolamins (< 20ppm).*

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Unfortunately, the quality of gluten-free products has not kept up with the rising demand. Gluten replacement in food presents several challenges.

As summarized by Elli et al. (2017) several studies have shown that the dietary habits of celiac patients did not differ basically from those of the general healthy population. However, because the derivates of gluten-rich grains are important sources of nutrients in the general diet, their exclusion can potentially affect the nutritional adequacy of a celiac patient's diet and in turn have a major effect on their nutritional status if such foods are not replaced with balanced alternatives. Although it remains difficult to date to draw conclusions about the nutritional adequacy of a GFD regimen because of conflicting study results, several studies have pointed out that celiac patients have a different intake of macro- and micro-nutrients as compared to healthy control subjects. Among the macronutrients, the major concern regards the higher intake of total and saturated fats recorded in the celiac patient's diet than in that of healthy control subjects. This has raised concerns also about the lipid content of commercial GF foods. This imbalance in the daily fat intake may lead to overweight and obesity in celiac patients, especially children and adolescents. Even though not all the studies reported a different fiber intake between celiac patients and the matched control group, it has been suggested that GFD is inadequate in terms of fiber content. This has been attributed to a decreased consumption of grain products, exacerbated by the fact that many GF foods are made with starches or refined flours with low fiber content. However, in recent years, manufacturers have improved the fiber content of breads, flour mixes and other GF products, as hydro-colloids and gums which, having colloidal properties, are used for replacing the gluten network and improving the technological properties of GF products. Regarding micro-nutrients, lower levels of vitamins, such as folate, niacin, vitamin B12, vitamin D were described in celiac individuals than in control subjects (Kinsey et al., 2008; Wild et al., 2010). Moreover, such a low intake has the consequence that, in many cases, celiac patients did not meet the recommended intake of these vitamins. Such a low intake can partly be due to the low content of folates in starches and low-protein flours (e.g., corn and rice), commonly used as main components of GF products (Pellegrini & Agostoni, 2015). Some studies reported lower intakes of several minerals in celiac patients than in the controls and inadequate intakes against the current recommendations. For instance, as compared to control

subjects, the daily intake of iron was significantly lower in celiac patients and that of calcium was significantly lower in female celiac patients. Lee et al. (2009), suggested that the inclusion of alternative GF grains (e.g., oats and quinoa) can significantly increase the nutritional quality of GFD including the levels of iron and calcium.

Wheat, rye, and barley belong to the grass family of monocotyledons (Liliopsida class), this family also includes oats and other cereals such as rice, millet, sorghum and teff which are generally considered nontoxic. However, the believed lack of toxicity for most of these cereals was based on their taxonomical classification rather than a direct evaluation of their immune stimulatory activity, but in the last two decades immunochemical, biochemical and genetic studies have confirmed their effective safety for celiac patients (Bergamo et al., 2011; Ciacci et al., 2007; Ellis et al., 1992; Pontieri et al., 2013, Maglio et al., 2011).

1.4. 3 Gluten-free foods: products

1.4.3.1. Products sorghum-based

In recent years, farmers in the United States have begun cultivating sorghum hybrids that produce white grain from a tan-color plant (often called "food-grade" sorghum) for production of wheat-free foods for persons with CD (Tuinstra, 2008).

Sorghum flour is an attractive alternative to wheat flour for the celiac market because of its neutral flavor and the use of hybrids with a white pericarp. These white grained sorghum lines produce a flour comparable to wheat flour in appearance and do not impart an unusual color to the flour. This means that it can be used in any kind of dish, from cakes to white bread. Many people believe that the taste of sorghum is much more similar to that of wheat than other gluten-free cereals. It is generally described as a pleasant taste that does not interfere and does not dominate the taste of the food in which it is used. This makes sorghum flour extremely versatile. Unlike rice flour, which is commonly used for the production of gluten-free foods, sorghum flour has a smooth and non-granular consistency. The granularity perceived in rice-based foods is the main objection by celiac patients. In addition, sorghum flour does not involve allergies over time. This is especially important given that the incidence of food allergies is growing, and it is not unusual that some people develop allergies to wheat not only, but also to maize, rice and soybeans, which are the main foods used to replace

wheat (De Mesa-Stonestreet et al., 2010). Because of the difficulty of finding a single flour capable of replicating the properties of the gluten present in the wheat, it is possible to mix the sorghum flour with other flours, so as to maximize their positive characteristics. Several studies have shown that sorghum flour quality could be improved, although the quality of the food made with sorghum is still lower than that of foods prepared from wheat flour. Therefore, it is still necessary to conduct studies to improve the quality of the products obtained from sorghum flour (Del Giudice et al., 2007).

In Asia and Africa, sorghum is traditionally prepared in different ways including porridges, flat breads, alcoholic beverages, and snacks (Murty & Kumar, 1995). Bread can be made with sorghum and millet grain. Perten et al. (1983) reported that the volume of bread made with sorghum or millet, was always smaller than that of bread made with wheat flour, but many consumers preferred it. The bread crumb was less elastic, drier and darker in breads made with sorghum or millet. These authors suggested that sorghum flour could not be considered breadmaking flour because it did not produce the elastic dough needed to obtain a large bread volume.

Breads made from sorghum bran contain more starch, sugar and dietary fiber and less ash and protein than breads made from wheat (Badi et al., 1990).

A traditional use for sorghum is in tortillas, although tortillas are generally made from maize, sorghum has been used in several Central American Countries, and due to its better drought tolerance, its importance has increased in recent decades (Murty & Kumar, 1995; Rooney & Waniska, 2000). Sorghum can partially or totally substitute for yellow maize in tortilla production when properly processed, i.e. decorticated to remove outer bran layers and cooked and steeped shorter than maize (Choto et al., 1985). Concerning snack foods, tortilla chips can be produced from white food-grade sorghum without problem, just by reducing lime concentration and cooking time of sorghum relative to maize. Sorghum tortilla chips have a bland taste, which might be advantageous in snack products in which a strong maize flavor is not desired (Serna-Saldivar et al., 1988). Young et al. (1990) studied parboiling of sorghum for rice-like product from decorticated sorghum. Parboiling increased the yield of decorticated grain, reduced kernel breakage, and caused increased firmness and reduced stickiness of the final cooked kernels. Microscopic examinations showed also that the soaking

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and boiling process did not permit sufficient hydration to allow complete cooking of the kernels, and that most kernels had ungelatinized centers. This is in agreement with the high resistance of hard sorghum endosperm to water penetration, due to hydrophobic matrix proteins, described by Munck (1995).

Sorghum flours were also used to produce biscuits, granolas and snack foods such as crisps and chips. Badi and Hoseney (1976) studied the eating quality of cookies made from sorghum and millet grains. Cookies made from these grains were tough, hard, gritty and mealy. Some wheat lipids, such as phosphatidyl ethanolamine, digalactosyl diglycerides and phosphatidyl choline, are not present in sorghum flour. These lipids, added to sorghum flour improved the cookie baking quality. The sorghum cookies were darker and more fragile than the wheat cookies. The grittiness of millet and sorghum cookies was reduced by increasing the pH of the cookie dough.

Moreover, sorghum noodles made from only decorticated sorghum flour, water and salt have been studied by Suhendro et al. (2000), who found that the timing of amylose dispersion (solubilization), formation of noodles, and amylose retrogradation was critical. Also, flour particle size was also critical with finer flour producing better quality noodles. Thus, good quality sorghum noodles could be produced when processing conditions were optimized and when the noodles were properly cooked. However, cooking sorghum, especially wet cooking, reduces its digestibility (Hamaker et al., 1986; Duodu et al., 2003; Emmambux & Taylor, 2009), making it less available for the body to use.

For celiac patients who typically suffer from malnutrition due to poor nutrient absorption, it is even more important for nutrients to be more readily available. Thus, a challenge exists to make sorghum proteins more digestible. Additionally, to make sorghum protein a commercially viable ingredient, it has to be concentrated and/or isolated at an industrial scale using processes and/or chemicals that are compatible with food grade applications, also for identify new developments in uses of sorghum in gluten-free foods, specifically in staples like bread and pasta (De Mesa Stonestreet et al., 2010).

Malting and brewing with sorghum to produce lager and stout, often referred to as clear beer as opposed to traditional African opaque beer, has been conducted on a large, commercial scale since the late 1980s, notably in Nigeria (Olori et al., 1996). In

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2005, Nigeria brews more than 900million liters of beer annually (Institute of Brewing and Distilling), most of this is brewed with at least some sorghum. Brewing with sorghum is now also taking place in east Africa, southern Africa and the USA. Taylor et al. (2006) detected extensive research and development works and several excellent reviews published covering enzymes in sorghum malting and brewing technology. In conclusion, however, sorghum's high-starch gelatinization temperature and low beta-amylase activity remain problems regarding complete substitution of barley malt with sorghum malt.

1.4.3.2 Products teff-based

Teff is cultivated in a few countries such as South Africa, India, USA, Eritrea and Ethiopia, although it is primarily used for human consumption only in the latter two. Although teff has been used for food in Ethiopia for many centuries, it is only recently that its use as a food ingredient has gained interest in other parts of the world. As technological challenges are over come in processing teff to make bread and other food products, demand for teff is likely to increase globally.

Teff flour has been incorporated into a range of food products, especially the gluten free products. Teff addition in wheat-based foods positively influences the nutritional properties of these products. The changes in the quality attributes of food products due to teff addition have been monitored, the lack of gluten-type protein in teff may negatively impacts on the product quality, and various techniques/additives have been employed to encounter such changes.

The main food product from teff flour is *injera*. Native to Ethiopia and Eritrea, *injera* is a traditional sourdough flatbread with teff as a major ingredient, made by mixing cereal flour with water to make dough and then triggering the fermentation process by inoculating with ersho, a starter obtained from previous fermentations (Baye et al., 2013). The fermentation lasts on average 2-3 days, after which the dough is thinned into a batter before steam baking. Teff is the preferred grain for making *injera*, primarily for its better sensory attributes (for example, taste, color, smell) and shelf life. Yetneberk et al. (2004) demonstrated the superiority of teff or the minimal force required to bend fresh, 24-, and 48- hour stored *injera* relative to *injera* made from other grain, indeed the ability to easily roll (softness) *injera* is an important quality

attribute since this allows easy wrapping of the sauces (wot) consumed with it. Similarly, incorporating teff flour into the sorghum flour has been shown to improve the sensory attributes of sorghum *injera* (Yetneberk et al. 2005).

Recent studies focused on the effect of fermentation on the phytic acid content of *injera* and how the changes may influence the nutritional properties of the product (Baye et al., 2013, 2014; Fischer et al., 2014). Teff contains a significant amount of phytic acid which tends to negatively affect the mineral adsorption. Fermentation hydrolysed 28% of the phytic acid in *injera* made of teff and white sorghum flour mixture, whereas the phytic acid were all hydrolyzed in *injera* made of barley and wheat as well as wheat and red sorghum flours (Baye et al., 2013). Indeed, blending teff with wheat has been found to be nutritionally beneficial, as it allows higher phytate degradation due to the higher endogenous phytase activity in wheat (Egli et al., 2004). However, the *in vitro* iron bioavailability of the *injera* was not affected by the fermentation process and the resulting lower content of phytic acid (Baye et al., 2014). Although used to a much lesser extent than for *injera*, teff can also be used for the making of porridges, unleavened breads (kitta), gruels (atmit), and traditional alcoholic beverages, like tella and arake (Gebremariam et al., 2014).

The incorporation of wholegrains in bread making is often challenging. This is further complicated when gluten-free ingredients are used, since gluten plays an essential role in producing leavened bread with a fine open structure (García Manzanares & Lucendo 2011).

The use of enzymes provides an alternative strategy to improve the texture and sensory properties of teff-enriched breads. Alaunyte et al. (2012) has shown that the application of a combination of enzymes, including xylanases, amylase, glucose oxidase, and lipase, improved the quality of teff-enriched breads. In contrast, treatment with glucose oxidase or protease did not show any improvement in the sensory and textural attributes of teff breads (Renzetti & Arendt, 2009). This suggests that the type, dose and combination of the enzymes use, determine their effects on the quality of teff-enriched breads. Further studies are needed to determine the optimal doses and conditions needed to improve the processing and sensory attributes of teff-enriched breads.

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The possibility of using teff for food products other than bread has begun to be evaluated. Coleman et al. (2013) confirmed the suitability of teff flours for biscuits and cake making. Similarly, studies evaluating the possibility of using teff in pasta formulations (Hager et al. 2012b, 2013), indeed gluten free egg spaghetti were made from teff flour and the product was compared with that of oat and wheat (Hager et al., 2013). The dietary fiber and mineral contents of teff and oat spaghetti were higher than those of wheat. Teff spaghetti had a lower predicted glycemic index (p GI) (45) than wheat spaghetti (67), and the p GI was higher than that of oat spaghetti (32). The texture of teff and oat spaghetti were similar to that of wheat spaghetti except for the lower elasticity. The overall sensory quality of teff spaghetti was inferior to that of wheat and oat, suggesting research opportunities for food technologists.

Teff grain has been used to produce malt for gluten free beverage application (Gebremariam et al., 2015; Gebremariam et al., 2013a; 2013b; 2013c). Effect of kilning conditions (drying temperature and time) on the activities of α- and β-amylases and limit dextrinase as well as the dimethylsulphide (MDS) level in teff malt was studied (Gebremariam et al., 2013b). The variety type instead of the storage length appeared to be the major factor affecting malt quality (Gebremariam et al., 2013c). Compared with standard barley malt flour, the teff malt samples from this study appeared to have much lower α-amylase activities, lower/similar β-amylase activities, higher wort color values, similar wort viscosity, and less total fermentable sugars (Gebremariam et al., 2013c). However, these studies showed that teff grains can be a good material for malt production. The teff malts produced remain to be applied in any alcoholic beverage production with good consumer acceptance. Finally, also gel-like food formulation (Abebe & Ronda, 2014) have shown promising results. These efforts show that teff can be used in various products familiar to Western culture, especially in the formulation of gluten-free products.

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CHAPTER 2: AIM OF THE WORK

The objective of this study was to evaluate five sorghum genotypes, one for zootechnical use and four food-grade hybrids, and two commercial teff genotypes (brown and white grain), for their technological, biochemical and nutritional traits, comparing them with a durum wheat cultivar as control in order to identify the most suitable for the formulation of innovative wholegrain, gluten free products of high qualitative value to develop a sustainable agri-food chain.

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CHAPTER 3: MATERIALS AND METHODS

3.1 PLANT MATERIALS

Grains from five sorghum genotypes, one for zootechnical (Zt) use (cultivar Aralba, RV-Venturoli, Bologna, Italy) and four food-grade (Fd) hybrids, i.e. PSE 7431 by Padana Sementi Elette (Padova, Italy) and three SW hybrids (Sem West, Semillas-Bolivia) kindly supplied by Dr. Alberto L. Chessa, and grains from two commercial white and brown teff (Teff Haeven, Larino, CB,Italy) and from the durum wheat, *cv* Iride, were used in this work.

The sorghum hybrids and the durum wheat were grown in Rome, at the experimental fields of the "'Inviolatella" of CREA-IT (Fig.15A), whereas the teff samples were purchased in three separate batches on the market (Fig. 15B-C).

The planting of sorghum was performed with parcel seed drill on May 2014, adopting an experimental block design with 3 repetitions and an investment of 30 plants/m^2 (Fig.15A). Relief irrigation was carried out immediately after sowing to encourage regular emergencies, whereas 120 units of nitrogen were distributed (60 for sowing + 60 for roofing); weeds were controlled with chemical weeding at the 2nd - 3rd leaf stage (S-Metolachlor + Terbutylazine). The harvest was conducted with parceling combine harvester on September 2014. The 2014 spring-summer weather trend was rather favorable to the crop, with not particularly high temperatures and well distributed rainfall during the entire cycle, with the exception of the initial phase in which the only irrigation intervention was done.

Figure 15. *Sorghum fields at Inviolatella (A) and commercial white (B) and brown (C) teff*

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3.1.1 Milling of sorghum and teff

Sorghum grains were manually selected and subjected to sieving for removal of dirty and impurities. All samples were milled to wholemeal flours (Fig.16) using a laboratory mill (Cyclotec, mod. 1093-Tecator/Hoganas, Sweden) equipped with a 0.5 or 1.0 mm sieve, depending on the further analyses to be performed, and kept at 4°C until their use. The wholemeal was thoroughly mixed to ensure uniformity and all analyses were performed in triplicate on two independent aliquots of each sample.

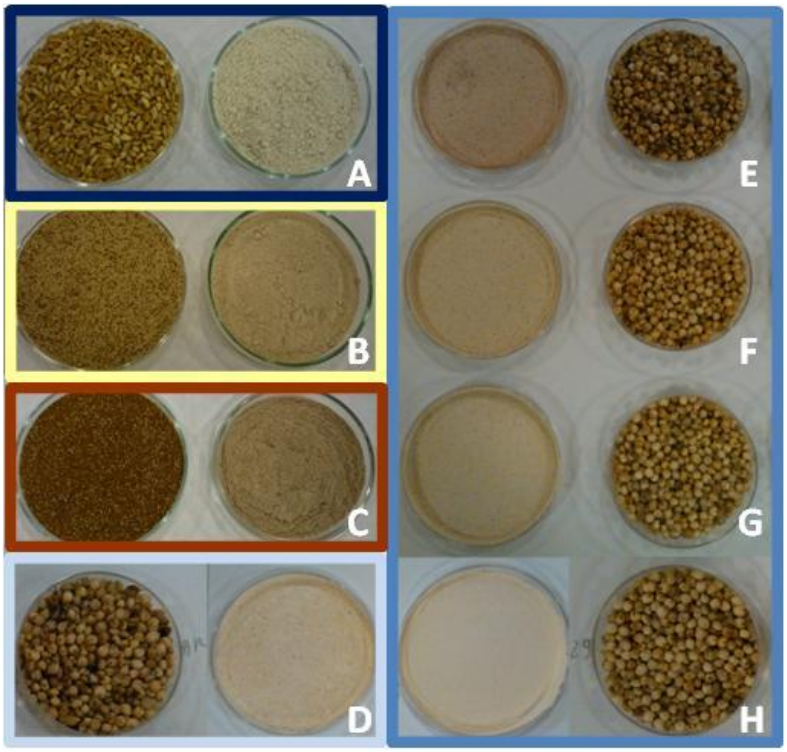

Figure 16. *Grains and wholemeal flours from durum wheat cv Iride (A), white teff genotype (B), brown teff genotype (C) and five sorghum hybrids, the zootechnical Aralba (D), and the food-grade PSE 7431 (E), SW6143W (F), SW6129 (G), SW6237W (H).*

3.2 PHYSICAL AND TECHNOLOGICAL ANALYSES

3.2.1 Thousand kernels weight

The EN ISO 520:2010 method was used to determine the 1,000-kernels weight (TWK). The purpose of the analysis is to evaluate the mass in grams of 1,000 kernels.

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The analytical procedure begins with the withdrawal of a sample aliquot after the separation from the broken kernels and impurities. Then the seeds were counted with a photoelectric seed counter (Contador Pfeuffer) up to the value of 1,000. Finally, the the 1,000 kernels were weighed with an accuracy of 0.01 g with a Gibertini E425 balance, the value thus obtained represents the mass of 1,000 seeds of examined sample.

3.2.2 Test weight

The test weight is the ratio between the mass of a cereal and the volume occupied after it has been poured into a container under well-defined conditions. It represents a measure of the filling degree of kernels and can be considered a global index of product quality. The reference method for the test weight is EN ISO 7971. The grain sample is poured into a filling cylinder and then weighed; in the reference method a cylinder with a capacity of 20 kg is used, but in practice, for operational purposes, the graduated cylinder used has a capacity of 1 kg or 250 g. These lower capacity cylinders must in any case be validated with the reference equipment and must produce comparable results. After having inserted the grains into the cylinder and weighed their mass in grams, a special weighing scale was used to obtain the value of the mass expressed in kg/hL.

3.2.3 Hardness index

Kernel hardness was evaluated on 300 kernels by the Perten Single Kernel Characterization System (SKCS) 4100 (Springfield, IL, USA) following the manufacturer's operating procedure. The instrument was set in a range of hardness between -40 and +120. The endosperm texture can be divided into four classes based on their average SKCS values, i.e. extra soft (SKCS <10), soft (10≤ SKCS <50), hard (50≤ SKCS <90) and extra-hard kernel texture with SKCS index ≥90.

3.2.4 The sodium dodecyl sulfate sedimentation test

The sedimentation test is based on the capacity of gluten proteins to swell under the influence of lactic acid; this method measures the relative gluten strength in wheat flour. Sedimentation volumes reflect differences in both protein quantity and protein

quality. There is a positive correlation between sedimentation volume and gluten strength, and loaf volume. The method is used as a screening test in wheat breeding. In commercial or experimental milling, it is used only for comparing lots of the same grade of flour milled by the same mill. The sodium dodecyl sulfate (SDS) sedimentation was assessed using a solution of 2% sodium dodecyl sulfate as described by the standard method AACC 56-70 (2003), and the sedimentation volumes were expressed in milliliters. Values above 40 for the sediment height are attributable to a good protein quality of the sample.
For each sample were used 6 g of wholemeal flours (ground with sieve 1mm) and three solutions were prepared:

- A: 29.4 grams of SDS (sodium dodecyl sulfate) were dissolved in one liter of distilled water
- B: 10 ml of 88% lactic acid in 80 ml of distilled water
- C: 1000 ml of solution A plus 18 ml of solution B

At time 0, the samples are placed in 100 ml cylinders with 50 ml of distilled water and were stirred for 15 times by placing the cylinders in a horizontal position.

- After 2 minutes the samples were inverted again 15 times and brought back to the initial position.
- After 4 minutes the 15 inversions were repeated, and 50 ml of the solution C are added. The samples were stirred 4 times and placed horizontally.
- After 6 minutes, 4 inversions were performed.
- After 8 minutes the previous operation was repeated.
- After 10 minutes the samples were inverted 4 times and left in a vertical position.
- After 25 minutes the sediment height was detected.

3.2.5 Falling Number

Falling Number (FN) system (Perten 1500) was used for the determination indirectly of alpha amylase activity in wheat flour, quantifying the rheological properties of starch hydrolyzed by the enzymes during the test (AACC 56-81B, 2000).

The α-amylase activity is evaluated using the starch present in the sample as a substrate. The determination is based on the ability of an aqueous suspension of flour, semolina or whole grain product to gelatinize rapidly in a boiling water bath, and on the measurement of starch liquefaction through the α-amylase present in the sample. The liquefaction influences the thickness of the starch gel and, therefore, the resistance of the viscometer agitator and the time taken to fall.

Process is the following:

- Fill the water bath with water up to the maximum possible level. Turn on the cooling system and ensure that cold water flows through the cooling lid. Turn on the equipment of the FN and bring the water to boil. The water in the bath must boil vigorously before any determination and throughout the entire test.
- Transfer the weighed sample, 7 grams at 14% moisture, into a clean and dry viscometer tube. Add 25 ml ± 0.2 ml of water at 22 °C ± 2 °C using the automatic dispenser and a pipette.
- Immediately close the viscometer tube with a stopper and shake vigorously up and down 20-30 times to obtain a uniform suspension. Ensure that dry flours or base materials are not trapped in the upper part of the tube towards the cap.
- Remove the cap, scrape the material still lying on the bottom of the cap into the tube and, with the viscometer shaker, scrape down any material that adheres to the pipe walls. Leave the stirrer in the tube.
- Immediately place the viscometer tube, together with the agitator, inside the lid hole in the bath with boiling water. Activate the agitator head in accordance with the manufacturer's instructions. The equipment can then carry out the operations automatically until the test is completed. The test is considered complete when the viscometer stirrer has reached the bottom of the gelatinized suspension. Record the time shown on the timer. This constitutes the Falling Number expressed in seconds.

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3.2.6 Color analysis: yellow and brown index

Colorimetric measurements on wholemeal flours were determined by means of a Tristimulus colorimeter (Chroma Meter CR-300; Minolta, Milan, Italy), using the CIE Lab color space coordinates L^* (lightness), a^* (red-green chromaticity), and b^* (yellow-blue chromaticity), and the D65 illuminant. For cereal products, browness generally expressed as 100-L is often used. The instrument was calibrated with a standard calibration plate.

3.3 BIOCHEMICAL AND NUTRITIONAL ANALYSES

3.3.1 Electrophoretic analyses

3.3.1.1 Extraction of total protein

Total proteins from individual crushed seeds (25 mg) were extracted with 0.5 ml of a solution containing 0.25 M Tris–HCl buffer (pH 6.8), 0.12% (w/v) SDS, 10% (v/v) glycerol, 0.2% (w/v) pyronine Y and 5% 2-mercaptoethanol and shaken for 1 h at room temperature. After incubation at 80 °C for 20 min and centrifugation at 15,000g for 10 min, an aliquot (20 µl) of the protein suspension was fractionated by SDS-PAGE.

3.3.1.2 Fractionation by SDS-PAGE

Protein fractionation by sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) was performed in an apparatus TV100 (VWR, Germany) at 200 V constant voltage until the dye reached the bottom of the gel. The separating gel (10 cm x 10 cm), 1 mm thick, was prepared with 15% (w/v) acrylamide (T =15% and C = 0.5%), 0.375 M Tris–HCl (pH 8.4) and 0.1% (w/v) SDS for SDS-PAGE running gels, whereas stacking gels contained 4.5% (w/v) acrylamide (T = 4.5% and C = 0.06%), 0.08 M Tris–HCl (pH 6.8) and 0.1% (w/v) SDS. The electrophoresis buffer was 0.025 M Tris–glycine (pH 8.3) and 0.1% (w/v) SDS. A 0.25% (w/v) solution of Coomassie Brilliant Blue R250 in 6% trichloroacetic acid was used to stain SDS-PAGE gels. SDS-PAGE electrophoretic patterns for High Molecular Weight Glutenin Subunits (HMW-GS) were determined according to the method described by Payne & Lawrence (1983).

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3.3.2 Moisture measurement

3.3.2.1 Flour moisture

Moisture was measured just before the chemical analyses on 3 g of milled sample by a Sartorius MA35 thermobalance (Muggiò MB, Italy) at 120 °C.

All analyses are reported on a dry weight basis, using the following formula:

$$\text{Dry weight} = \text{fresh weight} \times \frac{100\text{-moisture content (\%)}}{100}$$

3.3.2.2 Kernel moisture

Ten grams of kernels were placed in each of two tared moisture dishes. The dishes and contents have been weighed, then subtracted the weight of each dish from the total weight and recorded the weight of the sample. The dishes were placed in the oven. The oven temperature and heating period depend on the seed, 20h for barley,18h for sorghum and teff, and 19h for wheat, all these samples were dried at 130 °C. At the end of the heating period, the dishes were placed soon as possible in a desiccator. Finally, when the dishes reached room temperature, the weight was taken, and the percentage of moisture was calculated by dividing the loss in weight due to heating by the weight of the original sample and multiply by 100.

3.3.3 Determination of total protein content

Protein content (PC) was obtained by micro-Kjeldhal nitrogen analysis according to ICC 105/2 method (1994). PC was estimated using a conversion factor of 5.7 for durum wheat and 6.25 for sorghum and teff samples.

The Kjeldahl procedure involves three major steps: Digestion, Distillation and Titration.

a) Digestion: Organic nitrogen is converted into NH_4^+.The aim of the digestion procedure is to break all nitrogen bonds in the sample and convert all the

organically bonded nitrogen into ammonium ions (NH_4^+). For this purpose, 200 mg of sample were weighed and placed inside glass tubes.

In each tube were added 8 ml of H_2SO_4 96% w/v in mixture with 1% selenic solution (catalyst), and 2 ml of 30% v/v hydrogen peroxide. The tubes were placed in the appropriate mineralization unit (BUCHI digestion unit K-435) for about 2 hours at 450 °C. In this process the organic material carbonizes, and it can be visualized by the transformation of the sample into black foam. During the digestion the foam decomposes and finally a clear liquid indicates the completion of the chemical reaction. After digestion is completed, the sample is allowed to cool to room temperature, then transferred to the distillation unit.

b) Distillation: the solution is then distilled with a small quantity of sodium hydroxide, which converts the ammonium salt to ammonia. Three drops of methyl red indicator were added to the tubes and then the tubes were placed in the distillation unit (BUCHI distillation unit K-350). The distillation takes place in an alkaline environment by adding 50 ml of water and 50 ml of 32% (w/v) sodium hydroxide. Three drops of mixed indicator and 20 ml of 2% boric acid (v/v) were added in the 250 ml flasks used for the distillate collection. When NH_3 reacts with boric acid the solution turns from red violet to green (pH 4.4-5.8) due to the color change of the indicator from acid to basic medium.

c) Titration: Nitrogen is determined. The distilled samples were titrated with the appropriate burette, using sulfuric acid 0.05 M until the complete color change (from green-blue to pink-lilac). At the same time, a blank test is performed with 5 ml of a standard ammonium chloride solution to which the reagents are added following the same procedure described above, starting from the distillation step.

For quantification, the volume of sulfuric acid needed for the color change was multiplied by a conventional conversion factor.

The content in nitrogenous substances for 100g of product is obtained from the following formula:

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$$\%\, Total\ Nitrogen = \frac{v_1 * 5}{v_0} * \frac{F_c * 1 * 100}{C}$$

where:

5= volume in ml of ammonium chloride solution

V_0= 0.05 M sulfuric acid volume useful for titrate 5ml of ammonium chloride

V_1= 0.05 M sulfuric acid volume used to titrate the test sample

C= sample weight (200 mg)

100= percentage calculation

F_c= conversion factor of the analyzed matrix

3.3.4 Extraction of starch granules

To extract starch granules, 500 µl of distilled water were added to 1 g wholemeal of durum wheat, sorghum and teff samples. After 30 min at room temperature, samples were suspended in 5 ml of 0.1 M NaCl and gently mixed. An aliquot (3 ml) of the suspension was transferred to a fresh tube and an equal volume of 0.1M NaCl added to the sample. This operation was repeated twice. The three aliquots in the fresh tube were centrifuged at 5000 g for 5 min and the pellet washed three times with distilled water twice with 85% (v/v) methanol and air-dried. Then 100 mg of air-dried starch granules were resuspended in 5 ml of distilled water and an aliquot (50 µl) was injected into a counting chamber with the Thoma ruling (0.2 mm cell depth). The starch granules in the starch suspension injected into 20 random-chosen Thoma cells (50×50 μm^2) were photographed with a Leica DMLB100T optical microscope.

3.3.5 Determination of total starch content

Total Starch (TS) content was determined by enzymatic method using Megazyme (Bray, Ireland) kits K-TSTA according to McCleary et al. (1997). This method included a digestion of 100 mg of milled sample firstly with thermostable α-amylase diluted in MOPS (3-[N-morpholino] propane sulphonic acid) buffer and then with amyloglucosidase. The total starch was measured by adding GOPOD (glucose oxidase/peroxidase) reagent to 100 µl of digested sample. The absorbance was read against the blank, consisting of 100 µl distilled water plus 3 ml GOPOD reagent, at

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510 nm by a Lambda Bio20 (Perkin Elmer, Monza, Italy) spectrophotometer. A solution of D-glucose (100 µl of 1mg/ml D-glucose standard solution) was used as the control. The data were expressed as weight percentage (w/w) on a dry weight basis (% d.w.) and the analyses were performed in duplicate.

3.3.6 Determination of resistant starch content

Resistant Starch (RS) content was determined by enzymatic method using Megazyme (Bray, Ireland) kits K-RSTAR according to McCleary et al. (2002). This method included a digestion of 100 mg of milled sample with thermostable α-amylase and amyloglucosidase diluted in maleic acid buffer for 16 hours at 37°C. Then the residue obtained after two washing steps with ethanol 50%, which consists in the non-digestible starch, was hydrolyzed with KOH 2M at 4°C. The resistant starch was measured by adding GOPOD reagent to 100 µl of sample and following the procedure adopted for total starch described above.

3.3.7 Determination of total dietary fiber content

Total Dietary Fiber (TDF) content was measured using an enzymatic kit for fiber determination (Bioquant, Merck, Darmstadt, Germany) according to the Official Method 991.42 (AOAC, 1995), and an automatic filtration of the hydrolyzed products (Fibertec system, FossItalia, Italy). The procedure involves an initial enzymatic separation with heat resistant α-amylase (30 minutes at 95-100 °C) in order to convert the starch to a paste and achieve its partial breakdown. This is followed by protein digestion with proteases (30 minutes at 60 °C) and breakdown of the residual starch with amyloglucosidase (30 minutes at 60 °C). At the same time, the crucibles were prepared with inside 0.5 g of celite, weighed individually and placed at 525 °C overnight in the flask. Following enzymatic separation, 280 ml of 95% ethanol to each flask were added, previously heated to 60 °C, and left at room temperature for one hour. The precipitate was filtered using a Fibertec, by washing with ethanol 78%, then ethanol 96% and acetone to remove any residues. The samples were then dried in an oven at 105 °C overnight and subsequently weighed. The protein content of the residue of the first sample batch is determined by the Kjeldahl method, and the ash content of the residue of the second batch is determined. The mean weight of the two residues

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obtained after subtraction of the value for protein, ash and blank solution corresponds to the content of dietary fiber in the product. The dietary fiber content, weight percentage, was then obtained using the formula (Fig.17):

$$w = \frac{m_R - m_P - m_A - m_B}{m} \times 100$$

$$w = \frac{m_R - [(V_1 - V_2) \times 1.4007 \times 6.25] - m_A - [m_{RBlank} - [(V_1 - V_{2Blank}) \times 1.4007 \times 6.25] - m_{ABlank}]}{m} \times 100$$

$$m_B = m_{RBlank} - m_{PBlank} - m_{ABlank}$$

$$m_P = (V_1 - V_2) \times 1.4007 \times 6.25$$

w: total dietary fiber, by weight, in %
m_B: mass of the blank, in mg
m_P: mass of protein in the residue, in mg
m_R: mean of residue masses, in mg
m_A: mass of ash in the residue, in mg
V_1: amount of HCl 0.1 mol/l measured out, in ml
V_2: amount of NaOH 0.1 mol/l consumed, in ml
m: mean of the sample weights, in mg

Figure 17. *Total Dietary Fiber calculation from Merck analytical test kit*

3.3.8 Determination of β-glucan content

The β-glucan concentration was measured using a Megazyme Mixed-Linkage Beta-Glucan kit, according to the enzymatic method of McCleary and Codd (1991).
0.5 g of flour samples were added into polypropylene tubes to 1 ml of aqueous ethanol (50% v/v). Then 5 ml of sodium phosphate buffer (20 mM, pH 6.5) were added and the tubes stirred on a vortex mixer. After an incubation in a boiling water bath for approximately 2 min, the tubes were removed and vigorously stirred on a vortex mixer, and then heated again for a further 3 min in the boiling water bath (mixing after 2 min prevents formation of a lump of gel material).Then the tubes were cooled to 40°C and 0.2 ml of lichenase (10 U) were added to each tube, then capped, stirred and incubated at 40°C for 1h. After adjusting the volume of each tube to 30 ml by the addition of distilled water and a thoroughly mix of the tubes, they were centrifuged at 1,000 g for 10 min. Carefully and accurately aliquots (0.1 ml) from each sample were transferred to the bottom of three test tubes. Then, an aliquot (0.1 ml) of sodium acetate buffer (50 mM, pH 4.0) was added to one of these (the reaction blank), while to the other two (the reaction) was added with 0.1 ml of β-glucosidase (0.2 U) in 50 mM acetate buffer (pH 4.0). After incubation at 40°C for 15 min, GOPOD Reagent (3 ml) was added to each tube and incubated at 40°C for 20 min. The absorbance was measured at 510 nm

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for each reaction (EA) and reaction blank (EBl) and the β-glucan content was obtained using the formula (Fig. 18):

$$\beta\text{-glucan (\% w/w)} = \Delta A \times F \times 300 \times \frac{1}{1000} \times \frac{100}{W} \times \frac{162}{180}$$

$$= \Delta A \times \frac{F}{W} \times 27$$

Figure18. *β-glucan content calculation from Megazyme β-glucan kit*

Where:

ΔA	=	absorbance after β-glucosidase treatment (reaction) minus reaction blank absorbance.
F	=	factor for the conversion of absorbance values to µg of glucose.
	=	$\frac{100\ (\mu g\ \text{of D-glucose})}{\text{absorbance of 100 µg of D-glucose}}$
300	=	volume correction (i.e. 0.1 mL taken from 30.0 mL).
10,000	=	volume adjustment factor (0.1 mL was analysed but results are presented per litre of sample).
$\frac{1}{1000}$	=	conversion from µg to mg.
$\frac{100}{W}$	=	factor to express β-glucan content as a percentage of dry flour weight.
W	=	the calculated dry weight of the sample analysed, in mg (refer to example results sheet on page 6).
$\frac{5}{5}$	=	volume correction factor. For wort samples, 5.0 mL aliquots were treated with precipitant (ammonium sulphate) and the volume was readjusted to 5.0 mL (i.e. 4.8 mL + 0.2 mL lichenase).
$\frac{2}{5}$	=	volume correction factor. For beer samples, 5.0 mL aliquots were treated with precipitant (ammonium sulphate) and the volume was readjusted to 2.0 mL (i.e. 1.8 mL + 0.2 mL lichenase).
$\frac{162}{180}$	=	factor to convert from free D-glucose, as determined, to anhydro-D-glucose, as occurs in β-glucan.

Figure 19. *β-glucan content calculation from Megazyme β-glucan kit*

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3.3.9 Determination of fructo-oligosaccharides content

The analyses of fructo-oligosaccharides (FOS) were carried out by a preliminary extraction with 96% ethanol for 1h at 80°C, followed by a water extraction for 2 h at 105°C. The ethanol-soluble fraction mainly contained low molecular weight carbohydrates (e.g., mono- and di-saccharides); whereas high molecular weight carbohydrates (FOS) were mainly present in the water-soluble fraction. Glucose and fructose contents were determined in the two fractions using enzymatic (glucose oxidase/peroxidase kit; Sigma Diagnostic, St Louis, USA) or chemical (resorcine-HCl) procedures, respectively. Because fructans are exclusively composed of fructose and glucose, their quantification allowed the measurement of total fructan content. Fructans were investigated as described by D' Egidio et al. (1999). For alcoholic extraction, 96% ethanol was preheated in a bath at 40 °C. 100 mg of sample were inserted into 25 ml pirex tubes with screw cap and 10 ml of 96% pre-warmed ethanol was added to each sample. The tubes containing the samples were then placed in a bath at 80 °C for 1h, stirred every 30 minutes. Once cooled, the samples were transferred into 25 ml corex tubes and centrifuged at 14,000 rpm for 15 minutes at 4 °C. An alcoholic supernatant was obtained from the centrifuge, collected in 50 ml falcon tubes, and the pellet, transferred into 25 ml pirex tubes. Then 25 ml of distilled water were added to the pellet and the tubes were placed in an oven for 2 hours at 105 °C. The samples were shaken every 30 minutes. At the end of the two hours the samples were filtered using fast filter paper and collected in 50 ml falcon tube. For the determination of fructose, 0.1 ml of alcoholic and aqueous solution were taken and 5 ml of Resorcin reagent (100 mg of resorcin dissolved in 100 ml of EtOH 96% solution stock) were added to all the samples, including the standards. The samples were placed in a bath at 80 °C for 20 minutes. For the determination of glucose, 0.1 ml of aqueous and alcoholic solution were taken and 1 ml of 0.02 M HCl was added to each sample, including the standards and were incubated at 85 °C for two hours. Once the spectrophotometric analysis at 510 nm was performed, it was possible to quantify the fructan content by taking into consideration the aqueous fraction of glucose and fructose.

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3.4 BIOACTIVE COMPOUNDS ANALYSIS

3.4.1 Determination of total antioxidant capacity

The total antioxidant capacity (TAC) was determined according to the direct method used by Serpen et al. (2008). This method involves the direct contact of the flour with a solution containing the radical compound ABTS+ (2,2'- azino-bis3-ethylbenzthiazoline-6-sulforic acid). The radical was prepared following the procedure described by Re et al. (1999): 7 mM of ABTS together with 2.45 mM of KPS (potassium persulphate) are dissolved in water and the solution is left in the dark for 12-16 hours before use; subsequently the solution containing the radical is diluted with the solvent selected for the analysis (ethanol 50%) until obtaining an absorbance value of 0.7 OD at 734 nm wavelength. In the details, 90 mg of cellulose plus 10 mg of wholemeal, 1 mm granulometry, were dissolved in a radical solution containing ABTS and KPS, incubated for 50 minutes in an orbital shaker at 190 rpm at 25 °C. 2 ml of each sample were taken and centrifuged at 10,500 rpm for 10 minutes. The measurement of the absorbance was carried out at a wavelength of 734 nm and a Perkin Elmer Lambda 3B spectrophotometer was used. The total antioxidant capacity measurement was obtained by determining the absorbance decrease of the free radical in solution with respect to the initial absorbance value. Three replications were made per sample. The TAC is expressed in millimoles TEAC (Trolox Equivalent Antioxidant Capacity) per kg of sample.

3.4.2 Yellow colored pigment content

Yellow colored pigments (YCP) were determined by the method described by Fares et al. (1991). Samples (2 g) were added to 10 ml water-saturated n-butanol and mixed by handshaking and kept in the dark for 3h in an agitator at room temperature, to allow the extraction of the pigments. The extracted underwent a filtration process, before determining the absorbance at a wavelength of 435.8 nm. The pigment content was calculated directly from the absorbance using the conversion factor of 1.6632, as described in the AACC method 14.50 (AACC, 2000). The analysis was performed in triplicate and the results were expressed as milligrams of β-carotene per kilogram of dry weight (mg/kg d.w.).

3.4.3 Determination of total anthocyanin content

The extraction procedure involved the addition of 10 ml of solvent 1%HCl in methanol to 0.5 g of sample in 50 ml centrifuge tubes and shaking the samples for 2h in an orbital shaker at 50 rpm at 25 °C. Samples were then centrifuged at 4500g for 10 min and decanted. Residues were rinsed with two additional 10 ml volumes of solvent with shaking for 5 min, centrifuging at 4500g for 10 min, and decanting in each case. The three aliquots were combined and used for analysis. The pH differential method as reported by Fuleki and Francis (1968) and Giusti and Wrolstad (2001) was used for quantitative determination of anthocyanins with minor modifications (Awika, 2004b). One of two 0.2 ml aliquots was diluted with 2.8 ml of pH 1.0 buffer (125 ml of 0.2N KCl, and 385 ml of 0.2 N HCl) and the other with pH 4.5 buffer (400 ml of 1N sodium acetate, 240 ml of 1N HCl, and 360 ml of distilled water). The absorbance was measured by scanning with a UV-vis spectrophotometer from 300 to 700 nm. Total anthocyanin pigments were determined from absorbance in pH 1.0 buffer. Monomeric anthocyanins could not be estimated because a significant absorbance for purified monomeric sorghum 3-deoxyanthocyanidins in pH 4.5 buffer was releaved, as observed by Awika (2004b). Extinction coefficients for anthocyanin standards were determined using the formula described by Fuleki and Francis (1968) and expressing the results as cyanidin-3-glucoside equivalents for durum wheat and teff, and luteolinidin equivalents for sorghum samples.

3.4.4 Determination of total soluble poliphenol content

Total soluble poliphenols (TSP) were extracted using the following method: 250 mg of wholemeal flour was weighted and mixed with 1 ml of 80:20 (v/v) ethanol:water solution, the resulting mixture was sonicated for 10 min, maintaining the temperature at 4 °C to avoid starch gelatinization, and then centrifuged for 10 min at 10,000 rpm at 4 °C. The supernatant was transferred in a new vial. The extraction was repeated twice with the 80:20 (v/v) ethanol:water solution and the supernatants were combined, evaporated to dryness with gaseous nitrogen, and then lyophilized to avoid the oxidation of the extracted compounds. A 500 µl volume of 2% (v/v) aqueous acetic acid solution was added to the lyophilized sample produced, which was subsequently

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acidified to pH 2.0 with 12N HCl to enable extraction into organic solvent. After mixing, 500 µl of ethyl acetate was added and mixed at room temperature. The resulting mixture was centrifuged at 10,000 rpm for 2 min, and the upper organic layer was collected in a clean vial. The extraction with ethyl acetate was repeated twice, and the combined supernatants were evaporated to dryness with gaseous nitrogen and stored at −20 °C. The extracts were reconstituted in 100 µl 80:20 (v/v) methanol/water solution. The TSP content was determined using the Folin-Ciocalteau method as reported by Moore and Yu (2008): 50 µl sample was added to 3 ml of water and to 250 µl of Folin-Ciocalteu reagent and neutralized with 750 µl of sodium carbonate (20%, w/v). After incubation in a dark place at room temperature for 2h, the absorbance was measured at 765 nm using a spectrophotometer Lambda Bio20 (Perkin Elmer, Monza, Italy). A solution containing all the reagents, but without the sample, was used as a blank. TSP content was expressed as milligrams of ferulic acid equivalents per kilogram of dry weight (mg FAE/kg d.w.).

3.4.5 Determination of folate content

For the evaluation of the total folate content, the samples were extracted in duplicate in 50 ml of CHES-HEPES buffer at pH 7.85 at 100 °C in the presence of 2-mercaptoethanol and 10% ascorbic acid (Pfeiffer et al., 1997). An aliquot of each sample (1 ml) was treated with the enzyme deconiugase (Hog-Kidney aceton powder, SIGMA, Porcine, type 2 k7250-10G) at pH 4.6 for 4h at 37 °C in a water bath (Ruggeri et al., 2004). The total folate content was determined using the official microbiological method (DeVries et al., 2005), with *Lactobacillus casei* subsp. rhamnosus as a microorganism (ATCC 7469) and folic acid (PGA) as a standard evaluated in purity by the Kariluoto et al. (2004) method. To evaluate the accuracy of the analyses together with the samples, two certified samples were analyzed: Lyofilised Mixed Vegetable CRM- 485 (Institute of Reference Materials and Measurement, Geel, Belgium) and Haricots Verts Major Components (BCR 383).

3.5 FOOD AND BEVERAGE TECHNOLOGICAL TRANSFORMATIONS

3.5.1 Brewing attitude

Grain quality parameters important for malting are considered: 1,000-kernel weight, test weight, total protein content, total starch content, falling number, grain moisture, these last already described above, and the seed size, germinative capacity, germinative energy and mean germination time.

3.5.1.1 Seed sieving-Carter Dockage Tester

The screening of kernels was carried out using a mechanical separator (CARTER DOCKAGE TESTER). This procedure is important for the calibration of the grain for the malting tests for sorghum and barley, since a uniform size of kernels is essential for a homogeneous germination. For all cereals under study, the largest caliber was selected because the kernels size is directly proportional to the starch content. Furthermore, this practice allowed the removal and elimination of any foreign material.

3.5.1.2 Germination measurements

In order to evaluate Germinative Capacity (GC), Germinative Energy (GE) and Mean Germination Time (MGT) of the samples, 100 seeds are placed in a petri dish on a filter paper moistened with about 4 ml of water. During the test the petri dishes are kept at constant temperature (25 °C) and high humidity. Germinated seeds were counted after 24, 48 and 72 hours in the darkness. After three days, at least 96% of the seeds should be germinated, but in some cases the period of the test was extended to five days. For each sample, three replicates of 100 seeds were performed.

3.5.1.2.1 Germinative capacity

The germinative capacity (GC) is expressed as the percentage of kernels germinated under favorable conditions. Since the results of the germination tests are reproducible and comparable to each other it is necessary to use standardized methods which allow the control of the main factors (substrate, temperature, humidity, light) that influence seed germination.

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The germinative capacity (GC) is obtained from the following formula:

$$\mathrm{GC} = \frac{\mathrm{n}}{N} * \ 100$$

N= total number of seeds of the analyzed sample

n= number of seeds germinated after 72 hours

3.5.1.2.2 Germinative energy

The germinative energy (GE) considers the speed with which the seeds sprout. The germinative energy is a parameter that indicates the germination rapidity and is expressed as the weighted average of the germination days with respect to the number of germinated seeds. This parameter contains two values: the germination speed and the germination force (vigor, robustness, integrity of the shoots). Unfortunately, for this analysis there is no standardized and univocal methodology, so the results are not always comparable with each other. For the purposes of this work, the analysis of germinative energy makes it possible to obtain information on the most suitable methods for the wetting and germination phases. A seed with good germinative energy generally shows a homogeneous germination, fundamental in the malting process.

The germinative energy (GE) is obtained from the following formula:

$$\mathrm{GE} = \frac{100\mathrm{N}}{\sum(n1 * t1) + (n2 * t2) + (ni * ti)}$$

N= total number of seeds of the analyzed sample

n= number of seeds germinated in different time intervals

t= time when the seeds have germinated (i.e. 4 germination days)

3.5.1.2.3 Mean germination time

The determination of the mean germination time (MGT) considers the germination rate, only one of the two values that form the concept of germinative energy, which can be expressed in mathematical terms through the Pieper equation, which describes this parameter as the ratio between the summation of the number of seeds germinated in time t, divided by the total number of seeds.

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The mean germination time (MGT) is obtained from the following formula:

$$MGT = \frac{\sum(t * n)}{N}$$

N= total number of seeds of the analyzed sample

n= number of seeds germinated in different time intervals

t= time when the seeds have germinated (i.e. 4 germination days)

3.5.2 Micromalting procedure and malt analysis

Two malting experiments were carried out using different parameters (temperature and time) in steeping, germination and kilning process. The malting experiments for the brown teff followed the method exposed by Di Ghionno et al. (2017) and for the sorghum sample the method reported by Djameh et al. (2015) with slight modifications. In these methods, some of the factors that influence the malting characteristics such as steeping time, moisture content and germination time were optimized while the kilning temperature of the germinated samples (green malt) were altered in order to measure its effect.

3.5.2.1 Steeping and germination

A plastic container was used for the steeping and germination processes. About 2 Kg of the grain sample were used per set of malting. The sample was placed in the container, flushed with tap water and washed thoroughly. Dead grains were detected from the bulk sample during this operation because grains which floated were likely to be deficient in endosperm material. Actual steeping was carried out in tap water at a grain/water ratio of 1:1.4 (w/v) for teff and 1:1.5 (w/v) for sorghum at 24°C. The teff sample was steeped for 3h wet, 2h air-rest, 2h wet reaching a steeping degree of 40.5%, whereas the sorghum total steeping time was as longer as 12h; in particular 4 cycles of 3h wet were interspersed with 3 cycles one-hour air-rest. After steeping, germination was operated on moistened blotting paper in the plastic container for two days at 22 °C for teff sample and four days at 27°C for sorghum. The germinating grains were turned daily to expel heat and prevent root matting. Also, water was sprayed onto the germinating grains three times a day during the germinating period to keep it moist, maintain embryo growth and enzymatic modification of the

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endosperm. The steeping and germination were done in a dark cabinet at ambient temperature of about 22 – 27 ºC. At the end of germination, the moisture content of the green malt was determined before kilning.

3.5.2.2 Kilning

The green malt was spread on white tiles and kilned in a forced draft-air oven incorporating a ventilation device. Kilning for teff sample was performed in the dryer using the following program: 20h at 30 ºC, 2h at 60 ºC and 3h at 65 ºC, for a total period of 25 hours. Indeed, for sorghum green malt the kilning process was: 30 °C 1h and 35 °C for 1 hour, then 20h at 40 °C and finally 50 °C for the last 2 hours. Teff malt deculming was performed manually within nylon bags, whereas the sorghum grains were de-rooted by hand using sieves with different diameter: 2.0, 1.0 and 0.5 mm. The oven was pre-heated to each temperature regime before the green malt was loaded for kilning. The moisture content of the malt was determined immediately at the end of the kilning period in view of its hygroscopic nature.

3.5.2.3 Determination of the α- and β-amylase content

The malt-amylase assay procedure betamyl-3® ceralpha® methods kit was used. The method involves the extraction of the enzymes from the matrix followed by the determination of the content that is carried out separately for the two enzymes.

- Extraction phase: 50 g of malt were ground and reduced to a granulometry of 0.5 mm with laboratory mill (Laboratory mill LM 3100). Subsequently, 5 ml of Betamyl-3® buffer A was added to 0.5 g of the flour and the solution was stirred for a few seconds. The samples were incubated at room temperature for 1 hour under constant stirring. At the end of the incubation, the samples were centrifuged at 2000 rpm for 10 minutes. Then, the supernatants were taken and 4 ml of Betamyl-3® buffer B was added. The solution thus obtained was the A extract.
- β-amylase determination: 0.2 ml of malt extract (extract A) was placed into a 12 ml glass tube and pre-incubated at 40 °C for 5 minutes. The same treatment was performed for the Betamyl-3® substrate solution. Subsequently, 0.2 ml of Betamyl-3® substrate solution was added to the tube containing extract A. After being stirred

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for a few seconds, the sample was incubated at 40 °C for 10 minutes. After this time, 3 ml of stopping reagent were added and the solution was vigorously stirred. Finally, the measurement of the absorbance of the samples and of the control reagent against white (distilled water) at a wavelength of 400 nm was carried out with a spectrophotometric apparatus (Perkin Elmer Lambda 3B).

- α-amylase determination: 0.2 ml of extract A were diluted in 3 ml of Ceralpha® Buffer A. The solution thus obtained is extract B. In a 12 ml glass tube 0.2 ml of extract B and the tube was pre-incubated at 40 °C for 5 minutes. 0.2 ml of Ceralpha® substrate solution previously pre-incubated at 40 °C for 10 minutes were added to the tube containing extract B. The sample was incubated at 40 °C for 10 minutes and 3 ml of stopping reagent were added at the end of the incubation. The measurement of the absorbance of the sample and of the control reagent against white (distilled water) was carried out at a wavelength of 400 nm with a spectrophotometric apparatus (Perkin Elmer Lambda 3B).

The enzymatic units per gram of malt were obtained through the following formulas:

$$\frac{\alpha - \text{amylase unit}}{\text{g}} = \Delta\text{E400} * 315.6$$

$$\frac{\beta - \text{amylase unit}}{\text{g}} = \Delta\text{E400} * 19.7$$

3.5.3 Preparation of Italian tigelle bread

Tigelle, also called crescentine or crescenti, are typical focaccias from the mountain areas of Emilia Romagna based on water, salt, flour, lard and yeast. Tigelle can also be made without lard by adding the same amount of butter, seed oil or milk. For these peculiar characteristics it was decided to test teff flour in the production of these unleavened loaves of Italian tradition and two different trials were carried out to prepare 'tigelle', the first with 100% white teff wholemeal flour (WTF) and the second with an amount of ground brown teff malt (WTF+BTM) (Tab.6).

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Table 6. *Formulations of tigelle teff bread*

Ingredients (%)	**WTF**	**WTF+BTM**
White teff wholemeal flour	61	47
(% of total flour)	100%	77%
Brown teff malt ground	0	14
(% of total flour)	0%	23%
Yeast	1.7	1.7
Water	35	35
Extra virgin olive oil	1.5	1.5
Sea salt	0.8	0.8

WTF = 100% white teff flour, WTF+BTM = 77% WT + 23% Brown teff malt

The flour and the dehydrated yeast were mixed in a planetary, then oil and water were added, seasoned with salt and continued to mix until was obtained a compact dough, that could detach from the sides of the bowl. The dough was then worked with the hands on a support surface making it smooth and homogeneous. Then it was placed in a container, covered with transparent film, and left to rest for at least 2 hours at room temperature. After this time, the dough was worked on a floured surface and spread with a rolling pin (the thickness of the dough was about 5 millimeters), then discs with a pastry ring were made from dough. At the same time, the "tigelliera" has been heated over moderate heat on which the focaccia would have been cooked. When the tigelliera was boiling, the tigelle were placed on top and the lid was closed and cooked for 5 minutes. Once the discs have become golden on both sides, they have been removed from the pan and served hot.

3.5.3.1 Sensory Evaluation

A hedonic sensory evaluation was performed using the method of Lawless and Heymann (1999) with 14 not expert panelists, who were students and colleagues of the CREA-IT. Seven attributes were evaluated by the panel including crumb color, crust color, aroma, taste, softness, moistness and overall acceptance. A nine-items hedonic scale was used (9-like extremely, 8-like very much, 7-moderately like, 6-

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slightly like, 5-like/dislike,4-dislike slightly, 3-dislike moderately, 2-dislike very much and 1-dislike extremely).

3.6 STATISTICAL ANALYSIS

Three independent aliquots of composite wholemeal sample were considered as statistical replicates of each genotype. Analysis of variance (ANOVA) was performed with MSTATC program (Michigan State University, East Lansing, MI). Genotype means were compared using the Duncan's multiple range test ($p \leq 0.05$).

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CHAPTER 4: RESULTS

4.1 MERCEOLOGICAL AND TECHNOLOGICAL TRAITS

4.1.1 Thousand kernels weight and test weight

In order to evaluate the merceological parameters of samples under study, the thousand kernels weight (TKW) and the test weight (TW) have been carried out. On average, food-grade (Fd) sorghum reported the 1,000-kernel weight about half of *cv* Iride, durum wheat control (22.6 *vs* 51.9 g), from a minimum of 21.3 g (PSE 7431 hybrid) to a maximum of 23.5 g (SW6237W hybrid), whereas the zootechnical (Zt) *cv* Aralba presented the highest value of 24.3 g (Fig.20). Teff genotypes are characterized by an extremely low TWK, the mean values were 0.28 g in white teff and 0.26g in brown teff (Fig.20).

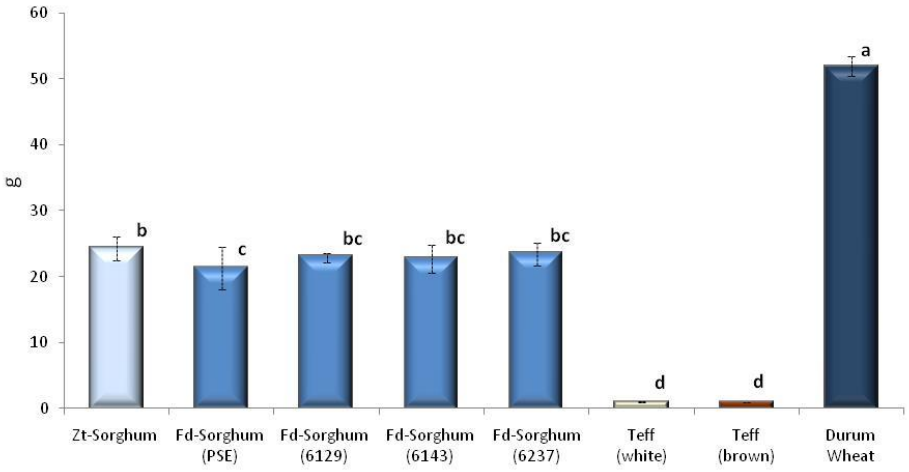

Figure 20. *Mean values ± standard deviation of TKW in five sorghum hybrids and two teff genotypes compared with durum wheat (cv Iride). In each histogram, means followed by the same letter do not differ significantly from one another (P<0.05).*

The sorghum test weight was, on average, 15% lower than control (73.2 *vs* 84.9 Kg/hL), and the lowest value was found in food-grade hybrid PSE7431 (67.3 Kg/hL). On the contrary, both teff genotypes highlighted values significantly higher among all the samples examined, with a mean test weight of 86.8 Kg/hL (Fig.21).

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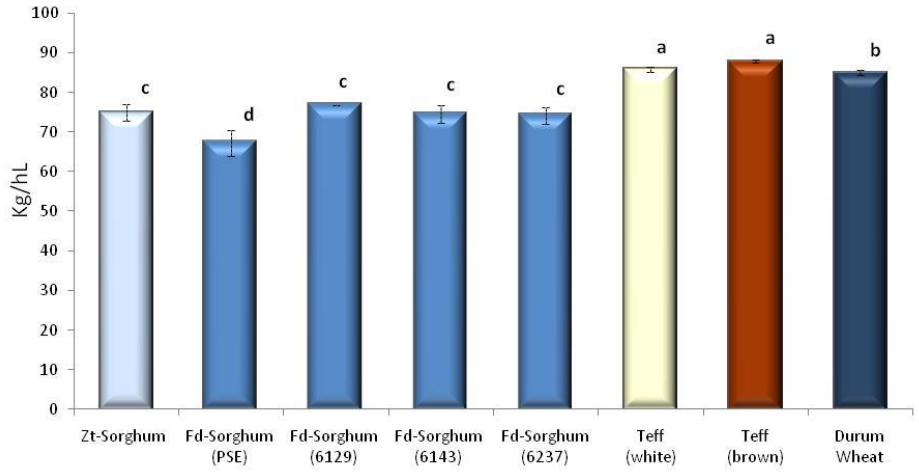

Figure 21. *Mean values ± standard deviation of TW in five sorghum hybrids and two teff genotypes compared with durum wheat (cv Iride). In each histogram, means followed by the same letter do not differ significantly from one another (P<0.05).*

4.1.2 Hardness index

The hardness index expressed by the Single Kernel Characterization System observed in sorghum samples was, on average, typical of that of hard common wheats or durum wheat (83.4) (Tab.7). The SW6129 food-grade hybrid showed an extra hard texture (96.1), whereas the PSE7431 food-grade hybrid presented the lowest value for hard texture (59.4).

Table 7. *Mean values ± standard deviation of SKCS in five sorghum hybrids compared with durum wheat. In column, means followed by the same letter do not differ significantly from one another (Duncan test at P<0.05).*

SAMPLES	SKCS Index
Zt-Sorghum	$81.7^{a} \pm 16.2$
Fd-Sorghum (PSE)	$59.4^{b} \pm 23.8$
Fd-Sorghum (6129)	$96.1^{a} \pm 13.2$
Fd-Sorghum (6143)	$89.4^{a} \pm 20.1$
Fd-Sorghum (6237)	$88.9^{a} \pm 15.1$
DurumWheat	$83.0^{a} \pm 5.2$

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4.1.3 Sedimentation Volume

The sedimentation test (SDS) showed both in the sorghum hybrids and in the teff genotypes values about half of those of the control (on average 15, 18 and 36mL, respectively), with the white teff being unique in showing an SDS sedimentation volume as high as 20 mL (Fig.22). This behavior was likely due to their lack of gluten, which is known to play an important role in the visco-elastic properties of dough.

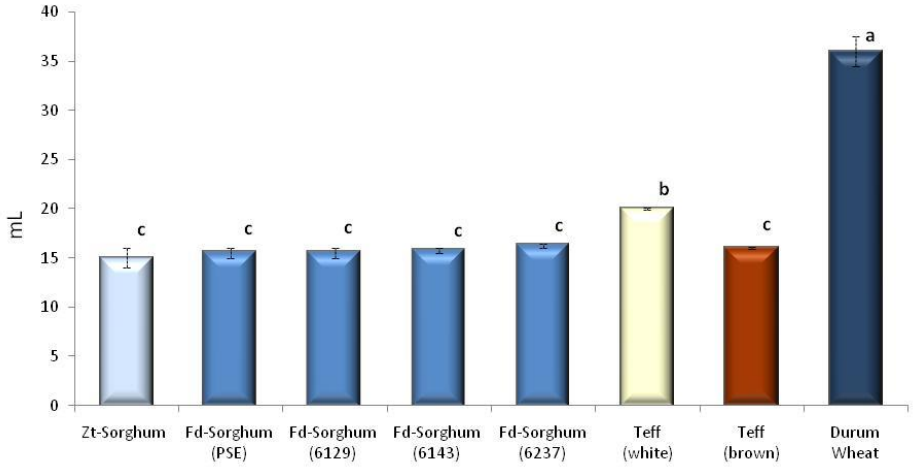

Figure 22. *Mean values ± standard deviation of SDS test in five sorghum hybrids and two teff genotypes compared with durum wheat (cv Iride). In each histogram, means followed by the same letter do not differ significantly from one another (P<0.05).*

4.1.4 Falling Number

The Falling Number (FN) presented very variable data within the two species, but on average both sorghum and teff samples were slightly lower than the control (546 and 531 vs 647 sec, respectively) (Fig.23). The lower values, indicative for a higher α-amylasic activity, were those of food-grade hybrid PSE (339 sec) and brown teff (461 sec), whereas food-grade hybrid SW6237W showed the highest FN (680 sec) and zootechnical Aralba presented an intermediate value (612 sec).

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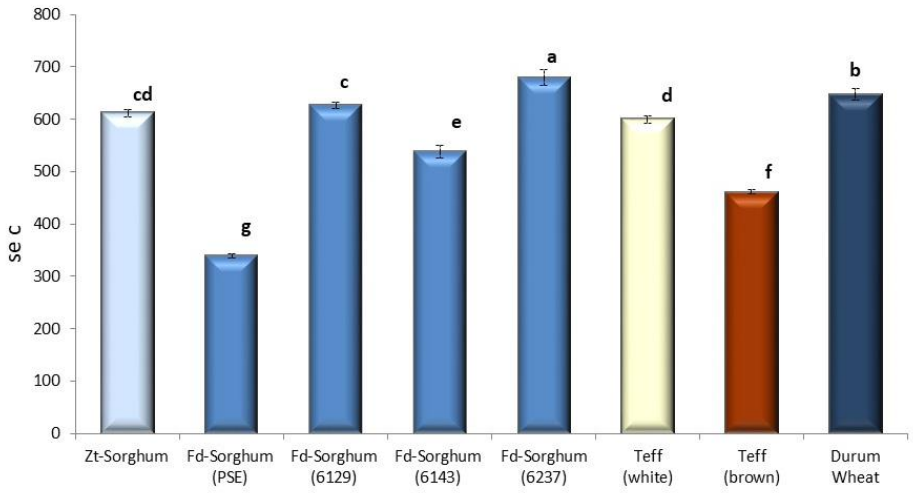

Figure 23. *Mean values ± standard deviation of FN in five sorghum hybrids and two teff genotypes compared with durum wheat (cv Iride). In each histogram, means followed by the same letter do not differ significantly from one another (P<0.05).*

4.1.5 Yellow and Brown index

The chromatic coordinate (b*) and the Luminosity (L*) of the different wholemeal flours are depicted in Fig.24. There were significant differences in the color depending on the type of flour (Fig.25). Both the food-grade sorghum hybrids and the teff genotypes showed on average values of yellow index (b*) lower than 10% of durum wheat (10.9 and 12.0 *vs* 13.5 b*) (Fig.25A), anyway the higher value was performed by white teff genotype (12.3 b*).On the other hand, the brown index (100-L*) highlighted the higher average values of sorghum and teff compared to the control (19.8 and 27.4 *vs* 14.4) with peaks on food-grade sorghum hybrids PSE 7431 (27.9) and brown teff (31.1) (Fig.25B).

Figure 24. *Photographs of whole grain flours of two food-grade sorghum hybrids (SW 6129 and PSE 7431) and two teff genotypes (white and brown) under study.*

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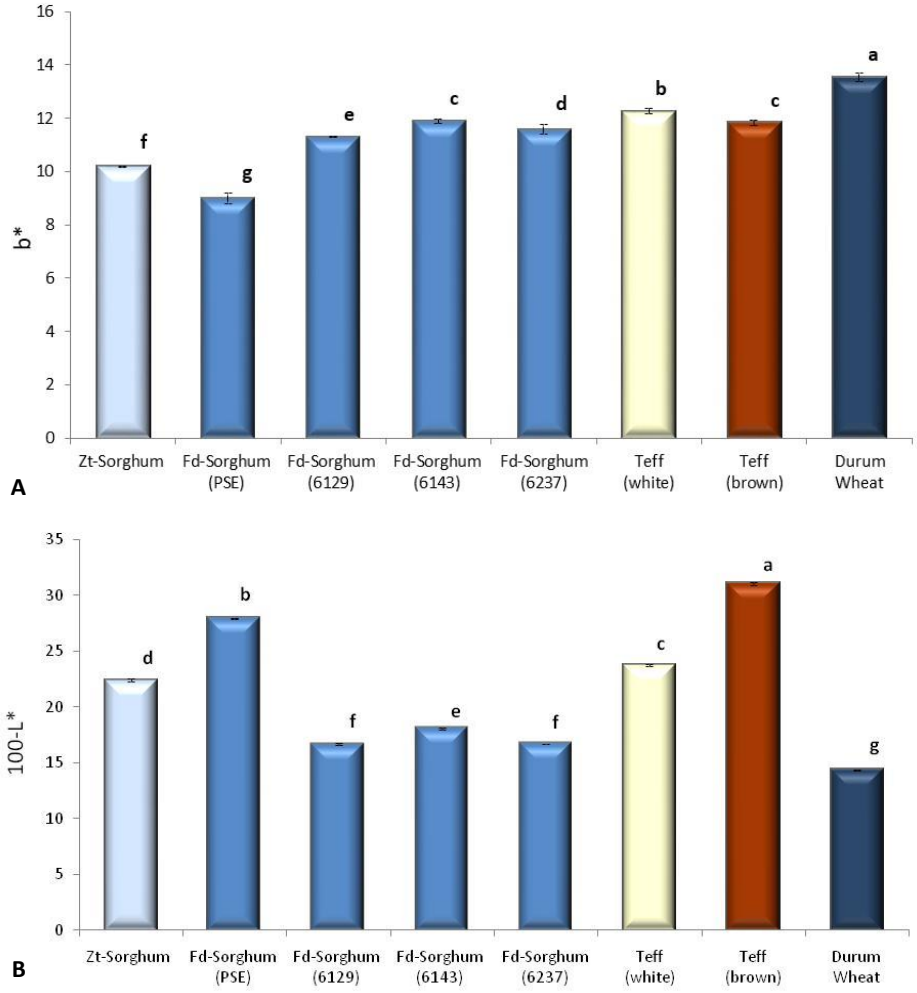

Figure 25. *Mean values ± standard deviation of Yellow(A) and Brown (B) index in five sorghum hybrids and two teff genotypes compared with durum wheat. In each histogram, means followed by the same letter do not differ significantly from one another (P<0.05).*

4.2 BIOCHEMICAL AND NUTRITIONAL TRAITS

4.2.1 SDS-PAGE of sorghum and teff prolamins

The SDS-PAGE fractionation allowed to identify the main component (70-80%) of storage proteins in sorghum kernel, the alcohol-soluble prolamins called kafirins, and in teff genotypes. Durum wheat *cv* Iride and two soft common wheat, *cvs* San Pastore and Centauro, the controls, showed in their elettroforetic pattern the high molecular weight glutenin subunits (HMW-GS), the low molecular weight glutenin subunits

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(LMW-GS) and the gliadins (Fig.26A-B), proteins totally absent in sorghum and teff lines (Figs.26-27).

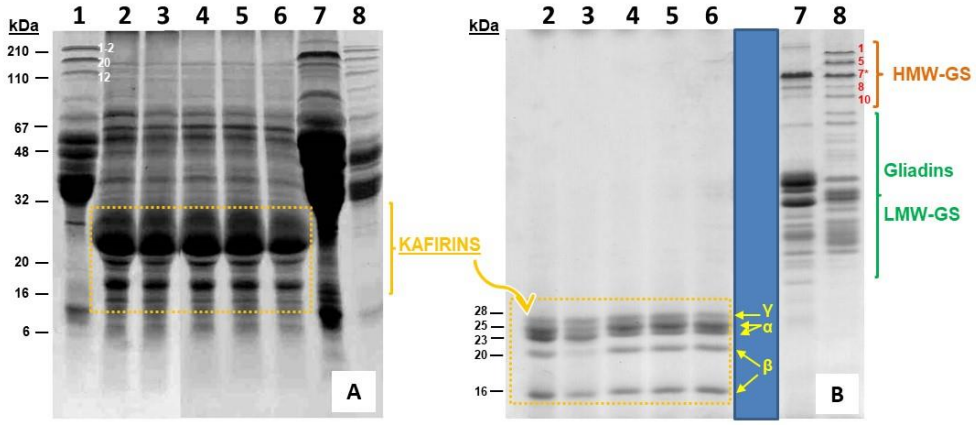

Figure 26. *SDS-PAGE fractionation under reducing conditions of total proteins (A) and kafirins (B) in 5 sorghum lines: Aralba (2), PSE 7431 (3), SW6129 (4), SW6143W (5), SW6237W (6) compared with the controls of common wheat cvs San Pastore (1), and Centauro (8), and durum wheat cv Iride (7). HMW glutenin subunits (HMW-GS) are numbered and arrowheads indicated kafirin proteins.*

Kafirin proteins were observed in sorghum hybrids, in form of dimers and trimers and oligomers. In Figure 26B are highlighted the different kafirins: γ-kafirine with a molecular weight around 28 KDa; α-kafirine with a molecular weight between 23 and 25 KDa, and β-kafirine, with a molecular weight between 16 and 20 KDa (Belton et al., 2006; El Nour et al., 1998; Shull et al., 1992). The Kafirins are located in the endoplasmic reticulum, where they form protein bodies rich in disulfide bonds responsible for the low digestibility of sorghum-based products.

The SDS-PAGE under reducing conditions showed teff prolamins with major bands approximately at 20 and 23 kDa, whereas other bands with higher molecular weights showed very faint signals, indicating that these polypeptides are disulphide bonded (Fig.27). Adebowale (2011) ascribed the presence of broad monomeric prolamin bands in teff even under non-reducing conditions to the observation that teff prolamins is less polymerized than sorghum prolamins.

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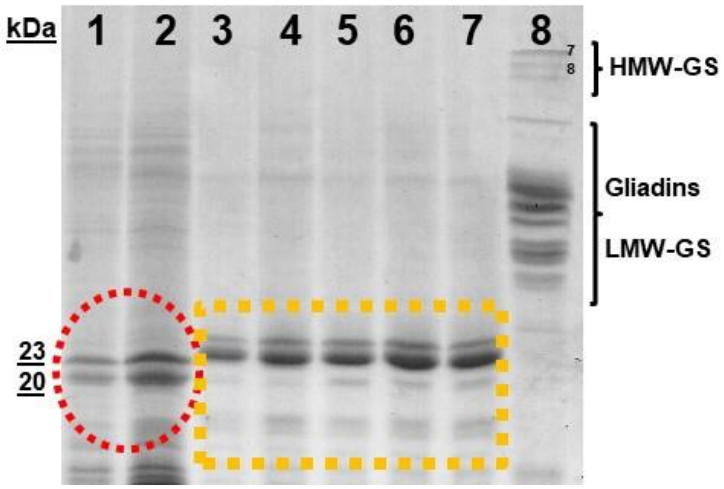

Figure 27. *SDS-PAGE fractionation of storage proteins under reducing conditions from two teff genotypes in the red circle: white (1) and brown (2); kafirins from five sorghum hybrids in the orange rectangle: Aralba (3), PSE 7431 (4), SW6129 (5), SW6143W (6), SW6237W (7); and glutenins and gliadins from durum wheat cv Iride (8). HMW glutenin subunits (HMW-GS) are numbered.*

4.2.2 Protein content

The total protein content (PC) showed a clear difference in protein content between the sorghum and teff lines analyzed and the durum wheat (Fig.28).

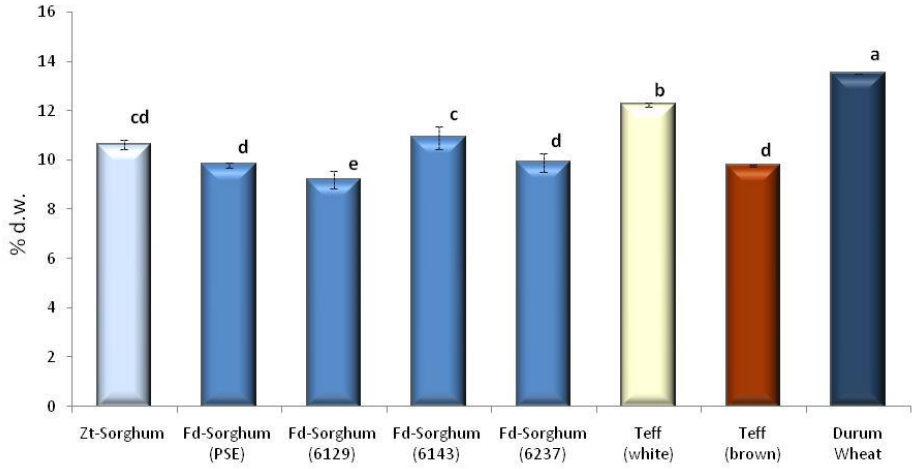

Figure 28. *Mean values ± standard deviation of PC in five sorghum hybrids and two teff genotypes compared with durum wheat. In each histogram, means followed by the same letter do not differ significantly from one another (P<0.05).*

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It is interesting to note that the sorghum hybrids, including zootechnical quality control, presented a homogeneous protein concentration ranged from a minimum content of 9.2% d.w. (SW6129 line) to a maximum of 10.9% d.w (SW6143W line), with an average value of 10% d.w. in food-grade hybrids, 3.5 percentage units lower than durum wheat (13.5%). Teff genotypes, instead, exhibited greater variability with an average value of 11% d.w., but with the highest value of all the samples analyzed in white teff genotype (12.2%) (Fig.28).

4.2.3 Starch granules

The teff and sorghum starch granules were compared for their diameter and surface area with durum wheat control (Fig.29). Teff starch granules are conglomerates of many polygonal simple granules. The individual starch granules in teff are very small (2–6 µm in diameter) and similar in size to rice starch granules (2–10 µm). The shape is polygonal, smooth with no surface pores (Fig.29C). The sorghum granules, conversely, have a large range of sizes (from 30 to 2-3 µm), with typical values of about 10-16 µm range (Fig.29B) and they are often misshapen due to the compressive effects of contact with the protein bodies and as a result may take on complex shapes (Gebremariam et al., 2014; Wolter et al., 2013).

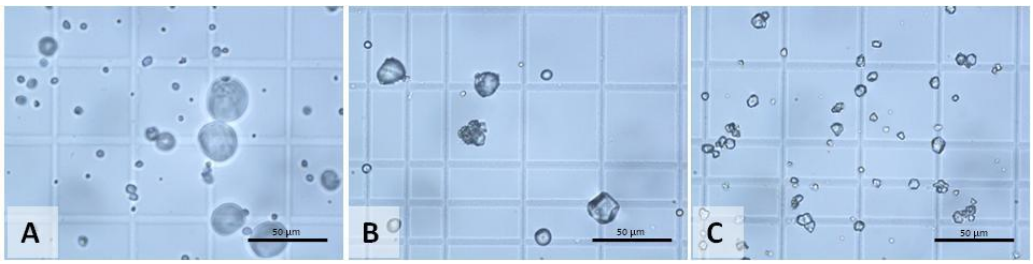

Figure 29. *Starch granules of the endosperm from durum wheat (A), sorghum hybrid (B) and teff genotype (C)*

4.2.4 Total starch content

From the analysis carried out to evaluate the total starch (TS) content, a significant variability among sorghum hybrids has been found, on the contrary the teff genotypes revealed homogeneous values (Fig.30).

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Above all, food grade sorghum hybrids showed, on average, a total starch content of 70.9% d.w., 3.8 percentage units higher than durum wheat control (67.1% d.w.), with a maximum of 74.9% reported in SW6237W line and a minimum value of 67.2% in PSE7431. Furthermore, the zootechnical quality Aralba presented a low total starch content (68.2% d.w.). Teff genotypes reported a mean value of total starch higher than both in food-grade sorghum hybrids and in durum wheat control (72.0 *vs* 70.9 and 67.1% d.w., respectively) (Fig.30).

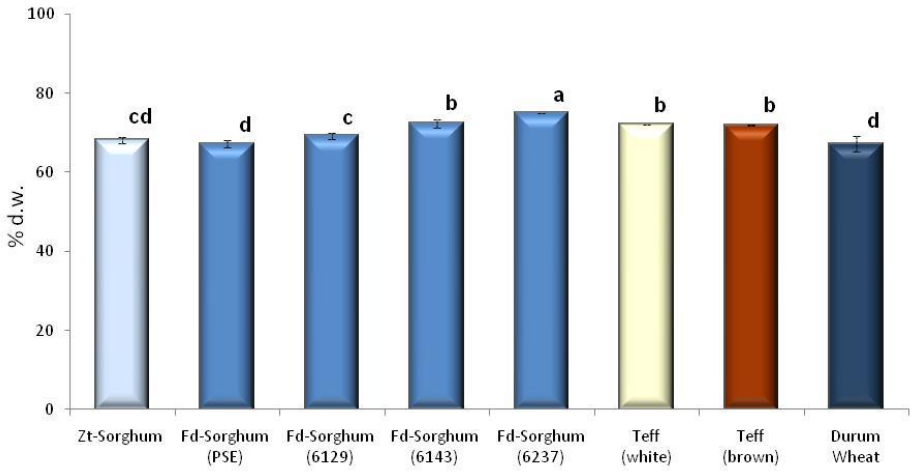

Figure 30. *Mean values ± standard deviation of TS in five sorghum hybrids and two teff genotypes compared with durum wheat. In each histogram, means followed by the same letter do not differ significantly from one another (P<0.05).*

4.2.5 Resistant starch content

The resistant starch (RS) content, exhibited, on average, very high values in the sorghum hybrids (1.714% d.w.),whereas in teff genotypes it was statistically comparable to the durum wheat (0.136 and 0.239% d.w., respectively) (Fig.31). In sorghum hybrids the highest and the lowest data were found in food-grade lines SW6129 and PSE7431 (2.320 and 0.660% d.w., respectively), whereas the zootechnical Aralba showed a still high value (1.920% d.w.) (Fig.31). In any case, among all the samples analyzed, the lowest resistant starch concentration was detected in white teff genotype (0.134% d.w.) (Fig.31).

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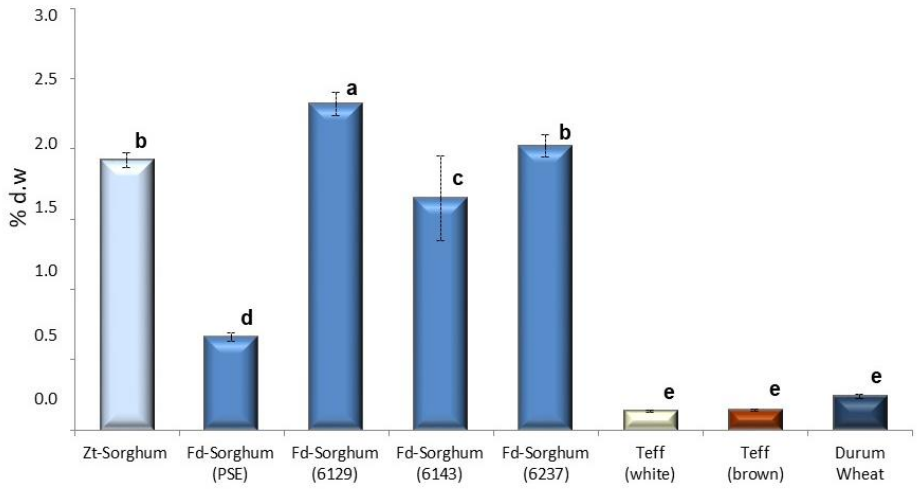

Figure 31. *Mean values ± standard deviation of RS in five sorghum hybrids and two teff genotypes compared with durum wheat. In each histogram, means followed by the same letter do not differ significantly from one another (P<0.05).*

Consequently, the resistant starch/total starch ratio (Fig.32) resulted on average higher in sorghum hybrids than those defined in teff genotypes and durum wheat (2.426 *vs* 0.188 and 0.356% respectively). Moreover, the food-grade hybrid SW6129 showed a value (3.36%) 10 times higher than control.

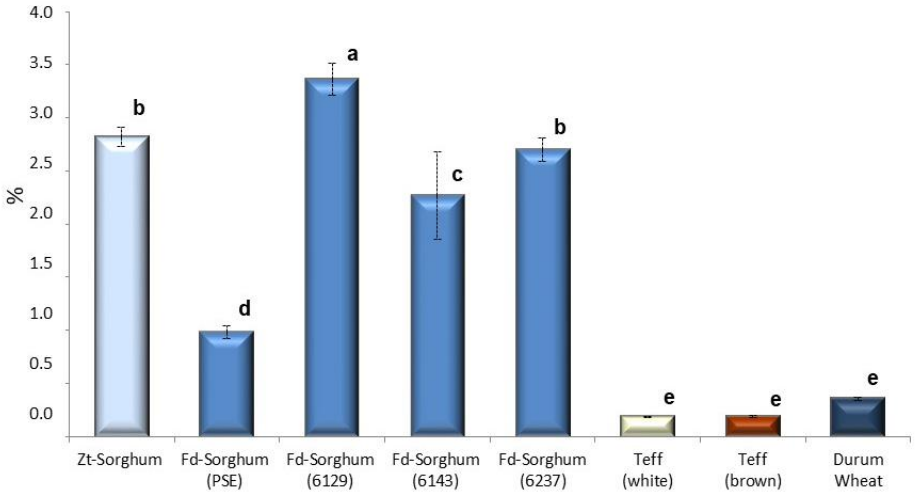

Figure 32. *Mean values ± standard deviation of RS /TS ratio in five sorghum hybrids and two teff genotypes compared with durum wheat. In each histogram, means followed by the same letter do not differ significantly from one another (P<0.05).*

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4.2.6 Total dietary fiber content

On average, total dietary fiber (TDF) content was about 20% lower in both the food-grade sorghum hybrids (9.68% d.w.) and the two teff lines (9.55% d.w.), except for the PSE hybrid (11.75% d.w.) comparable to durum wheat (11.95% d.w.), followed by the SW6143W line (10.22%d.w.) (Fig.33). Unlike these, the SW6129 and SW6237W hybrids showed the lowest values (8.52 and 8.23% d.w., respectively). The zootechnical quality Aralba exhibited intermediate value (9.40% d.w.), statistically similar to the white (9.36% d.w.) and to the brown teff genotypes (9.74% d.w.) (Fig.33).

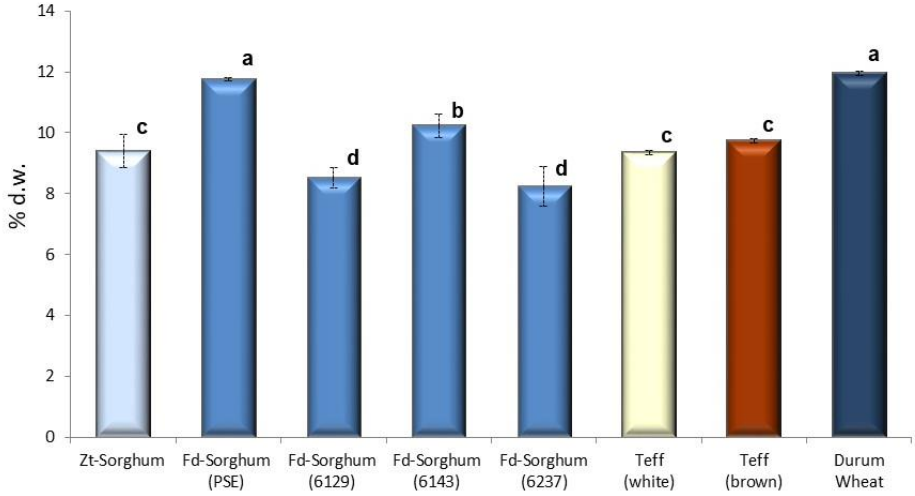

Figure 33. *Means values ± standard deviation of TDF content in five sorghum hybrids and two teff genotypes compared with durum wheat. In each histogram, means followed by the same letter do not differ significantly from one another (P<0.05).*

4.2.7 β-glucan content

The β-glucan content was low in all the examined samples, indeed, on average, the food-grade sorghum hybrids showed value (0.256% d.w.) equal to one third of the durum wheat (0.753% d.w.), whereas the teff genotypes (0.383% d.w.) equal to half of the control (Fig.34). Among all the sorghum hybrids, the zootechnical quality Aralba showed the highest value (0.320% d.w.), however lower than those of white (0.354% d.w.) and brown teff genotypes (0.411% d.w.) (Fig.34).

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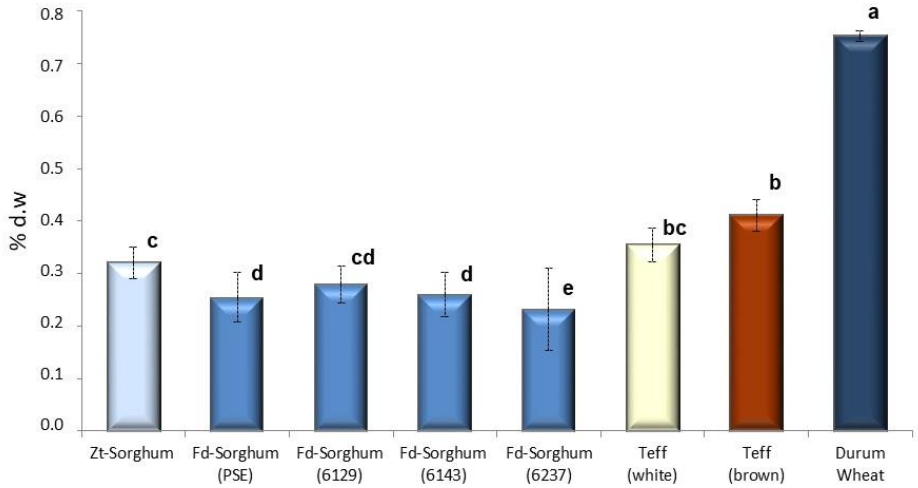

Figure 34. *Means values ± standard deviation of β-glucan content in five sorghum hybrids and two teff genotypes compared with durum wheat. In each histogram, means followed by the same letter do not differ significantly from one another (P<0.05).*

4.2.8 Fructo-oligosaccharides content

As shown in the graph below (Fig.35), the analysis of the fructo-oligosaccharides (FOS) presented a wide range of values between the sorghum hybrids analyzed (from 0.56 to 1.90% d.w.). These values are on average lower than that of durum wheat (1.24 *vs* 1.49% d.w.).

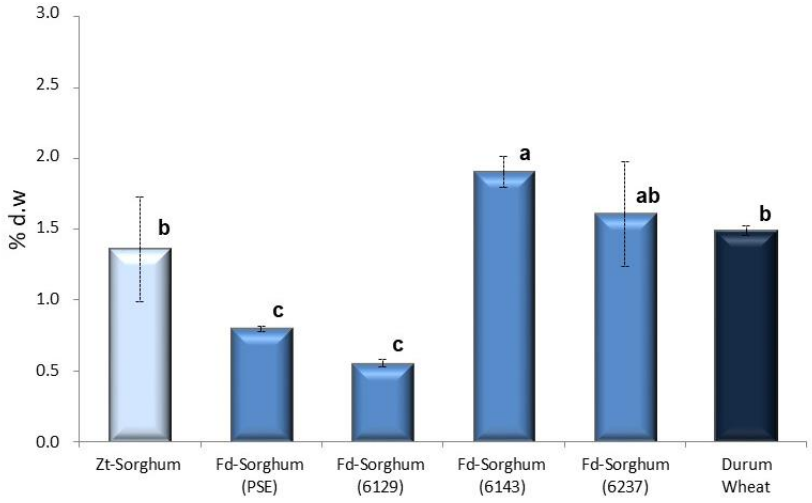

Figure 35. *Means values ± standard deviation of FOS content in five sorghum hybrids compared with durum wheat. In each histogram, means followed by the same letter do not differ significantly from one another (P<0.05).*

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Among sorghum samples, SW6143W and SW6237W showed higher fructan content, with mean values of 1.90% and 1.61%, respectively (Fig.35). Lower values were obtained in the food-grade hybrids PSE 7431 (0.80% d.w.) and SW6129 (0.56% d.w.). The zootechnical control Aralba showed a mean value of 1.36% (Fig.35).

4.3 BIOACTIVE COMPOUNDS

4.3.1 Total antioxidant capacity

The total antioxidant capacity (TAC) showed significantly lower values ($P < 0.05$) both in food-grade sorghum hybrids and in commercial teff compared to durum wheat (on average 30.4 and 35.0 *vs* 43.1 mmol TEAC/Kg d.w.) (Fig.36).

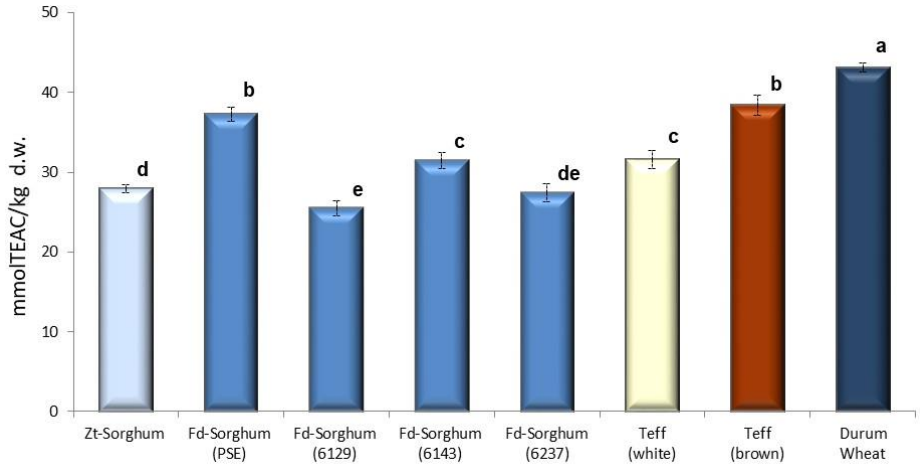

Figure 36. *Means values ± standard deviation of TAC in five sorghum hybrids and two teff genotypes compared with durum wheat. In each histogram, means followed by the same letter do not differ significantly from one another (P<0.05).*

All sorghum values were observed very various and lower than the control, among food-grade sorghum samples, PSE7431 line showed a greater total antioxidant capacity (37.3 mmol TEAC/Kg d.w.), followed by SW6143W (31.5 mmol TEAC/Kg d.w). The lowest values of total antioxidant capacity were obtained in food-grade hybrids SW6129 and SW3237W (25.5 and 27.5 mmol TEAC/Kg d.w., respectively), and in the zootechnical sorghum control (27.9 mmol TEAC/Kg d.w.) (Fig.36).The brown teff commercial genotype revealed the highest TAC (38.4 mmol TEAC/Kg d.w.) among all the samples examined, whereas its white counterpart gave a lower value (31.6 mmol TEAC/Kg d.w.) (Fig.36).

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4.3.2 Yellow colored pigment content

The yellow colored pigment (YCP) content, on average, did not exhibit significant differences in white and brown teff genotypes (3.3 and 3.8 mg β-carotene/kg d.w., respectively) compared with durum wheat (5.3 mg β-carotene/kg d.w.), conversely the total average of all sorghum samples was found higher than control (35.9 mg β-carotene/kg d.w.) (Fig.37).

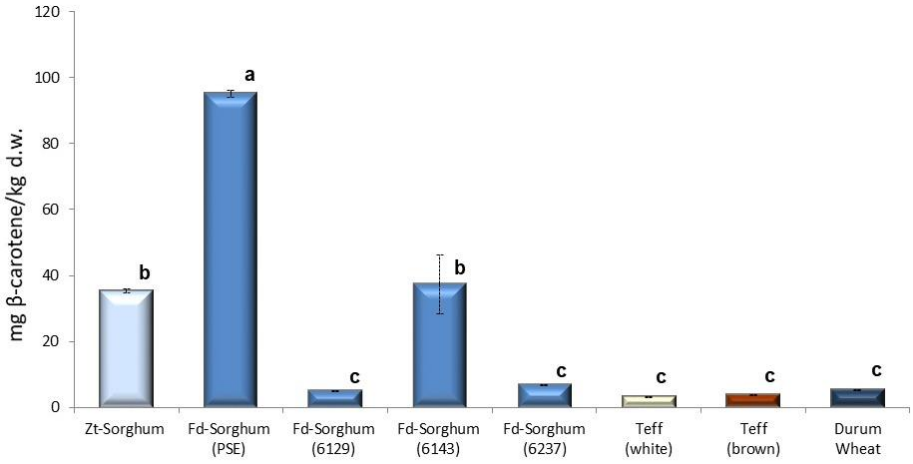

Figure 37. *Means values ± standard deviation of YCP content in five sorghum hybrids and two teff genotypes compared with durum wheat. In each histogram, means followed by the same letter do not differ significantly from one another (P<0.05).*

The highest YCP content among sorghum samples was in the hybrid PSE 7431 (95.3 mg β-carotene/kg d.w.), followed by the SW6143W (37.3 mg β-carotene/kg d.w.) and the zootechnical witness Aralba (35.3 mg β-carotene/kg d.w.) (Fig.37). Among the sorghum lines, the SW6129 and SW6237W hybrids showed the lowest values (6.7 and 5.0 mg β-carotene/kg d.w., respectively), analogous to the control (Fig.37).

4.3.3 Anthocyanin content and total antioxidant capacity in anthocyanin extracts

The white and brown commercial teff genotypes showed anthocyanin (ANT) content (4.2 and 3.7 mg/g d.w. respectively) statistically similar to durum wheat (4.0 mg/g d.w.) (Fig.38). All sorghum lines, instead, revealed data higher than control, for food-grade sorghum hybrids the mean value was more than double (9.2 *vs* 4.0 mg/g d.w.). In particular, in food grade hybrids the maximum value was found in PSE7431 (13.7 mg/g d.w.) and the minimum in SW6237W (6.4 mg/g d.w.) lines (Fig.38). The

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zootechnical quality Aralba showed a content three times higher (11.8 mg/g d.w.) than control (Fig.38).

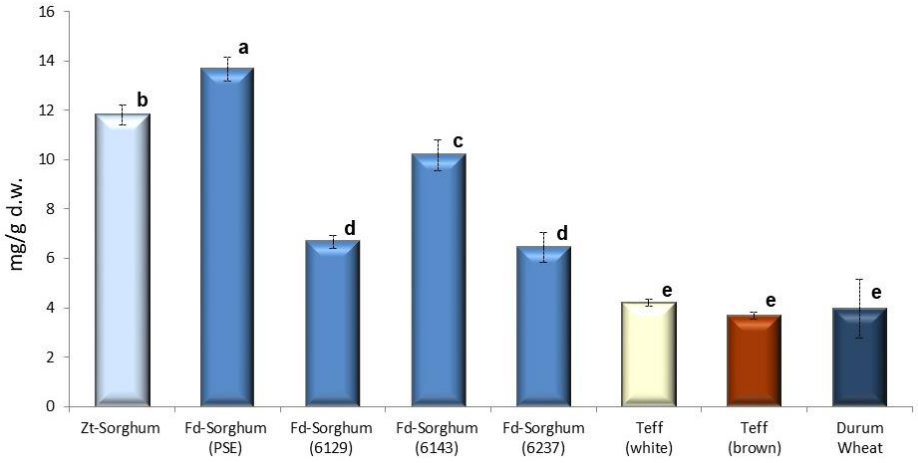

Figure 38. *Means values ± standard deviation of ANT content in five sorghum hybrids and two teff genotypes compared with durum wheat. In each histogram, means followed by the same letter do not differ significantly from one another (P<0.05).*

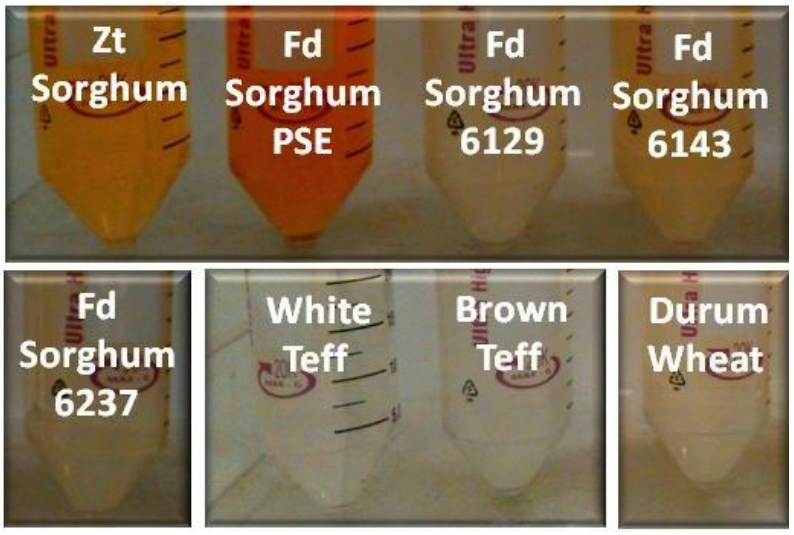

Figure 39. *Photos of methanol extracts of five food-grade sorghum hybrids and two teff genotypes compared with durum wheat.*

The 3-deoxyanthocyanidins, the most common anthocyanin type present in sorghum, produce a yellow (apigeninidin) and orange (luteolinidin) color in acidic solvents (Fig.39) and they are distinctly different from the anthocyanins and their aglycons, which are mostly reddish to purple in acidic media. The total antioxidant capacity

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performed on the anthocyanin extracts (Fig.39) was found in SW sorghum hybrids, on average, 50% lower than durum wheat (0.62 *vs* 1.31 mmol TEAC/kg d.w.), statistically similar to the brown teff genotype (0.67 mmol TEAC/kg d.w.). Conversely, the white teff genotype (1.67 mmol TEAC/kg d.w.), PSE food-grade sorghum hybrid (1.56 mmol TEAC/kg d.w.) and the zootechnical quality Aralba (1.47 mmol TEAC/kg d.w.) exhibited, respectively, values about 30%, 20% and 10% higher than durum wheat (Fig.40).

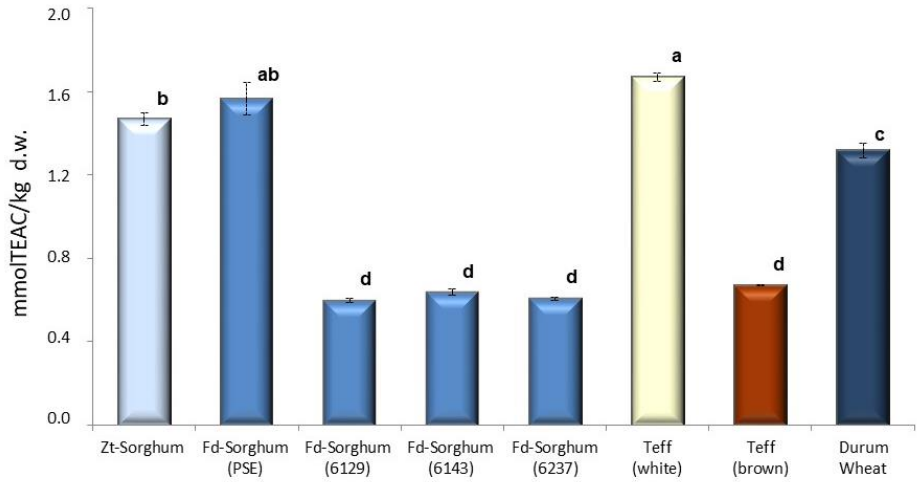

Figure 40. *Means values ± standard deviation of TAC of anthocyanin extracts in five sorghum hybrids and two teff genotypes compared with durum wheat. In each histogram, means followed by the same letter do not differ significantly from one another (P<0.05).*

4.3.4 Total soluble polyphenol content

The analysis carried out for the determination of the total soluble polyphenol (TSP) content revealed a significant great amount in sorghum hybrids (Fig. 41).

On average, food-grade sorghum lines presented TSP content five times higher than durum wheat (1252 *vs* 231 mg FAE/kg d.w.), among these the highest value was detected in PSE7431 hybrid (1933 mg FAE/kg d.w.) and the lowest in SW6143W (909 mg FAE/kg d.w.); in the same way the zootechnical sorghum Aralba showed a mean value of 1052 mg FAE/kg d.w. (Fig.41).

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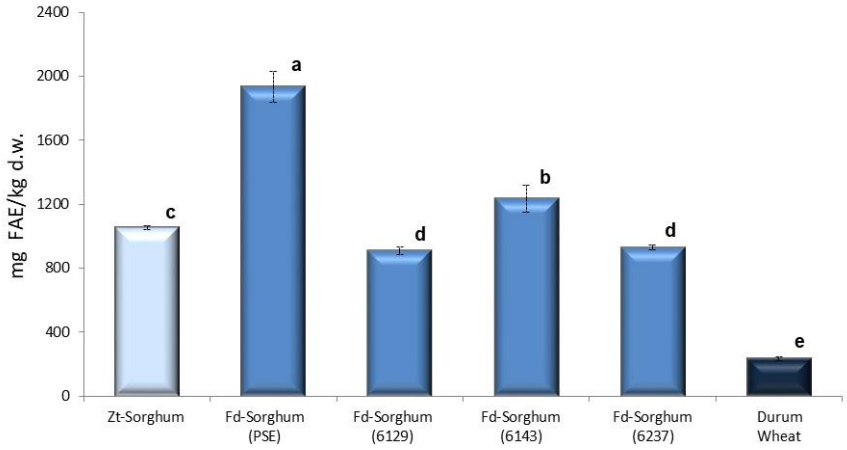

Figure 41. *Means values± standard deviation of TSP content in five sorghum hybrids compared with durum wheat; FAE=ferulic acid equivalents. In each histogram, means followed by the same letter do not differ significantly from one another (P<0.05).*

4.3.5 Folate Content

Folate, an essential component in the human diet, is involved as a cofactor in metabolic reactions (e.g. the biosynthesis of nucleotides) and plays a critical role in the prevention of neural tube defects. The total folate content was found in both teff genotypes 50% higher than durum wheat, on average 59.7 *vs* 41.8 µg/100g; on the contrary, both food-grade SW6237W and zootechnical quality Aralba showed values about 20% lower than the control (32.4 and 33.5 µg/100g) (Fig.42).

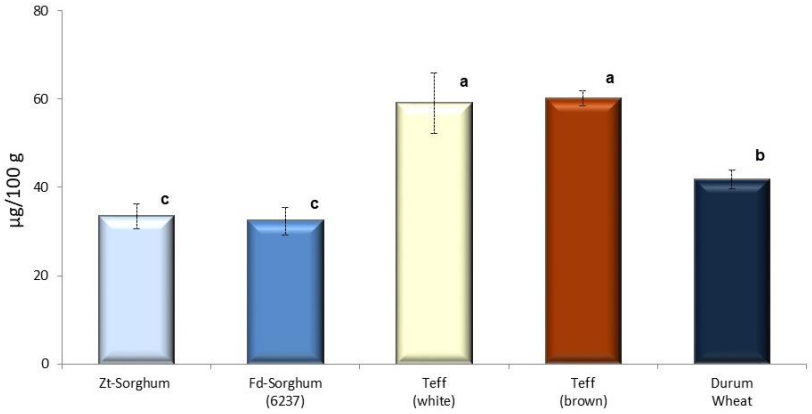

Figure 42. *Means values ± standard deviation of total folate content in two sorghum hybrids and two teff genotypes compared with durum wheat. In each histogram, means followed by the same letter do not differ significantly from one another (P<0.05).*

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4.4 FOOD AND BEVERAGE TECHNOLOGICAL TRANSFORMATIONS

4.4.1 Brewing attitude

In order to investigate the ability of gluten-free cereals to be malted and used as brewing raw material, the brown teff genotype was chosen, which in the analysis of biochemical and nutritional characterization presented the most interesting values for its transformation into alcoholic beverage (rather low protein, β-glucan contents, falling number, and TW and TS with relative high values). Therefore, the brown teff genotype was compared with a sample of the last harvest grains of a commercial sorghum variety and with a control of barley, *cv* Tea, for parameters related to preliminary evaluation of malting attitude. The unmalted grain quality parameters investigated and compared were: 1,000-kernels weight, test weight, total protein content, total starch content, falling number, germinative capacity, germinative energy and mean germination time.

Both brown teff and sorghum commercial genotypes, as highlighted in Table 8, showed unmalted grain quality parameters suitable to be tested as brewing raw material.

Table 8. *Means values ± standard deviation of TKW, TW, PC, TS content and FN in barley, cv Tea, sorghum and brown teff commercial genotypes. In column, means followed by the same letter do not differ significantly from one another (Duncan test at P<0.05).*

	TKW (g)	TW (Kg/hL)	PC (% d.w.)	TS (% d.w.)	FN (sec)
Barley	26.0^{a} ±0.1	73.4^{c}±0.3	10.6^{a}±0.1	64.4^{c}±0.1	303^{b}±7
Sorghum	24.2^{a}±0.4	78.1^{b}±0.2	10.5^{a}±0.3	79.5^{a}±0.9	473^{a}±11
Teff (brown)	0.26^{b}±0.02	87.8^{a}±0.4	9.8^{b}±0.1	71.9^{b}±0.1	461^{a}±4

Among these, one of the most important trait for the production of beer is the protein content, that should not exceed 11%, because an excessive protein content could negatively affect the chemical-physical stability of the final product; so the brown teff genotype, for its lowest value (9.8% d.w.) resulted the sample more interesting. Furthermore, the high starch content is an important prerogative for the malt

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production and it is related to the quantity of fermentable sugars that will be used by yeast during fermentation, as well as the alcohol content of the resulting beverage. As reported in Table 8, sorghum and teff exhibited starch content higher than that of barley control (79.5 and 71.9 *vs* 64.4% d.w.).

The sorghum and brown teff commercial genotypes obtained, on average, falling number values of 473 and 461 seconds respectively (Tab.8), higher than barley control (303 sec) but lower than all the samples previously analyzed (Fig.23), however index of low enzymatic activity. Afterwards the falling number was carried out also on the malts to evaluate the increase of the amylase activity during the malting process. All the malts showed a FN value of 62 seconds, indicating the presence of amylase activity, and therefore the occurrence of the malting process, although this evaluation did not allow to discriminate the level of enzymatic activity of each malt.

4.4.1.1 Seed sieving- Carter Dockage Tester

A homogeneous kernels size is necessary because also the germination times are homogeneous. Therefore, a sieving process of the sorghum kernels was carried out, whereas for the teff genotype this was not possible due to the reduced dimensions of the seed itself. The selected sizes for sorghum was between 3.5 and 3.7 mm.

4.4.1.2 Mean germination time, germinative capacity and germinative energy

The germination (Fig.43) resulted faster in the barley seeds, indeed, after the first day, on average, the 77% of barley seeds germinated, whereas for teff genotype only the 47%, and no seed for the sorghum sample (Fig.44).

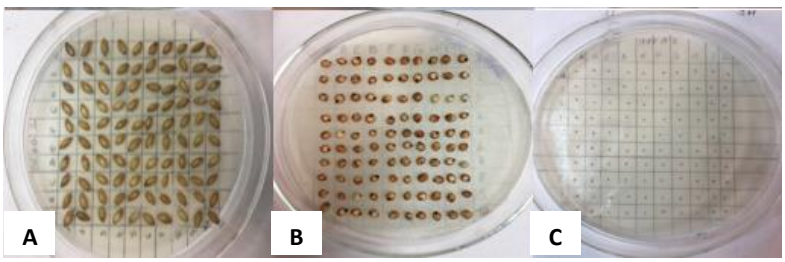

Figure 43. *Germination and germinative energy test of barley cv Tea (A), sorghum (B) and brown teff (C).*

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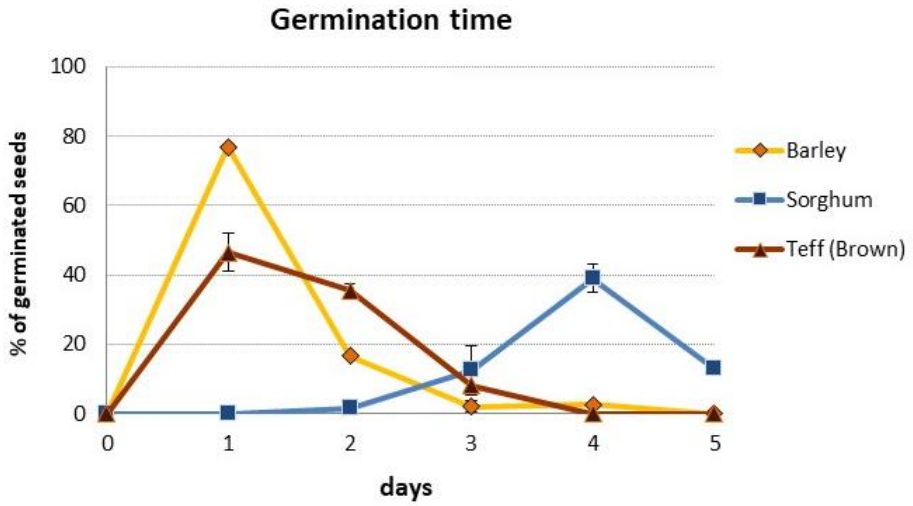

Figure 44. *Means values ± standard deviation of **g**ermination time of barley cv Tea (yellow), sorghum (blue) and brown teff (brown) commercial genotypes.*

Germinative capacity (GC) is a measure of the ability of kernels to germinate in 72 hours and it is considered an index of seed vitality. Usually, values greater than or equal to 96% indicate excellent germination. The results obtained have been reported in figure 45; the brown teff sample showed good germination capacity (90%), similar to barley (96%), whereas sorghum presented a very low value (14%).

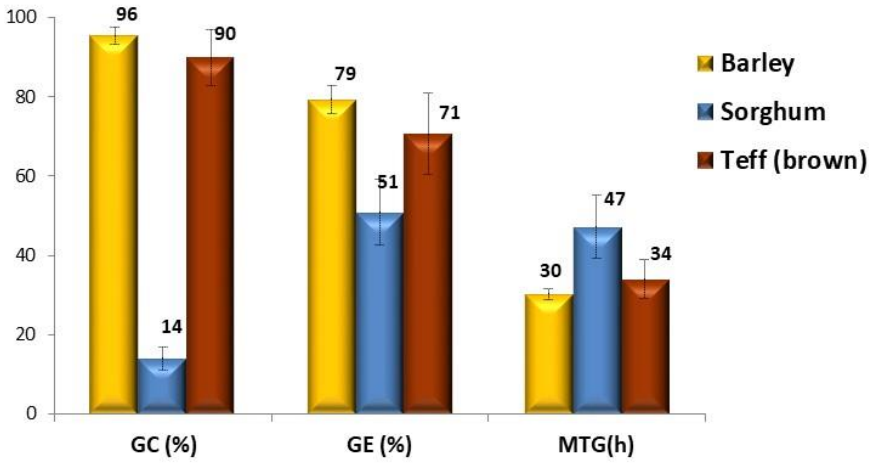

Figure 45. *Means values ± standard deviation of germinative capacity (GC), germinative energy (GE) and mean germination time (MTG) of barley cv Tea (yellow), sorghum (blue) and brown teff (brown) commercial genotypes.*

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The germinative energy (GE) is a parameter that expresses the rapidity of germination and, consequently, the ability of the kernel to overcome the dormant phase. A seed with good germinative energy generally shows a homogeneous birth, fundamental in the malting process. The germinative energy, calculated over a period of 4 days, was higher in barley *c*v Tea (79%), followed by brown teff (71%), and finally sorghum (51%) (Fig. 45), consequently the mean germination time estimated on 4 days, was higher in sorghum (47 hours) than in brown teff and barley samples (34 and 32 hours respectively).

Therefore, under this experimental condition the germinative capacity and the germinative energy in sorghum were insufficient for malting process. The values obtained, in effect, are strictly related to the experimental conditions of the test, so it was decided to modify the parameters most influencing the germination (temperature, time and moisture) to obtain better germinative energy values and to develop a specific malting protocol for each cereal.

4.4.1.3 Micromalting

Two malting experiments were performed using different parameters (temperature and time) in steeping, germination and kilning programs for teff and sorghum (Fig.46), partly modifying the trials of germination tests.

Figure 46. *Teff (A) and sorghum(B) malting processes.*

In fact, the sorghum grains required longer times both for the steeping (12h wet) and for the germination (4 days at 27°C) phases, whereas for the teff grains were enough

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2 days of germination at 22 °C after a steeping period of 5h water rest. According to the literature, the optimum steeping out moisture for sorghum and teff should be respectively 61% and 48% (Dewar et al., 1997, Di Ghionno et al., 2017). In this study, lower results were found both in sorghum (42.3%) and in teff (40.5%) samples. Moisture was determined on raw grains and after steeping, before and after kilning. Results from the two malting trials are showed in Table 9.

Table 9. *Means ± SD of moisture variation during malting steps of teff and sorghum.*

Samples	Moisture (%)			
	Raw grain	**After steeping**	**Before kilning**	**After kilning**
Teff	13.7±0.2	37.7±0.3	40.5±0.5	7.2±0.1
Sorghum	13.3±0.1	40.1±0.5	42.3±0.6	5.6±0.1

The differences in water up-take values, compared to the findings in the aforementioned literature, could be explained by variations in the biochemical characteristics among different cereal and pseudo-cereal cultivars that may affect their malting behavior. This has been supposed by Blaise and Alexander, moreover it has been reported by Owuama that the differences in biochemical characteristics among different sorghum cultivars affected their optimal malting conditions.

4.4.1.4 Amylase activity

Amylase activity is fundamental for the degradation of starch to fermentable sugars which will be used by yeast during fermentation for the production of ethyl alcohol and CO_2.The α-amylase activity is expressed in Ceralpha Units/g dry substance (CU/g d.w.) whereas the β-amylase activity is expressed in Betamyl-3 Units/g dry weight (B3U/g d.w.).The results obtained, reported in Figure 47, showed in all the samples an α-amylase and β-amylase activity considerably lower than those of the control malt. "Basic malt" is a type of malt that, thanks to its high content of amylolytic enzymes, can supply a sufficient quantity of fermentable sugars and, consequently, can also be used as the only malt for the production of beer. The α-amylase activity was found higher in the barley *cv* Tea (194 U/g) than in sorghum and teff malts (79 and 4 U/g respectively). The β-amylase activity compared to barley malt control was 35% and

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80% less in *cv* Tea and brown teff malts, and it was present only in traces in sorghum sample (0.56 U/g).

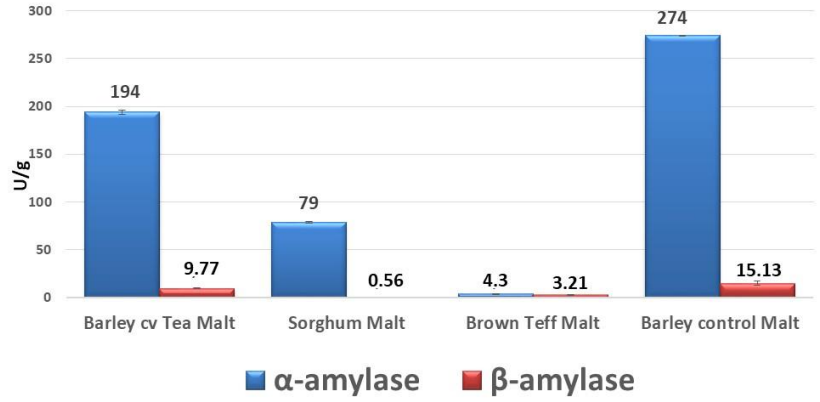

Figure 47. *Means values ± standard deviation of α-amylase and β-amylase content in malt from barley cv Tea, sorghum and brown teff commercial genotypes.*

The malts analyzed did not show an amylase activity comparable to that found in malt obtained from beer barley, therefore the activity was found insufficient to guarantee a suitable quantity of fermentable sugars. As a consequence, the drinks obtained from this type of malts, could show a reduced alcohol content. However, as the light beer market is a growing market, this type of beverage could still be interesting for this sector. Otherwise, the malts produced could be used in combination with other malts for the production of innovative beers, not yet available on the market.

4.4.2 Italian tigelle bread teff-based and sensory evaluation

"Tigelle" are typical focaccias from Emilia Romagna based on water, salt, flour, lard and yeast. For their peculiar characteristics it was decided to test teff flour in the production of these unleavened loaves of the Italian tradition and two different trials were carried out to prepare 'tigelle' (Fig.48), the first dough with 100% white teff flour (WTF) and the second (WTF+BTM) adding 23% of brown teff ground malt obtained after the malting process described above. In fact, considering that the malt enzymes may influence the technological quality of the end products, as noted by Makinen (2013) the fortification of wheat bread with unconventional malted grains has been

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studied previously on malted quinoa, oat, sorghum and brown rice, and it is interesting to study their suitability for use in gluten free baking.

Figure 48. *Phases of preparation of the dough and baking tigelle teff-bread WTF (A) and WTF+BTM (B).*

At first impact the dough without the addition of malt was more elastic, perhaps due to a different absorption of water by the malt.

The use of 100% teff flour (WTF) and teff flour with malt (WTF+BTM) were acceptable according to the overall acceptance scores of the panelists. The radar chart relating to the tasting session carried out by not-expert people are reported below in Figure 49.

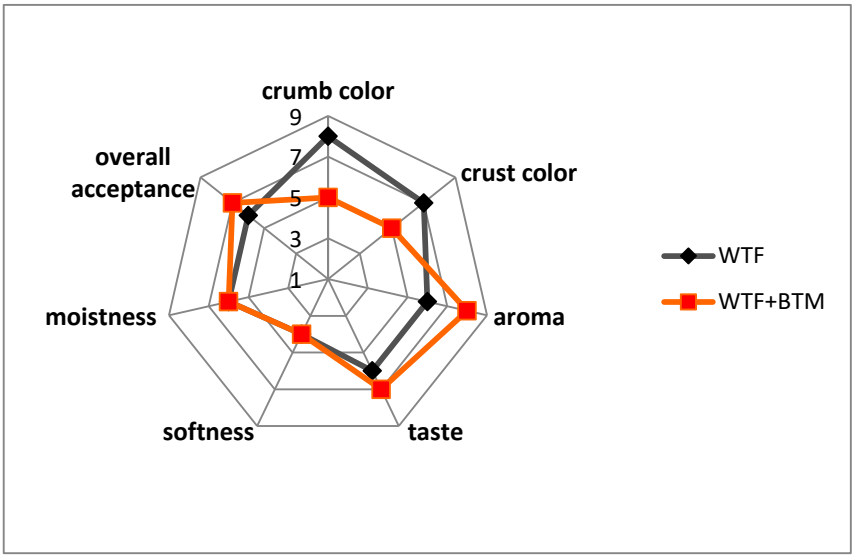

Figure 49. *Sensory evaluation of WTF and WTF+BTM tigelle:9-like extremely, 8-like very much, 7-moderately like, 6-slightly like, 5-like/dislike, 4-dislike slightly, 3-dislike moderately, 2-dislike very much and 1-dislike extremely.*

The results obtained from the hedonic analysis highlighted in WTF higher scores for crumb and crust color, whereas the bread with malt was preferred for its aroma and taste (Fig.49).

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CHAPTER 5: DISCUSSION

The main goal of the present study was to compare the merceological, technological, biochemical and nutritional traits and the bioactive compounds of four food-grade sorghum hybrids and two teff genotypes with durum wheat and zootechnical sorghum cultivars as controls, and to analyse them for their food and beverage technological transformations attitude to evaluate the possibility of introducing these two gluten free cereals and their products in Italian market.

The acquisition of good quality grain is fundamental for the production of acceptable food products from sorghum and teff both for growing population in many developing countries, particularly in West Africa, and for production of wheat-free foods for persons with celiac disease in Western Countries.

Many thousands of sorghum accessions have been developed and are represented in seed collections around the world, particularly collections in Ethiopia, China, USA, and the International Crops Research Institute for the Semi-Arid Tropics (ICRISAT) (Rosenow & Dahlberg, 2000). There is a need for further characterization of the sorghum and teff collections with respect to food and other quality attributes.

Kernel properties including texture and size, impact both the particle size of the final flour as well as starch damage, these attributes in turn playing a large role in final product quality. The kernel texture is worldwide considered a main determinant in wheat end product use and quality, because of its strong effects on milling conditions, granularity of flour and starch granules integrity, flour yield and water absorption, rheological and baking properties. The Single Kernel Characterization System (SKCS) has been widely used in the wheat industry, and SKCS parameters have been linked to end-use quality. As highlighted by Bean et al. (2006), the SKCS was designed to analyze wheat, which has a different kernel structure from sorghum, however comparing it with hardness measured by abrasive decortications (AHI values), despite a moderate correlation ($r = 0.61$) between the two hardness indices, it was concluded that SKCS hardness can be used to predict sorghum grain hardness without modification.

The results concerning the kernel weight (from 21.3 to 24.3 mg) and hardness (from 96.1 to 59.4 SKCS index) of the sorghum food-grade analyzed here, reported data

belonged to the classes described by Bean et al. (2006), in which the higher values of hardness corresponded to lower kernel weight classes, despite the great variability occurred within the classes themselves confirming also the results described by Gazza et al. (2008) in common wheat. On average the Bolivian hybrids (SW) presented a very high SKCS index (91.4); a higher hardness index corresponds to a higher compactness of the kernel structure and thus a greater resistance to mechanical stress, consequently more resistant to breakage during decortication (dehulling) and milling than grain with a high proportion of floury endosperm. Kernel hardness (endosperm texture) is the proportion of corneous (vitreous or hard) fraction of the endosperm with respect to the floury or soft endosperm fraction. During milling hard grains tend to yield proportionally cleaner endosperm of large particle size than soft grains, this is because the corneous endosperm is easily separated from intact starchy endosperm giving a higher yield. Sorghum grains with corneous endosperm are preferred for stiff porridge and grits making, moreover in the field, hard grains, are also more resistant to insect and mould damage than soft grains.

Sorghum food-grade hybrids showed the test weight (73.2 Kg/hL) lower (15% less) than durum wheat (84.9 Kg/hL), whereas the high test weight presented by both teff genotypes (86.8 Kg/hL) was indicative of a full kernel; consequently, the yields in flour will be greater. Their average TKW (0.268 g) was not significantly different from data reported by Bultosa (2007).

As expected, the breadmaking attitude measured by SDS sedimentation test of the wholemeal flour was poor in gluten free cereals, on average about half of wheat flour sedimentation value (16.3 *vs* 36.0 ml).

The Falling Number is an indication of amylase enzyme activity, Mohammed et al. (2009) found that substitution of wheat flour by teff flour caused significant increase in Falling Number (from 536 to 943 sec), indicating less amylase activity, and Hugo et al. (2000) referred value for sorghum flour and sorghum malt equal to 507 and 62 sec respectively, confirming present data, on average FN of 546 sec for sorghum hybrids and 531 for teff genotypes, comply with what is shown in literature.

Kernel color determination is important because the information obtained helps in anticipating end product color quality. In Africa, white or light sorghums are more generally preferred for porridge making. Red colored sorghums are generally preferred

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for brewing traditional beer whereas for industrial production of lager beer white sorghums are generally selected. White tan-plant sorghums with tan glumes and pericarp have essentially no colored pigments and produce grains that are evenly pale or essentially white used in the production of baked products, tortillas, noodles, extruded snacks. In general sorghums kernel color can be red, lemon, yellow, brown or white, the color is used in grain trade. In the USA for instance, one of the bases of sorghum classification is color, sorghum is divided into four classes, namely: Sorghum, Tannin sorghum, White sorghum and Mixed sorghum. As discussed before the sorghum kernel color is genetically controlled and is due to phenolic flavonoid pigments anthocyanins and flavan-4- ols which are located in the pericap.

In this thesis, the brown index value (100-L*), colorimetric measurements on wholemeal flours determined by means of a Tristimulus colorimeter, indicated highest values for food-grade sorghum hybrids PSE 7431 (27.9) and brown teff (31.1). As regards the sorghum hybrids, a positive correlation between the data of brown index and bioactive compounds was found (Fig.50). In particular, the highest correlation was detected with the content of anthocyanins (R^2=0.843) and yellow pigments (R^2=0.884) (Fig.50C-D). As observed by Awika et al. (2004a) black sorghums generally have high levels of anthocyanins and may be a useful source of these compounds, moreover they reported that the high antioxidant capacity of black sorghums and their brans were correlated with their anthocyanin contents, and in sorghum, phenol content correlates most strongly with antioxidant activity measured by various methods indicating the phenols are largely responsible for this activity (Awika et al., 2003).

In the same way, data show a strong relationship especially between the total antioxidant capacity and both the content of total soluble polyphenols (R^2=0.956) and yellow pigments (R^2=0.914) (Fig.51A-B).

Summing up, on average, despite the total antioxidant capacity was 30% lower than durum wheat, sorghum food-grade hybrids showed higher contents of yellow pigments, free polyphenol and anthocyanin contents (+581%, +442% and +130%) than the control, respectively.

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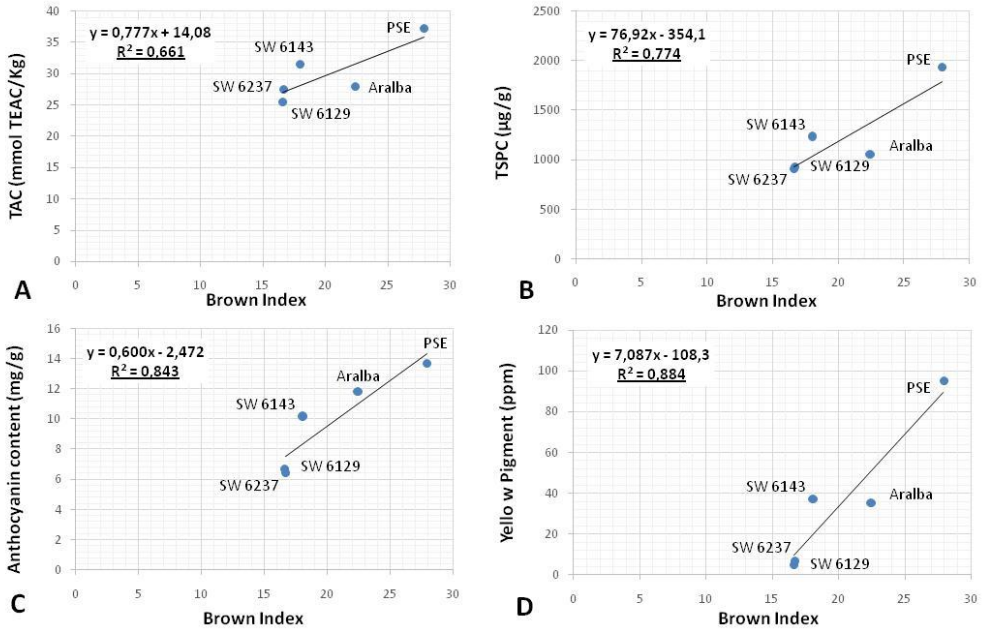

Figure 50. *Correlation between Brown Index and level of total antioxidant capacity (TAC) (A), total soluble polyphenol content (TSPC)(B), anthocyanin content (C) and yellow pigment (D) in five sorghum hybrids*

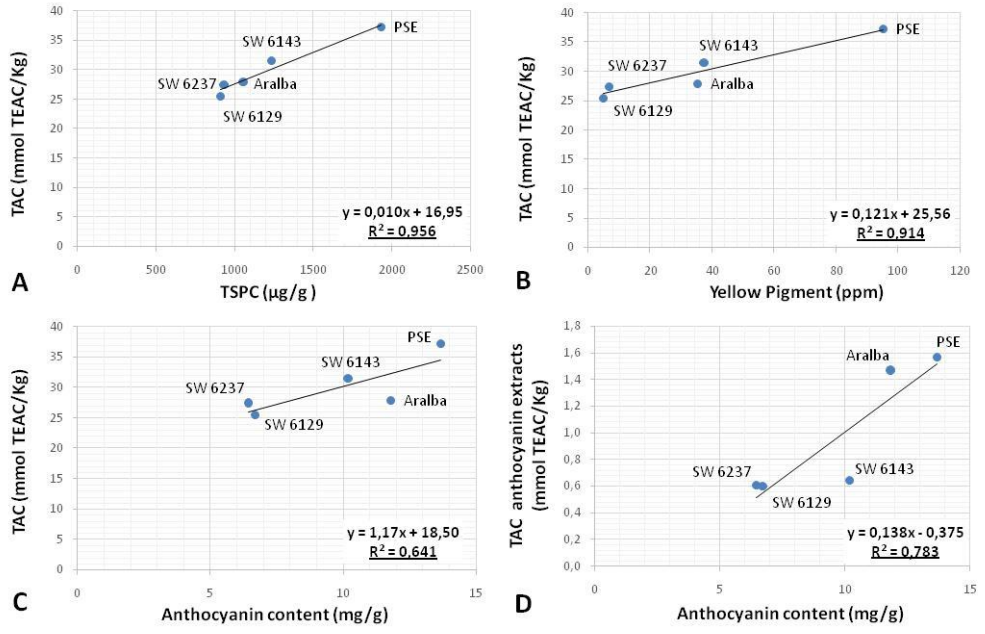

Figure 51. *Correlation between total antioxidant capacity (TAC) and total soluble polyphenol content (TSPC)(A), yellow pigment (B) and anthocyanin (C) content, and correlation between total antioxidant capacity in anthocyanin extracts and anthocyanin content (D) in five sorghum hybrids.*

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Anthocyanins are becoming increasingly important not only as food colorants, but also as antioxidants. Anthocyanins are reported to have some therapeutic benefits including vasoprotective and anti-inflammatory properties, anti-cancer and chemoprotective properties (Karaivahnova et al., 1990). Anthocyanins are, therefore, considered to contribute significantly to the beneficial effect of consuming fruits and vegetables (Wang et al., 1997). There is a rising demand for natural source of food colorants with nutraceutical benefits (Boyd, 2000) and alternative sources of natural anthocyanins are becoming increasingly important. The 3-deoxyanthocyanidins present in sorghum were also reported to be more stable in acidic solutions relative to the anthocyanidins (Awika et al., 2004b) commonly found in fruits, vegetables and other cereals (Sweeny & Iacobucci, 1981). This suggests a potential advantage of sorghum as a viable commercial source of anthocyanins. Additional studies are necessary to establish the color stability of the anthocyanins in actual food systems.

The content in bioactive compounds in the two teff genotypes was statistically not different from the durum wheat either as regards the content in anthocyanins (3.9 *vs* 4.0 mg/g d.w.) or in yellow pigments (3.5 *vs* 5.3 mg β-carotene/kg d.w.), but on average the antioxidant capacity was 19% lower than the control (35.0 *vs* 43.1 mmol TEAC/Kg d.w.), however overall higher than that identified in the sorghum (on average 30.4 mmol TEAC/Kg d.w.). Surprisingly, little is known about the phenolic profile of teff; and as yet, the compounds responsible for the intense pigmentation in the brown teff pericarp are unknown. Ravisankar et al. (2018) underline how the few studies available on teff still provide little structural and unreliable information on the antioxidant compounds present, thus determining little agreement on the type of compounds identified in the studies. In their recent detailed study on phenolic compounds in white and brown teff varieties, it is revealed that the extractable phenolic compounds in teff grain are almost exclusively flavones, primarily their C-glycosides and differences exist between flavone profile of white *versus* brown teff, where white teff contains only apigenin glycosides, whereas brown teff contains mostly luteolin glycosides; this indicates key differences in their flavonoid biosynthetic pathways.

They concluded that the differences in pericarp color between white and brown teff could not be attributed to the phenolic profile, but condensed tannins were probably present as procyanidins in unextractable form only in brown teff. Furthermore, they

found that teff grains contain particularly high levels of flavones, much higher than those reported in other cereals or other food products. This makes teff a valuable source of relatively rare dietary flavones and also an excellent model for studying the bioactive properties of grain flavones in a natural food matrix. The nutritional and health implications of tannins not extractable in the brown teff warrant further studies. As highlighted by Afify (2012c), epidemiological studies have suggested that increased consumption of whole grains, fruits and vegetables is associated with reduced risks of chronic diseases. This association may be attributed to the natural antioxidants from plant foods such as vitamin C, tocopherols, carotenoids, polyphenolics and flavonoids which prevent free radical damage by modulating the effects of reactive oxidants. Also, some plants are promising sources of potential antioxidants and may be efficient as preventive agents in the pathogenesis of some diseases. It can be also used in stabilizing food against oxidative deterioration because these phenolic compounds possess structural features favorable for radical scavenging and/or metal chelation, which would enable them to be effective antioxidants. Currently, antioxidant activity is the most common *in vitro* parameter used to assess or predict potential benefits of plant phytochemical compounds. However, correlations between *in vitro* antioxidant activity and actual health benefits are unknown. Antioxidant activity data are also hard to compare since there are no standardized methods; the methods currently used do not always agree in terms of ranking samples for antioxidant efficacy. However, antioxidant activity data still provide useful information for screening plant materials and products with desirable compounds and properties that can be used for further biological testing (Awika & Rooney, 2004).

Different factors affect the bioavailability of phenolic compounds in humans, including environmental factors, food processing, food matrix, and interaction with other compounds and polyphenols (D'Archivio, et al., 2010).

As regards chemical and nutritional values, the proximate composition of sorghum food-grade hybrids, compared to durum wheat flour, presented, on average, lower concentrations of protein (- 26 %), total dietary fiber (- 19 %), β-glucans (- 65 %), fructo-oligosaccharides (- 18 %), folate (- 22 %), whereas the highest contents were highlighted as total starch (+ 5.7 %) and resistant starch (+ 600 %), consequently, the resistant starch/total starch ratio resulted on average 6.5 times the control. The higher

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starch content of this type of cereals should be considered with caution by individuals with problems of hyperglycaemia /diabetes. So, the very high amount of resistant starch could influence the digestibility of foods and intestinal functions, as well as could affect the technological properties of food production.

When teff genotypes were compared to wheat flour, they presented lower concentrations of protein (-18%), total dietary fiber (-20%), β-glucans (-50%), whereas the highest values (59.7 µg/100g) were found in folate content (+42%). On average the total starch in teff was 7.3 % higher than control, but resistant starch was lower (-43%), thus, the resistant starch/total starch ratio resulted about half the control.

Our research results about folate content were partially in agreement with Hager et al. (2012b), who observed big variations of folate content between the samples of different flours: in particular, wheat and wholemeal wheat flour contained low levels (18 and 34 µg/100g respectively), whereas sorghum (77 µg/100 g) and teff (96 µg/100 g) contain notably higher levels.

Cereals usually comprise of about 50-80% carbohydrate on a dry weight basis and starch is the main cereal polysaccharide and a major food reserve, providing a bulk nutrient and energy source in the human diet (Dewettinck et al., 2008). It is stored in granular form of variable size and shapes characteristic of the species. In contrast to wheat starch, granules found in the other cereals have a simple size distribution, being of similar shape and diameter. Our micrographs of sorghum and teff starch granules comply with what is shown by scanning electron micrographs of the gluten free and wheat flours (magnification x2000) from Hager et al. (2012a) where sorghum starch granules are polygonal of approximately 10 µm surrounded by smaller spherical bodies of only a few micrometers, likely to be protein bodies and where teff granules are polygonal in shape (between 2 and 7 µm diameter) packed together and protein seems to attach outside of the compound starch granule. The milling of grains causes physical damage to a proportion of the starch granules, their altered properties are of technological significance, as damaged starch granules increase water absorption and are also more susceptible to enzyme hydrolysis, thereby promoting yeast fermentation. Hager et al. (2012a) reported that teff flour contained the lowest amounts of damaged starch (2.08 g/100 g), about half of wholemeal wheat, concluding that this variation was not only due to difference in biological origin of the flours, but resulted also from

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the different milling procedures applied and equipment used. Therefore, for example, larger starch granules are subject to greater starch damage (Dubois, 1949). Cereals have always been considered chiefly as dietary energy sources because of their high content of hydrolysable polysaccharides, but recently they have received attention as sources of compounds with added health benefits as fructose polymers (fructans and fructo-oligosaccharides). In particular, fructans appear to exert favorable effects on mineral absorption and on pre-biotic activity in experimental animals and in humans (Gibson, 1999; Niness, 1999), on reducing the levels of circulating lipids and glucose (Delzenne & Kok, 1999) and on modulating the immune response (Schley & Field, 2002). Mature wheat contains low concentration of fructans (1– 4%), but is the most important dietary source of fructans, due to the high content of wheat products in the human diet (Van Loo et al., 1995). Among sorghum hybrids examined, the food-grade SW6143W presented the highest value 1.9%, belonging to the relative range of mature wheat mentioned above. Nevertheless, in cases of individuals with gastrointestinal disorders associated with reductions in some gut bacteria and greater mucosal inflammation, fructans and fructo-oligosaccharides have emerged negative effects. Prebiotic supplementation studies have shown some promise at low doses for modulation of the gut bacteria and reduction of symptoms in irritable bowel syndrome (IBS) and in Crohn's disease; however, larger doses may have neutral or negative impact on symptoms (Wilson & Whelan, 2017).

Knowledge of the bioavailability of phenolic compounds in sorghum, including dietary factors able to modulate it, is essential for analysis of their functional potential in humans and, in the long term, for the implementation of therapeutic measures for health professionals. The contribution of intestinal tissue and the microbiota impact on absorption and anthocyanin bioavailability is also highlighted (Faria et al., 2013). It has been suggested that this is likely due to the spontaneous degradation under physiological conditions or following microbial metabolism (Woodward et al., 2009). In fact, colonic microbiota hydrolyzes glycosides into aglycones and degrades them to simple phenolic acids, which can be further fermented in the colon (Williamson & Clifford, 2010).

The electrophoretic analysis in SDS-PAGE confirmed the absence of storage proteins constituent of gluten in sorghum and teff samples analyzed, showing, under reducing

conditions, teff and sorghum prolamins, with major bands approximately at 20-23 kDa in teff and from 16 to 28 kDa in sorghum.

Moreover, as far as the sorghum and teff applications in the food sector are concerned, the beverage transformations attitude of these two gluten free cereals revealed that both, brown teff and sorghum commercial genotypes, presented grain quality parameters suitable to be tested as brewing raw material (rather low protein, β-glucan contents, falling number, and TW and TS with relative high values); the malts analyzed did not show an amylase activity comparable to that found in malt obtained from beer barley, as the activity was found insufficient to guarantee a suitable quantity of fermentable sugars. As a consequence, the drinks obtained from this type of malts, could show a reduced alcohol content. However, as the light and gluten-free beer market is a growing market, this type of beverage could still be interesting for this sector. Otherwise, the malts produced could be used in combination with other malts for the production of innovative fermented beverages, not yet available on the market. Finally, in order to explore the potential application of gluten-free flour, the teff-based "Tigelle", typical Italian unleavened focaccias from Emilia Romagna, were produced from 100% white teff flour (WTF) and white teff flour (77%) added with 23% of brown teff ground malt (WTF+BTM). Both the products presented good overall acceptance scores. Indeed, the results obtained from the hedonic analysis carried out by not-expert people highlighted in WTF higher scores for crumb and crust color, whereas the bread sample with malt was preferred for its aroma and taste.

It must be underline that it is necessary to develop specific transformation processes for the two gluten-free cereals, because they are interesting raw materials for the production of typical Italian foods like beer and non-leavened bakery products as tigelle, not only for the celiac market. To date, the possibility of using teff for food products other than bread has begun to be evaluated. Coleman et al. (2013) confirmed the suitability of teff flours for biscuits and cake making. Similarly, studies evaluating the possibility of using teff in pasta formulations (Hager et al., 2013), beer making (Gebremariam et al., 2014), and gel-like food formulation (Abebe & Ronda, 2014) have shown promising results. These efforts show that teff can be used in various products familiar to Western culture, especially in the formulation of gluten-free products.

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Furthermore, the introduction of food-grade sorghum hybrids, beyond their importance as a cereal naturally gluten-free, can be a viable alternative for example to corn, whose hygienic conditions are increasingly threatened by the presence of mycotoxins which affect the use of it both in human nutrition and in the livestock sector.

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CHAPTER 6: CONCLUSIONS

Sorghum and teff are attractive raw materials and a good source of protein for gluten-free products due to the neutral flavor and color of specific varieties, low allergenicity and their ability to grow in drought-like conditions. In conclusion, the data of this study showed that the four food-grade sorghum hybrids and the two teff genotypes examined have technological and nutritional characteristics comparable with the durum wheat, used as control, for the most of the parameters examined. When the analyzed lines showed lower values than wheat, they are still acceptable for the transformation processes. Moreover, the food-grade sorghum hybrids showed higher contents in free polyphenols, yellow pigments, anthocyanins and resistant starch, whereas commercial teff lines were very interesting for total folate content. Sorghum and teff, therefore, for their potential can also be used to produce gluten-free bakery products, such as snacks and pizza, or beer, foods more similar to Western tastes and more suitable for the Italian market. They could also be a natural source of bioactive compounds in gluten-free foods, and, above all, in fiber and folate, of which these latter products are often deficient.

Present and future researchers and investors must ensure the continuity of long-term research coupled with more-effective breeding programmes in order to unlock the maximum potential of these cereals, because their resilience to both drought and heat stresses could be the answer to the grim state of food security and also to the nutritional demands of a growing population; these aims are in line with the current global sustainability agenda on food and nutrition security.

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CHAPTER 7: REFERENCES

AACC (2000). *Method 14-50.* Approved Methods of the American Association of Cereal Chemists, tenth ed. American Association of Cereal Chemists, St.Paul, MN.

AACC (2000). *Method 56-81B –Falling Number.* Approved Methods of the American Association of Cereal Chemists St. Paul, MN, USA.

AACC (2003). *Method 56-70 – SDS sedimentation test.* Approved methods of the American Association of Cereal Chemists, 10th ed. AACC International, St Paul, MN, USA.

Abebe W., Ronda F. (2014). *Rheological and textural properties of teff [Eragrostis tef (zucc.) Trotter] grain flour gels.* Journal of Cereal Science. 60: 122–130.

Abebe Y., Bogale A., Hambidge K.M., Stoecker B.J., Bailey K., Gibson R.S. (2007). *Phytate, zinc, iron and calcium content of selected raw and prepared foods consumed in Rural Sidama, Southern Ethiopia, and implications for bioavailability*. Journal of Food Composition and Analysis, 20: 161-168.

Abraha M.T., Shimelis H., Laing M., Assefa K., Amelework B. (2016). *Assessment of the genetic relationship of tef (Eragrostis tef) genotypes using SSR markers.* South African Journal of Botany 105:106-10.

Acosta-Estrada B., Gutierrez-Uribe, J., Serna-Saldivar, S.O. (2014). *Bound phenolics in foods, a review*. Food Chemistry, 152: 46–55.

Adebowale A.R.A., Emmambux M.N., Beukes M., Taylor J. (2011). *Fractionation and characterization of teff proteins.* Journal of Cereal Science, 54: 380-386.

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Adish A.A., Esrey S.A., Gyorkos T.W., Johns T. (1999). *Risk factors for iron deficiency anaemia in preschool children in Northern Ethiopia.* Public Health Nutrition, 2: 243–252.

Afify A. E.-M. M. R., El-Beltagi H. S., Abd El-Salam S. M., Omran A. A. (2012a). *Oil and fatty acid contents of white sorghum varieties under soaking, cooking, germination and fermentation processing for improving cereal quality*. Notulae Botanicae Horti Agrobotanici, 40: 86-92.

Afify A. E.-M. M. R., El-Beltagi H. S., Abd El-Salam S. M., Omran A. A. (2012b). *Protein solubility, digestibility and fractionation after germination of sorghum varieties*. PLoS ONE 7: e31154.

Afify A. E.-M. M. R., El-Beltagi H. S., El-Salam S. M. A., Omran, A. A. (2012c). *Biochemical changes in phenols, flavonoids, tannins, vitamin E, β–carotene and antioxidant activity during soaking of three white sorghum varieties.* Asian Pacific Journal of Tropical Biomedicine 2:203-209.

Alaunyte I., Stojceska V., Plunkett A., Ainsworth P., Derbyshire E. (2012). *Improving the quality of nutrient-rich teff (Eragrostis tef) breads by combination of enzymes in straight dough and sourdough breadmaking*. Journal of Cereal Science. 55: 22-30.

Althwab S., Carr T.P., Weller C.L., Dweikat M., Schlegel V. (2015). *Advances in grain sorghum and its co-products as a human health promoting dietary system.* Food Research International, 77: 349–359.

Ambaw A. (2013). *Determination of iron fractions of laboratory threshed and field threshed teff injera.* Addis Ababa University

Elena Galassi

Anderson J.W., Baird P., Davis R.H., Ferreri S., Knudtson M., Koraym A., Waters V., Williams C.L. (2009). *Health benefits of dietary fiber*. Nutritional Review, 67:188–205

Anglani C. (1994). *Digestibility and phenolic substances from meals of sorghum without a pigmented testa*. Agricoltura Mediterranea 124: 229–237.

AOAC (1995). *Association of Official Analytical Chemists. Official Methods of Analysis 991.42*, 16th ed.; Cunniff P., Ed.; AOAC, Gaithersburg, MD (USA).

Assefa K., Yu J.K., Zeid M., Belay G., Tefera H., Sorrells M.E. (2010). *Breeding tef [Eragrostistef (Zucc.) trotter]: conventional and molecular approaches.* Plant Breeding, 130: 1-9

Assefa K., Cannarozzi G., Girma D., Kamies R., Chanyalew S., Plaza-Wüthric S., Blösch R., Rindisbacher A., Rafudeen S., Tadele Z. (2015). *Genetic diversity in tef [Eragrostis tef (Zucc.) Trotter]*. Frontiers in Plant Science, 6: 1–13.

Assefa K., Yu J.K., Zeid M., Belay G., Tefera H., Sorrells M.E. (2011). *Breeding tef [Eragrostistef (Zucc.) trotter]: conventional and molecular approaches*. Plant Breeding, 130: 1–9.

Awika J.M., Rooney L.W., Wu X., Prior R.L., Cisneros-Zevallos L. (2003). *Screening methods to measure antioxidant activity of sorghum (Sorghum bicolor) and sorghum products.* Journal of Agricultural and Food Chemistry, 51: 6657–6662.

Awika J.M., Rooney L.W. (2004). *Sorghum phytochemicals and their potential impact on human health*. Phytochemistry, 65: 1199-1221.

Awika J.M., Rooney L.W., Waniska R.D. (2004a). *Anthocyanins from black sorghum and their antioxidant properties.* Food Chemistry, 90: 293–301.

Elena Galassi

Awika J.M., Rooney L.W., Waniska R.D. (2004b). *Properties of 3-deoxyanthocyanins from sorghum.* Journal of Agricultural and Food Chemistry, 52: 4388–4394.

Awika J. M., McDonough C. M., Rooney L. W. (2005). *Decorticating sorghum to concentrate healthy phytochemicals.* Journal of Agricultural and Food Chemistry 53:6230-6234.

Badi S., Pedersen B., Monowar L., Eggum B.O. (1990). *The nutritive value of new and traditional sorghum and millet foods from Sudan.* Plant Foods Human Nutrition, 40: 5–19.

Badi S.M., Hoseney R.C. (1976). *Use of sorghum and pearl millet flours in cookies*. Cereal Chemistry, 53: 733–738.

Barros F., Awika J.M., Rooney L.W. (2012). *Interaction of tannins and other sorghum phenolic compounds with starch and effects on in vitro starch digestibility.* Journal of Agricultural and Food Chemistry, 60: 11609-11617.

Baye K., Mouquet-Rivier C., Icard-Vernière C., Picq C., Guyot J.P. (2014). *Changes in mineral absorption inhibitors consequent to fermentation of Ethiopian injera: implications for predicted iron bioavailability and bioaccessibility*. International Journal of Food Science & Technology, 49: 174-180.

Baye K., Mouquet-Rivier C., Icard-Vernière C., Rochette I., Guyot J.P. (2013). *Influence of flour blend composition on fermentation kinetics and phytate hydrolysis of sourdough used to make injera.* Food Chemistry, 138: 430-436.

Baye K. (2014). *Teff: nutrient composition and health benefits*. International Food Policy Research Institute and Ethiopian Development Research Institute (Vol.67), Washington, USA.

Elena Galassi

Bean S.R., Chung O.K., Tuinstra M.R., Pedersen J.F., Erpelding J. (2006). *Evaluation of single kernel characterization system (SKCS) for measurement of sorghum grain attributes*. Cereal Chemistry, 83: 108-113.

Bean S.R., Ioerger B.P., Smith B.M., Blackwell D.L. (2011). *Sorghum protein structure and chemistry: implications for nutrition and functionality*. Advances in Cereal Science: Implications to Food Processing and Health Promotion, 7: 131-147.

Becker R., Hanners G.C. (1991). *Carbohydrate composition of cereal grain.* In: Lorenz KJ, Kulp K (eds) Handbook of cereal science and technology. Marcel Dekker, New York: 482-483.

Bedane G. M., Saukuru A. M., George D. L., Gupta M. L. (2015). *Evaluation of teff (Eragrostis tef [Zucc.] Trotter) lines for agronomic traits in Australia.* Australian Journal of Crop Science, 9: 242–247.

Bekele E., Fido R. J., Tatham A. S., Shewry P. R. (1995). *Heterogeneity and polymorphism of seed proteins in teff (Eragrotis tef)* Hereditas, 122: 67-72.

Belton P.S., Delgadillo I., Halford N.G., Shewry P.R. (2006). *Kafirin structure and functionality.* Journal of Cereal Science 44: 272-286.

Bergamo P., Maurano F., Mazzarella G., Iaquinto G., Vocca I., Rivelli A. R., De Falco E., Gianfrani C., Rossi M. (2011). *Immunological evaluation of the alcohol-soluble protein fraction from gluten-free grains in relation to celiac disease.* Molecular Nutrition & Food Research, 55: 1266–1270.

Bezwoda W.R., Bothwell T.H., Torrance J.D., MacPhail A.P., Charlton R.W., Kay G., Levin J. (1979). *The relationship between marrow iron stores, plasma ferritin concentrations and iron absorption*. Scandinavian Journal of Haematology. 22: 113-120.

Elena Galassi

Blakeley M.E., Rooney L.W., Sullins R.D., Miller F.R. (1979). *Microscopy of the pericarp and the testa of different genotypes of sorghum.* Crop Science, 19: 837–842.

Blaise P., Alexander M. (2010). *Processing of a top fermented beer brewed from 100% buckwheat malt with sensory and analytical characterisation*. Journal of the Institute of Brewing, 116: 265-274.

Bonciarelli F., Bonciarelli U. (1994). *Cotivazioni erbacee.* Edagricole. Edizioni Agricole, 81-85.

Boren B., Waniska R.D. (1992). *Sorghum seed color as an indicator of tannin content.* Journal of Applied Poultry Research, 1: 117–121.

Boyd W. (2000). *Natural colors as functional ingredients in healthy foods*. Cereal Foods World, 45: 221–222.

Bucci C., Zingone F., Russo I., Morra I., Tortora R., Pogna N., Scalia G., Iovino P., Ciacci C. (2013). *Gliadin does not induce mucosal inflammation or basophil activation in patients with nonceliac gluten sensitivity.* Clinical Gastroenterology and Hepatology, 11: 1294-1299.

Bultosa G., Taylor J.N.R. (2004). *Teff.* In: Wringley C., Corke H., Walker C. (eds) Encyclopedia of grain science. Academic, Oxford: 281–289

Bultosa G. (2007). *Physicochemical characteristics of grain and flour in 13 tef [Eragrostis Tef (Zucc.) Trotter] grain varieties.* Journal of Applied Science Research, 3: 2042-2051.

Bultosa G. (2016). *Teff: Overview*. In C. Wrigley, H. Corke, K. Seetharaman, & J. Faubion (Eds.), Encyclopedia of food grains (2nd ed., 209–220). Oxford: Elsevier

Elena Galassi

Cannarozzi G., Plaza-Wuthrich S., Esfeld K., Larti S., Wilson Y. S., Girma D., De Castro E., Chanyalew S., Blösch R., Farinelli L., Lyons E., Schneider M., Falquet L., Kuhlemeier C., Assefa K., Tadele, Z. (2014). *Genome and transcriptome sequencing identifies breeding targets in the orphan crop tef (Eragrostis tef).* BMC Genomics, 15: 581.

Cardoso L.d.M., Montini T.A., Pinheiro S.S., Queiroz V.A.V., Pinheiro Sant'Ana H.M., Martino H.S.D., Moreira A.V.B. (2014). *Effects of processing with dry heat and wet heat on the antioxidant profile of sorghum (Sorghum bicolor L.).* Food Chemistry, 152: 201-217

Cardoso L.d.M., Pinheiro S.S., Martino H.S.D., Pinheiro-Sant'Ana H.M. (2017) *Sorghum (Sorghum bicolor l.): nutrients, bioactive compounds, and potential impact on human health*. Critical Reviews in Food Science and Nutrition, 57: 372–390

Cheng A., Mayes S., Dalle G., Demissew S., Massawe F. (2017). *Diversifying crops for food and nutrition security –a case of teff.* Biology Review, 92: 188–198.

Chiremba C., Taylor J. R. N., Rooney L. W., Beta T. (2012). *Phenolic acid content of sorghum and maize cultivars varying in hardness.* Food Chemistry 134:81-88.

Choto C.E., Morad M.M., Rooney L.W. (1985). *The quality of tortillas containing whole sorghum and pearled sorghum alone and blended with yellow maize.* Cereal Chemistry, 62: 51–55.

Chufo A., Yuan H., Zou D., Pang Y., Li X. (2015). *Biomethane production and physicochemical characterization of anaerobically digested teff (Eragrostis tef) straw pretreated by sodium hydroxide*. Bioresource Technology, 181: 214–219.

Ciacci C., Maiuri L., Caporaso N., Bucci C., Del Giudice L., Rita Massardo D., Pontieri P., Di Fonzo N., Bean S. R., Ioerger B., Londei M. (2007). *Celiac disease:*

Elena Galassi

In vitro and in vivo safety and palatability of wheat-free sorghum food products. Clinical Nutrition, 26:799-805.

Coleman J., Abaye A.O., Barbeau W., Thomason W. (2013). *The suitability of teff flour in bread, layer cakes, cookies and biscuits.* International Journal of Food Sciences and Nutrition, 64: 877-881.

Comino I., de Lourdes Moreno M., Real A., Rodríguez-Herrera A., Barro F., Sousa C. (2013). *The gluten-free diet: testing alternative cereals tolerated by celiac patients.* Nutrients, 5: 4250-4268.

Costanza S. H., Dewet J. M. J., Harlan J. R. (1979). *Literature review and numerical taxonomy of Eragrostistef (tef).* Economic Botany, 33: 413–424.

CSA, 2005-2013. Central Statistics Agency. Agricultural Sample Survey. Report on Area Production and Yield of Major Crops (Private Peasant Holdings. Meher Season). Addis Ababa, Ethiopia.

CSA, 2014. Central Statistics Agency. Agricultural Sample Survey. Report on Area Production and Yield of Major Crops (Private Peasant Holdings. Meher Season). Addis Ababa, Ethiopia.

Cummings D.P, Axtel J.D. (1973). *Relationships of pigmented testa to nutritional quality of sorghum grain in inheritance and improvement of protein quality in sorghum.* Research Progress Report, Purdue University, West Lafayette, IN, 112.

D'Archivio M., Filesi C., Varì R., Scazzocchio B., Masella R. (2010). *Bioavailability of the polyphenols: status and controversies*. International Journal Of Molecular Sciences, 11:1321-1342.

D'Egidio M.G., Cecchini C., Corradini C., Canali G., Cervigni T., De Vita P., (1999). *Fructo-oligosaccharides from cereal crops as ingredient for innovative foods.* Proceedings of Eighth Seminar on Inulin, Lille (France), 128-136.

Dawit T., Hirut K. (1995). *Germplasm collection, conservation and characterization of tef.* Plant Genetic Resources Centre/Ethiopia. Internal Report. Addis Ababa, Ethiopia.

De Mesa-Stonestreet N.J., Alavi S., Bean S.R. (2010). *Sorghum proteins: the concentration, isolation, modification, and food applications of kafirins.* Journal of Food Science, 75: 90-104

Del Giudice F., Massardo D.R., Pontieri P., Maddaluno L., De Vita P., Fares C., Ciacci C., Bucci C., Tortora R., Zingone F, Ruaao I., Del Giudice L. (2007). *A sorghum chain in the italian campania region*. Health, Research, and Entrepreneurship: Sorghum Food for Celiac Patients, 104-117.

Delzenne N.M., Kok N.N. (1999). *Biochemical basis of oligofructose-induced hypolipedemia in animal models*. Journal of Nutrition, 129: 1467S–1470S.

Demissie A. (2001). *Tef genetic resources in Ethiopia*. In: H. Tefera, G. Belay, and M. Sorrells (eds), Narrowing the Rift, 27—31, Proceedings of the International Workshop on Tef Genetics and Improvement, 16–19 October 2000, Tef Research and Development, Debre Zeit, Ethiopia.

Dewar J., Taylor J.R.N., Berjak P. (1997). *Determination of improved steeping conditions for sorghum malting.* Journal of Cereal Science, 26: 129-131

DeVries J.W., Rader J.I., Keagy P.M., Hudson C.A., Angyal G., Arcot J., Castelli M., Doreanu N., Hudson C., Lawrence P., Martin J., Peace R., Rosner L., Strandler H.S., Szpylka J., van den Berg H., Wo C., Wurz C. (2005).

Elena Galassi

Microbiological assay-trienzyme procedure for total folates in cereals and cereal foods: collaborative study. Journal of AOAC International, 88: 5-15.

Dewettinck K., Vanbockstaele F., Kuhne B., Vandewalle D., Courtens T., Gellynck X., (2008). *Nutritional value of bread: influence of processing, food interaction and consumer perception*. Journal of Cereal Science, 48: 243-257.

Dicke W.K. (1950). *Coeliac disease. Investigation of the harmful effects of certain types of cereal on patients with coeliac disease,* in Medicine. University of Utrecht, Utrecht, Thesis

Di Ghionno L., Sileoni V., Marconi O., De Francesco G., Perretti G. (2017). *Comparative study on quality attributes of gluten-free beer from malted and unmalted teff [Eragrostis tef (Zucc.) Trotter]*. LWT-Food Science and Technology, 84:746–752

Djameh C., Saalia, F. K., Sinayobye A. B., Essilfie G., Mensah-Brown H., Sefa-Dedeh S. (2015). *Optimization of the sorghum malting process for Pito production in Ghana*, Journal of the Institute of Brewing, 121: 106–112.

Doudu K.G., Taylor J.R.N., Belton P.S., Hamaker B.R. (2003). *Factors affecting sorghum protein digestibility*. Journal of Cereal Science, 38: 117–131.

Dubois M. (1949). *Incidences du réglage de la mouture sur les propriétés des farines*. Bulletin Ensmic, 113: 170-187.

Dykes L., Rooney L.W. (2007). *Phenolic compounds in cereal grains and their health benefits*. Cereal Foods World, 52:105–111.

Dykes L., Rooney L.W. (2006). *Sorghum and millet phenols and antioxidants*. Journal of Cereal Science, 44: 236–251.

Elena Galassi

Dykes L., Seitz L. M., Rooney W. L., & Rooney L. W. (2009). *Flavonoid composition of red sorghum genotypes*. Food Chemistry, 116: 313-317.

Dykes L., Rooney L. W., Waniska R. D., Rooney W. L. (2005). *Phenolic compounds and antioxidant activity of sorghum grains of varying genotypes*. Journal of agricultural and food chemistry, 53: 6813-6818.

Earp C.F., McDonough C.M., Rooney L.W. (2004). *Microscopy of pericarp development in the caryopsis of Sorghum bicolor (L.) Moench.* Journal of Cereal Science, 39: 21-27

Egli I., Davidsson L., Zeder C., Walczyk T., Hurrell R. (2004). *Dephytinization of a complementary food based on wheat and soy increases zinc, but not copper, apparent absorption in adults.* The Journal of Nutrition, 134: 1077-1080.

El Nour N.A., DalBelin Peruffo A., Curioni A. (1998). *Characterisation of sorghum kafirins in relation to their cross-linking behaviour.* Journal of Cereal Science, 28: 197-207.

El-Alfy T.S., Ezzat S.M., Sleem A.A. (2012). *Chemical and biological study of the seeds of Eragrostis tef (zucc.) trotter.* Natural Product Research, 26: 619-629.

Elli L., Villalta D., Roncoroni L., Barisani D., Ferrero S., Pellegrini N., Bardella M.T, Valiante F., Tomba C., Carroccio A., Bellini M., Soncini M., Cannizzaro R., Leandro G. (2017). *Nomenclature and diagnosis of gluten-related disorders: A position statement by the Italian Association of Hospital Gastroenterologists and Endoscopists (AIGO).* Digestive and Liver Disease, 49: 138–146.

Ellis H.J., Doyle A.P., Sturgess R.P., Ciclitira P.J. (1992). *Coeliac disease: characterisation of monoclonal antibodies raised against a synthetic peptide corresponding to amino acid residues 206−217 of α- gliadin*. Gut, 33: 1504−1507.

Elena Galassi

Emmambux M.N., Taylor J.R.N. (2009). *Properties of heat-treated sorghum and maize meal and their prolamin proteins*. Journal of Agricultural Food Chemistry 57: 1045–50.

Emmambux M.N., Taylor J.R.N. (2013). *Morphology, physical, chemical, and functional properties of starches from cereals, legumes, and tubers cultivated in Africa: A review*. Starch/Stärke, 65: 715–729.

EN ISO 520:2010. *Cereals and pulses-Determination of the mass of 1000 grains*, p.10.

EN ISO 7971-1:2009. *Cereals-Determination of bulk density, called mass per hectolitre*-Part1: Reference method, p.8.

Espinosa-Ramírez J., Garza-Guajardo I., Pérez-Carrillo E., Serna-Saldívar S.O. (2017). *Differences in the functionality and characterization of kafirins extracted from decorticated sorghum flour or gluten meal treated with protease.* Journal of Cereal Science 73: 174-182.

Ethiopian Agricultural Transformation Agency – ATA (2016). www.ata.gov.et.

Evangelisti F., Restani P. (2011). *Prodotti dietetici. Chimica Tecnologia ed Impiego.* Seconda edizione. Piccin: 335-350.

Ezeogu L.I., Duodu K.G., Emmambux M.N., Taylor J.R.N. (2008). *Influence of cooking conditions on the protein matrix of sorghum and maize endosperm flours.* Cereal Chemistry 85: 397–402.

Fardet A., Leenhardt F., Lioger D., Scalbert A., Rémésy C. (2006). *Parameters controlling the glycaemic response to breads.* Nutrition Research Reviews, 19: 18-25.

Elena Galassi

Fares C., Platani C., Tamma G., Leccese F. (1991). *Microtest per la valutazione del colore in genotipi di frumento duro.* Molini d'Italia, 12: 19-21.

Faria A., Fernandes I., Mateus N., Calhau C. (2013). *Bioavailability of Anthocyanins*. In: Natural Products: 2465-2487. K. G. Ramawat & J.-M. Mérillon Eds., Springer Berlin Heidelberg,

Fischer M.M., Egli, I.M., Aeberli, I., Hurrell, R.F., Meile L. (2014). *Phytic acid degrading lactic acid bacteria in tef-injera fermentation*. International Journal of Food Microbiology, 190: 54–60.

Foreign Agricultural Service/USDA, December 2018 report (https://ipad.fas.usda.gov/)

Forsido S.F., Rupasinghe H.P.V., Astatkie T. (2013). *Antioxidant capacity, total phenolics and nutritional content in selected Ethiopian staple food ingredients.* International Journal of Food Sciences and Nutrition, 64: 915–920.

Fuleki T., Francis F.J., (1968). *Quantitative methods for anthocyanins. 2. Determination of total anthocyanin and degradation index for cranberry juice*. Journal of Food Science 33: 78–83.

García-Manzanares Á., Lucendo A.J. (2011). *Nutritional and dietary aspects of celiac disease.* Nutrition in Clinical Practice, 26: 163-173.

Gazza L., Taddei F., Corbellini M., Cacciatori P., Pogna N.E. (2008). *Genetic and environmental factors affecting grain texture in common wheat*. Journal of Cereal Science, 47: 52-58.

Gebremariam M.M., Abegaz K., Zarnkow M., Becker T. (2015). *Studies on the mashing conditions of teff (Eragrosti stef) malt as a raw material for lactic acid*

fermented gluten-free beverage. International Journal of Food Science and Technology, 50: 2032–2037.

Gebremariam M.M., Zarnkow M., Becker T. (2013a). *Thermal stability of starch degrading enzymes of teff (Eragrostis tef) malt during isothermal mashing*. Process Biochemistry, 48: 1928–1932.

Gebremariam M.M., Zarnkow M., Becker T. (2013b). *Effect of drying temperature and time on alpha-amylase, beta-amylase, limit dextrinase activities and dimethyl sulphide level of teff (Eragrostis tef) malt.* Food and Bioprocess Technology, 6: 3462–3472.

Gebremariam M.M., Zarnkow M., Becker, T. (2013c). *Effect of teff (Eragrostis tef) variety and storage on malt quality attributes.* Journal of the Institute of Brewing, 119: 64–70.

Gebremariam M.M., Zarnkow, M., Becker, T. (2014). *Teff (Eragrostis tef) as a raw material for malting, brewing and manufacturing of gluten-free foods and beverages: A review*. Journal of Food Science and Technology, 51: 2881–2895.

Gianfrani C., Maglio M., Rotondi Aufiero V., Camarca A., Vocca I., Iaquinto G., Giardullo N., Pogna N., Troncone R., Auricchio S., Mazzarella G. (**2012).** *Immunogenicity of monococcum wheat in celiac disease patients*. American Journal of Clinical Nutrition, 96: 1339–1345.

Gibson G.R. (1999). *Dietary modulation of the human gut microflora using the prebiotics oligofructose and inulin*. Journal of Nutrition 129: 1438S–1441S.

Giusti M.M., Wrolstad R.E. (2001). *Anthocyanins. characterization and measurement with uv visible spectroscopy.* In R.E. Wrolstad (Ed), Analyticla Chemistry. New York: Wiley. Unit F1.2.1-13.

Elena Galassi

Goodall M.A., Campanella O.H., Ejeta G., Hamaker B.R. (2012). *Grain of high digestible, high lysine (HDHL) sorghum contains kafirins which enhance the protein network of composite dough and bread*. Journal of Cereal Science, 56: 352-357.

Guiducci M. (2000). *Manuale di corretta prassi per la produzione integrata di sorgo*. In: Progetto per la Valorizzazione delle Produzioni Agroalimentari Umbre.

Gujer R., Magnolato D., Self R. (1986). *Glucosylated flavonoids and other phenolic compounds from sorghum.* Phytochemistry, 25:1431–1436.

Hager A.S., Czerny M., Bez J., Zannini E., Arendt E.K. (2013). *Starch properties, in vitro digestibility, and sensory evaluation of fresh egg pasta produced from oat, teff and wheat flour*. Journal of Cereal Science; 58: 156-163.

Hager A. S., Lauck F., Zannini E., Arendt E. K. (2012b). *Development of gluten-free fresh egg pasta based on oat and teff flour*. European Food Research and Technology, 235: 861–871.

Hager A. S., Wolter A., Jacob F., Zannini E., Arendt E. K. (2012a). *Nutritional properties and ultra-structure of commercial gluten free flours from different botanical sources compared to wheat flours.* Journal of Cereal Science, 56: 239–247.

Hahn D.H., Rooney L.W., Earp C.F. (1984). *Tannins and phenols of sorghum.* Cereal Foods World, 29: 776–779.

Hamaker B.R., Bugusu B.A. (2003). *Overview: Sorghum proteins and food quality.* Paper 8 in: Afripro Workshop on the Proteins of Sorghum and Millets. P. S. Belton and J. R. N. Taylor, eds. Available online at www.afripro.org.uk.

Hamaker B.R., Kirleis A.W., Mertz E.T., Axtell J.D. (1986). *Effect of cooking on the protein profiles and in vitro digestibility of sorghum and maize*. Journal of Agricultural and Food Chemistry, 34: 647–649.

Helbing J (2009). *Konfokale laser scanning-mikroskopie und rasterelektronenmikroskopie zur beobachtung der mälzung verschiedener zerealien und pseudozerealien.* Technische Universität München, Germany.

Hicks C., Bean S.R., Lookhart G.L., Pedersen J.F., Kofoid K.D., Tuinstra M.R., (2001). *Genetic analysis of kafirins and their phenotypic correlations with feed quality traits, in vitro digestibility and seed weight in grain sorghum*. Cereal Chemistry, 78: 412–416.

Hill H., Slade Lee L., Henry R.J. (2012). *Variation in sorghum starch synthesis genes associated with differences in starch phenotype*. Food Chemistry, 131:175-183.

Hopman E., Dekking L., Blokland M.-L., Wuisman M., Zuijderduin W., Koning F., Schweizer J. (2008). *Teff in the diet of celiac patients in the Netherlands* Scandinavian Journal of Gastroenterology, 43: 277-282.

Hugo, L.F., Rooney, L.W., Taylor, J.R.N. (2000). *Malted sorghum as a functional ingredient in composite bread.* Cereal Chemistry, 77: 428-432.

Iacomino G., Di Stasio L., Fierro O., Picariello G., Venezia A., Gazza L., Ferranti P., Mamone G. (2016). *Protective effects of ID331 Triticum monococcum gliadin on in vitro models of the intestinal epithelium*. Food Chemistry, 212: 537-542.

ICC (1994). *ICC -Standard No.105/2. Determination of Crude Protein in Cereals and Cereals Products for Food and Feed.* International Association for Cereal Science and Technology. Approved 1980, Revised 1994, Vienna, Austria.

Ingram A.L., Doyle J.J. (2003). *The origin and evolution of Eragrostis tef (Poaceae) and related polyploids: evidence from nuclear waxy and plastid rps16.* American Journal of Botany, 90: 116–122.

ISTAT (2017). Annata agraria 2016-2017. www.istat.it

Italian celiac association website: *celiac census of the Annual Report 2016 to the Parliament.* www.celiachia.it

Jansen G.R., Di Maio L.R., Hause N.L. (1962). *Amino acid composition and lysine supplementation of teff.* Journal of Agricultural and Food Chemistry, 10:62–64

Jones B. M. G., Ponti J. A., Tavassoli A., Dixon P. A. (1978). *Relationship of the Ethiopian cereal tef (Eragrostistef (Zucc.) Trotter): evidence from morphology and chromosome number.* Annals of Botany, 42: 1369–1373.

Jordon W.R., Sullivan C.Y. (1980). *Reaction and resistance of grain sorghum to heat and drought.* In: House LR, Mughogho LK, Peacock JM (eds) Sorghum in the eighties, ICRISTAT, Patancheru, India, 1: 131-142.

Kagnoff M.F. (2005). *Overview and pathogenesis of celiac disease.* Gastroenterology, 128: 10–18.

Kangama C.O., Rumei X. (2005). *Introduction of sorghum (Sorghum bicolor (L.) Moench) into China.* African Journal of Biotechnology, 4: 575-579.

Karaivanova M., Drenska D., Ovcharov R. (1990). *A modification of the toxic effects of platinum complexes with anthocyanins.* Eksperimetnalna Meditsna I Morfologiia, 29: 19–24.

Kariluoto S., Vahteristo L., Salovaara H., Katina K., Liukkonen K., Piironen V. (2004). *Effect of baking method and fermentation on folate content of rye and white breads.* Cereal Chemistry, 81: 134-139.

Kean G.E., Ejeta G., Hamaker B.R., Ferruzzi M.G. (2007). *Characterization of Carotenoid Pigments in Mature and Developing Kernels of Selected Yellow-*

Endosperm Sorghum Varieties. Journal of Agricultural and Food Chemistry, 55: 2619-2626.

Khan I., Yousif A., Johnson S., Gamlath S. (2013). *Effect of sorghum flour addition on resistant starch content, phenolic profile and antioxidant capacity of durum wheat pasta*. Food Research International, 54: 578-586.

Kinsey L., Burden S.T., Bannerman E. (2008). *A dietary survey to determine if patients with coeliac disease are meeting current healthy eating guidelines and how their diet compares to that of the British general population*. European Journal of Clinical Nutrition, 62:1333–42.

Koletzko B., Baker S., Cleghorn G., Neto U. F., Gopalan S., Hernell O., Hock Q. S., Jirapinyo P., Lonnerdal B., Pencharz P., Pzyrembel H., Ramirez-Mayans J., Shamir R., Turck D., Yamashiro Y., Zong-Yi D. (2005). *Global Standard for the Composition of Infant Formula: Recommendations of an Espghan Coordinated International Expert Group.* Journal of Pediatric Gastroenterology and Nutrition, 41: 584-599.

Kotaskova E., Sumczynski D., Mlcek J., Valasek P., (2016). *Determination of free and bound phenolics using HPLC-DAD, antioxidant activity and in vitro digestibility of Eragrostis tef.* Journal of Food Composition and Analysis, 46: 15–21.

Kushi L.H., Meyer K.A., Jacobs D.R. (1999). *Cereals, legumes, and chronic disease risk reduction: evidence from epidemiologic studies.* The American Journal of Clinical Nutrition, 70: 451S-458S.

Lamacchia C., Camarca A., Picascia S., Di Luccia A., Gianfrani C. (2014). *Cereal-based gluten-free food: how to reconcile nutritional and technological properties of wheat proteins with safety for celiac disease patients*. Nutrients; 6:575–90.

Elena Galassi

Lawless H.T., Heymann H. (1999). *Sensory evaluation of food: principles and practices*. 1stEdn., Springer Science and Business Media, New York: 848.

Lee A.R., Ng D.L., Dave E., Ciaccio E.J., Green P.H. (2009). *The effect of substituting alternative grains in the diet on the nutritional profile of the gluten-free diet.* Journal of Human Nutrition and Dietetics: The Official Journal of the British Dietetic Association; 22:359–63.

Lee B.H., Weller C.L., Cuppett S.L., Carr T.P., Walter J., Martìnez I., Schlegel V.L. (2011). *Grain sorghum lipids: extraction, characterization, and health potential* American Chemical Society, 1089: 149-170.

Liu R.H. (2007). *Whole grain phytochemicals and health.* Journal of Cereal Science, 46: 207–219.

Lo S.C.C., De Verdier K., Nicholson R.L. (1999). *Accumulation of 3-deoxyanthocyanidin phytoalexins and resistance to Colletotrichum sublineolum in sorghum*. Physiological and Molecular Plant Pathology, 55: 263–273.

Maki M., Mustalahti K., Kokkonen J., Kulmala P., Haapalahti M., Karttunen T., Ilonen J., Laurila K., Dahlbom I., Hansson T., Hopfl P., Knip M. (2003). *Prevalence of celiac disease among children in Finland*. New England Journal of Medicine, 348:2517–24

Makinen O, Zannini E, Arendt E. (2013). *Germination of oat and quinoa and evaluation of the malts as gluten free baking ingredients*. Plant Foods Human Nutrition, 68:90–5.

Maglio M., Mazzarella G., Barone M.V., Gianfrani C., Pogna N., Gazza L., Stefanile R., Camarca A., Colicchio B., Nanayakkara M., Miele E., Iaquinto G., Giardullo N., Maurano F., Santoro P., Troncone R., Auricchio S. (2011).

Elena Galassi

Immunogenicity of two oats varieties, in relation to their safety for celiac patients. Scandinavian Journal of Gastroenterology, 46: 1194-1205.

Marietta E.V., Camilleri M.J., Castro L.A., Krause P.K., Pittelkow M.R., Murray J.A. (2008). *Transglutaminase autoantibodies in dermatitis herpetiformis and celiac sprue.* Journal of Investigative Dermatology,128: 332-5.

Martino H.S.D., Alves Tomaz P., Aguiar Moraes E., Lopes da Conceicao L., Da Silva Oliveira D., Aparecida Vieira Queiroz V., Avelino Santos Rodrigues J., Ribeiro Pirozi M., Pinheiro-Sant'ana H.M., Machado Rocha Ribeiro S. (2012). *Chemical characterization and size distribution of sorghum genotypes for human consumption*. Revista do Instituto Adolfo Lutz (Impresso), 71: 337-344

McCleary B. V., Codd R. (1991). *Measurement of (1-3), (1-4)- beta-D-glucan in barley and oats-a streamlined enzymatic procedure,* Journal of the Science of Food and Agriculture, 55: 303–312.

McCleary B.V., Gibson T.S., Lugford, D.C. (1997). *Measurement of total starch in cereal products by amyloglucosidase-α-amylase method: collaborative study.* Journal of AOAC International, 80: 571-579.

McCleary B.V., McNally M., Rossiter P. (2002). *Measurement of resistant starch by enzymatic digestion and selected plant materials: collaborative study.* Journal of AOAC International, 5:1103-1111.

McDonough C.M., Rooney L.W. (2000). *The millets*. In: Kulp K, Ponte JG Jr (eds) Handbook of cereal science and technology. Marcel Dekker, New York, pp 177–201.

Mengesha M.H. (1966). *Chemical composition of teff (Eragrostis tef) compared with that of wheat, barley and grain sorghum*. Economic Botany, 20: 268-273.

Elena Galassi

Mkandawire N.L., Kaufman R.C., Bean S.R., Weller C.L., Jackson D.S., & Rose D.J. (2013). *Effects of sorghum (Sorghum bicolor (L.) Moench) tannins on α-amylase activity and in vitro digestibility of starch in raw and processed flours.* Journal of agricultural and food chemistry, 61: 4448-4454.

Mohammed M.I.O., Mustafa A.I., Osman, G.A.M. (2009). *Evaluation of wheat breads supplemented with Teff (Eragrostis tef (ZUCC.) Trotter)* Grain flour. Australian Journal of Crop Science 3: 207-212.

Moore J., Yu L.L. (2008). *Methods for antioxidant capacity estimation of wheat and wheat-based food products.* In: Yu L. (Ed.), Wheat Antioxidants. John Wiley & Sons, Inc., Hoboken, Nj, 118-172.

Moraes É. A., Queiroz V. A. V., Shaffert R. E., Costa N. M. B., Nelson J. D., Ribeiro S. M. R., Martino H. S. D. (2012). *In vivo protein quality of new sorghum genotypes for human consumption.* Food Chemistry, 134:1549-1555.

Munck L. (1995). *New milling technologies and products: Whole plant utilization by milling and separation of the botanical and chemical components*. In: Dendy, D.A.V. (Ed.), Sorghum and Millets: Chemistry and Technology. American Association of Cereal Chemists, St. Paul, MN, USA: 223–281.

Murty D.S., Kumar K.A. (1995). *Traditional uses of sorghum and millets.* In: Dendy DAV, editor. Sorghum and millets: chemistry and technology. St. Paul, Minn, American Association of Cereal Chemists: 185–221.

National Institute. of Diabetes and Digestive and Kidney Diseases. [NIDDK] (2009). *Celiac disease*. Bethesda, Md.: NIDDK Clearing Houses Publications Catalog; c2009. Available from: www.digestive.niddk.nih.gov. Accessed 2009 February.

Nguyen H.T., Xu W., Rosenow D.T., Mullet J.E., McIntyre L. (1997). *Use of biotechnology in sorghum drought resistance breeding, part A.* In: Proceedings of the

Elena Galassi

International Conference on Genetic Improvement of Sorghum and Pearl Millet. Lubbock, TX, 2-424.

Niness K.R. (1999). *Inulin and oligofructose: what are they?* Journal of Nutrition, 129: 1402S–1406S.

Ochanda S.O., Onyango C., Mwasaru A., Ochieng J., Mathooko F. (2010). *Effects of malting and fermentation treatments on group B-vitamins of red sorghum, white sorghum and pearl millets in Kenya*. Journal of Applied Biosciences, 34: 2128-2134.

Olori M.O., Makinwa E.O., Irefin I.A. (1996). *Indigenous technological capability development in the brewing industry in Nigeria: an engineering economic assessment and policy implications.* Food Reviews International, 12: 511–523.

Oom A., Pettersson A., Taylor J.R.N., Stading M. (2008). *Rheological properties of kafirin and zein prolamins.* Jornal of Cereal Science, 47:109–16.

Oria M.P., Hamaker B.R., Axtell J.D., Huang C.-P. (2000). *A highly digestible sorghum mutant cultivar exhibits unique folded structure of endosperm protein bodies.* PNAS USA, 97: 5065–5070.

Osagie A.U. (1987). *Total lipids in sorghum grain.* Journal of Agricultural and Food Chemistry, 35: 601-604.

Owuama C. I. (1999). *Brewing beer with sorghum*. Journal Institute of Brewing, 105: 23-34.

Parr A.J., Bolwell G.P. (2000). *Phenols in the plant and in man. The potential for possible nutritional enhancement of the diet by modifying the phenols content or profile.* Journal of Science Food Agricultural, 80: 985-1012.

Paterson A.H., Bowers J.E., Bruggmann R., Dubchak I., Grimwood J., Gundlach H., Haberer G., Uffe H., Mitros T., Poliakov A., Schmutz J., Spannagl M., Tang

Elena Galassi

H., Wang X., Wicker T., Bharti A.K., Chapman J., Feltus F.A., Gowik Udo., Grigoriev I.V., Lyons E., Maher C.A., Martis M., Narechania A., Otillar R.P., Penning B.W., Salamov A.A., Wang Y., Zhang L., Carpita N.C., Freeling M., Gingle A.R., Hash C.T., Keller B., Klein P., Kresovich S., McCann M.C., Ming R., Peterson D.G., Mehboob-ur-Rahman, Ware D., Westhoff P., Mayer K.F.X., Messing J., Rokhsar D.S. (2009). *The Sorghum bicolor genome and the diversification of grasses.* Nature, 457: 551-556.

Payne P.I., Lawrence G.J. (1983). *Catalogue of alleles for the complex gene loci, GLU-Al, GLU-BI, and GLU-Dl which code for high-molecular-weight subunits of glutenin in hexaploid wheat.* Cereal Research Communications, 11:29-35

Pellegrini N., Agostoni C. (2015). *Nutritional aspects of gluten-free products*. Journal of the Science of Food and Agriculture, 95:2380–5.

Perten H. (1983). *Practical experience in processing and use of millet and sorghum in Senegal and Sudan*. Cereal Foods World, 28: 680–683.

Pfeiffer C.M., Rogers L.M., Gregory J.F. (1997). *Determination of folate in cereal-grain food products using trienzyme extraction and combined affinity and reserved-phase liquid chromatography*. Journal of Agricultural and Food Chemistry, 45: 407-413.

Picariello G., Mamone G., Nitride C., Addeo F., Ferranti P. (2013). *Integrated platforms to study food-protein digestion and derived functional and active peptides,* Trends in Analytical Chemistry, 52: 120-134.

Pontieri P., Mamone G., De Caro S., Tuinstra M.R., Roemer E., Okot J., De Vita P., Ficco D.B.M., Alifano P., Pignone D., Massardo D.R., Del Giudice L. (2013). *Sorghum, a healthy and gluten-free food for celiac patients as demonstrated by genome, biochemical and immunochemical analyses*. Journal of Agricultural and Food Chemistry, 61:2565-2571.

Elena Galassi

Price M.L., Van Scoyoc S., Butler L.G. (1978). *A critical evaluation of vanillin reaction as an assay for tannin in sorghum*. Journal of Agricultural and Food Chemistry, 26: 1214–1218.

Qiang H., Yuanping L., Kai Y. (2006). *Effects of tea polyphenols on the activities of α-amylase, pepsin, trypsin and lipase.* Food Chemistry 101:1178–1182.

Ragaee S., Abdel-Aal E.-S. M., Noaman M. (2006). *Antioxidant activity and nutrient composition of selected cereals for food use.* Food Chemistry, 98: 32-38.

Ravisankar S., Abegaz K., Awika J. M. (2018). *Structural profile of soluble and bound phenolic compounds in teff (Eragrostis tef) reveals abundance of distinctly different flavones in white and brown varieties.* Food Chemistry, 263: 265–274.

Re R., Pellegrini N., Proteggente A., Pannala A., Yang M., Rice-Evans C. (1999). *Antioxidant activity applying an improved ABTS radical cation decolorization assay.* Free Radical Biology and Medicine, 26: 1231-1237.

Reddy B.V.S., Ramesh S., Longvah T. (2005). *Prospects of breeding for micronutrients and β-carotene-dense sorghums.* International Sorghum and Millets Newsletter, 46: 10–14.

Renzetti St., Arendt E.K. (2009). *Effects of oxidase and protease treatments on the breadmaking functionality of a range of gluten-free flours*. European Food Research and Technology, 229: 307-317.

Rooney L.W., Miller F.R., Mertin J.V. (1982). *Proceedings of the International Symposium on Sorghum Grain Quality.* ICRISAT, Patancheru, AP, India, 143–162.

Elena Galassi

Rooney L.W., Waniska R.D. (2000). *Sorghum food and industrial utilization*. In: Smith, C.W., Frederiksen, R.A. (Eds.), Sorghum: Origin, History, Technology, and Production. Wiley, New York: 689–729.

Rosenow DT, Dahlberg JA. (2000). *Collection, conversion, and utilization of sorghum*. In: Smith CW, Frederiksen RA, editors, Sorghum: origin, history, technology, and production. New York (NY): John Wiley & Sons: 309-28.

Ruggeri S., Aguzzi A., Carnovale E. (2004). *Adequacy of trienzyme treatment in determination of folate in food.* In: First International Conference of Folates: analysis, bioavalability and health, 11-14 February 2004, Warsaw, Poland: 27-32.

Salas Fernandez M.G., Hamblin M.T., Li L., Rooney W.L., Tuinstra M.R., Kresovich S. (2008). *Quantitative trait loci analysis of endosperm color and carotenoid content in sorghum grain.* Crop Science, 48:1732-1743.

Salawu S. O., Bester M. J., Duodu K. G. (2014). *Phenolic composition and bioactive properties of cell wall preparations and whole grains of selected cereals and legumes*. Journal of Food Biochemistry, 38: 62–72.

Sang Y., Bean S., Seib P.A., Pedersen J., Shi Y.C. (2008). *Structure and functional properties of sorghum starches differing in amylose content.* Journal of Agricultural and Food Chemistry, 56: 6680-6685.

Saturni L., Ferretti G., Bacchetti T. (2010). *The gluten-free diet: safety and nutritional quality: review*. Nutrition, 2:16–34.

Schley P.D., Field C.J. (2002). *The immune-enhancing effects of dietary fibers and prebiotics.* British Journal of Nutrition, 87: s221–s230.

Elena Galassi

Seitz L.M. (2004). *Effect of plant-type (purple vs. tan) and mold invasion on concentrations of 3-deoxyanthocyanidins in sorghum grain*. AACC Annual Meeting Abstracts.

Serna-Saldivar S., Rooney L.W. (1995). *Structure and chemistry of sorghum and millets*. D.A.V. Dendy (Ed.), Sorghum and Millets, Chemistry and Technology, American Association of Cereal Chemists, St Paul, MN: 69-124

Serna-Saldivar S.O., Tellez-Giron A., Rooney L.W. (1988). *Production of tortilla chips from sorghum and maize*. Journal of Cereal Science, 8:275–284.

Serpen A., Gokmen V., Pellegrini N., Fogliano V. (2008). *Direct measurament of the total antioxidant capacity of cereal products.* Journal of Cereal Science, 48: 816-820.

Seyfu Ketema (1997). *Tef, Eragrostis tef (Zucc.) Trotter*, pp. 52. Rome, Italy: Institute of Plant Genetics and Crop Plant Research, Gatersleben/InternationalPlant Genetic Resources Institute.

Shan L., Molberg Ø., Parrot I., Hausch F., Filiz F., Gray G. M., Sollid L.M., Khosla C. (2002). *Structural basis for gluten intolerance in celiac sprue*, Science, 297: 2275-2279.

Shegro A., Shargie N., Biljon A., Labuschagne M. (2012). *Diversity in starch, protein and mineral composition of sorghum landrace accessions from Ethiopia*. Journal of Crop Science and Biotechnology,15: 275-280.

Shih C.-H., Siu Ng R., Wong E., Chiu L. C. M., Chu I. K., Lo C. (2007). *Quantitative analysis of anticancer 3-deoxyanthocyanidins in infected sorghum seedlings.* Journal of Agricultural and Food Chemistry, 55:254-259.

Elena Galassi

Shull J.M., Watterson J.J., and Kirleis A.W. (1991). *Proposed nomenclature for the alcohol-soluble proteins (kafirins) of Sorghum bicolor (L.) Moench based on molecular weight, solubility and structure.* Journal of Agricultural and Food Chemistry, 39: 83-87.

Shull J.M., Watterson J.J., Kirleis A.W. (1992). *Purification and immunocytochemical localization of kafirins in Sorghum bicolor (L. Moench) endosperm*. Protoplasma, 171: 64-74.

Shumoy H., Gabaza M., Vandevelde J., Raes K. (2017). *Soluble and bound phenolic contents and antioxidant capacity of tef injera as affected by traditional fermentation.* Journal of Food Composition and Analysis, 58: 52–59.

Shumoy H., Raes K. (2016). *Antioxidant potentials and phenolic composition of tef varieties: An indigenous Ethiopian cereal*. Cereal Chemistry, 93: 465–470.

Silva C.S., Queiroz V.A.V., Simeone M.L.F., Guimarães C.d C., Schaffert R. E., Rodrigues J.A.S., Miguel R.d.A. (2012). *Teores de mineraisemlinhagens de sorgo para uso naalimentaçãohumana.* In XXIX Congresso Nacional de Milho e Sorgo, 2776-2782. Água de Lindóia.

Singh J., Dartois A., Kaur L. (2010a). *Starch digestibility in food matrix: A review.* Trends in Food Science & Technology, 21: 168-180.

Singh H., Sodhi N.S., Singh N. (2010b). *Characterisation of starches separated from sorghum cultivars grown in India.* Food Chemistry, 119: 95-100.

Slavin J. (2004). *Whole grains and human health*. Nutritional Research Review, 17: 99-110.

Smith C.W., Frederiksen R.A. (2000). *Sorghum: origin, history, technology, and production.* Wiley Series in Crop Science. Series Editor: 660-662.

Elena Galassi

Sollid L.M. (2002). *Coeliac disease: dissecting a complex inflammatory disorder*. Nature Reviews Immunology, 2: 647-655.

Spaenij-Dekking L., Kooy-Winkelaar Y., Koning F. (2005b). *The ethiopian cereal teff in celiac disease.* New England Journal of Medicine, 353: 1748-1749.

Spaenij-Dekking L., Kooy-Winkelaar Y., Van Veelen P., Drijfhout J.W., Jonker H., Soest L., Smulders M.J.M., Bosch D., Gilissen L.J.W.J., Koning F. (2005a). *Natural variation in toxicity of wheat: potential for selection of nontoxic varieties for celiac disease patients*. Gastroenterology, 129: 797-806.

Stallknecht G. F., Gilbertson K. M., Eckoff, G. L. (1993). *Tef: food crop for humans and animals.* In New Crops (eds J. Janick and J. E. Simon), 231–234. Wiley, New York, USA.

Stefoska-Needham A., Beck E.J., Johnson S.K., Chu J.Y., Tapsell L.C. (2016). *Flaked sorghum biscuits increase postprandial GLP-1 and GIP levels and extend subjective satiety in healthy subjects*. Molecular Nutritional and Food Research, 60: 1118–1128.

Svensson L., Sekwati-Monang B., Lutz D. L., Schieber A., Gänzle M. G. (2010). *Phenolic acids and flavonoids in nonfermented and fermented red sorghum (Sorghum bicolor (L.) Moench).* Journal of agricultural and food chemistry, 58:9214-9220.

Suhendro E.L., Kunetz C.F., McDonough C.M., Rooney L.W., Waniska R.D., (2000). *Cooking characteristics and quality of noodles from food sorghum*. Cereal Chemistry, 77: 96–100.

Sweeny J.G., Iacobucci G.A. (1981). *Synthesis of anthocyanidins-III: Total synthesis of apigeninidin and luteolinidin chlorides.* Tetrahedron, 37: 1481-1483.

Elena Galassi

Tadesse B., Teju E., Megersa N. (2015). *The Teff straw: A novel low-cost adsorbent for quantitative removal of Cr(VI) from contaminated aqueous samples.* Desalination and Water Treatment, 56: 2925–2936.

Tatham A.S., Fido R.J., Moore C.M., Kasarda D.D., Kuzmicky D.D., Keen J.N., Shewry P.R. (1996). *Characterisation of the major prolamins of teff (Eragrostis Tef) and finger millet (Eleusine Coracana).* Journal of Cereal Science, 24: 65-71.

Taylor J.R.N., Belton P.S. (2002). *Sorghum*. Pseudocereals and Less Common Cereals, 25-91.

Taylor J.R.N., Schober T J., Bean S.R. (2006). *Novel food and non-food uses for sorghum and millets.* Journal of Cereal Science, 44: 252–271.

Taylor J.R.N., Schossler L., Van der Walt W.H. (1984). *Fractionation of proteins from low-tannin sorghum grain.* Journal of Agricultural Food Chemistry, 32: 149-154.

Taylor J.R.N., Emmambux M. (2010). *Developments in our understanding of sorghum polysaccharides and their health benefits.* Cereal chemistry, 87: 263-271.

Tefera M.M. (2011). *Land use/land-cover dynamics in Nonno District, Central Ethiopia*. Journal of Sustainable Development in Africa, 13: 123–141.

Thaddi B.N., Nallamilli S.M. (2014). *Estimation of total bioactive compounds in pigmented and non pigmented genotypes of sorghum (Sorghum bicolor (L.) Moench).* International Journal of Advanced Research in Science and Technology. 3: 86-92.

Tuinstra M.R. (2008). *Food-grade sorghum varieties and production considerations: a review*. Journal of Plant Interactions, 3: 69-72.

U.S. Grains Council. (2004). *Sorghum Handbook: White Sorghum, the New Food Grain.* U.S. Grains Council, Washington, DC.

Elena Galassi

Udachan I. S., SahuA. K., Hend F. M. (2012). *Extraction and characterization of sorghum (Sorghum bicolor L. Moench) starch*. International Food Research Journal, 19: 315-319.

USDA Food Composition Databases. (2017). https://ndb.nal.usda.gov/ndb/search>. Accessed 14.06.17.

Van Loo J., Coussement P., de Leenheer L., Hoebregs H., Smits, G. (1995). *On the presence of inulin and oligofructose as natural ingredients in the western diet*. Critical Reviews in Food Science and Nutrition, 35: 525–552.

Vavilov N.I. (1951). *The origin, variation, immunity and breeding of cultivated plants: selected writings*, Chronica Botanica Co, Waltham, MassUSA.

Wang H., Cao G., Prior R. L. (1997). *Oxygen radical absorbing capacity of anthocyanins*. Journal of Agricultural and Food Chemistry, 45: 304–309.

Wang Y., Tilley M., Bean S., Sun X.S., Wang D. (2009). *Comparison of methods for extracting kafirin proteins from sorghum distillers dried grains with solubles*. Journal of Agricultural and Food Chemistry, 57: 8366-8372.

Waniska R.D., Rooney L.W. (2000). *Structure and chemistry of the sorghum caryopsis.* In: Sorghum: production, agronomy, chemistry and utilization, 649-688. W. Smith & R.A. Frederiksen Eds., Wiley & Sons, New York.

Watson L., Dallawitz M.J. (1992). *The Grass Genera of the World*. CAB International, Wallingford, Oxon, UK.

Wild D., Robins G.G., Burley V.J., Howdle P.D. (2010). *Evidence of high sugar intake, and low fibre and mineral intake, in the gluten-free diet.* Alimentary Pharmacology & Therapeutics, 32: 573–81.

Elena Galassi

Williamson G., Clifford M. N. (2010). *Colonic metabolites of berry polyphenols: the missing link to biological activity?* British Journal of Nutrition, 104: 48-66.

Wilson B., Whelan K. (2017). *Prebiotic inulin-type fructans and galacto-oligosaccharides: definition, specificity, function, and application in gastrointestinal disorders.* J Gastroenterology and Hepatology, 32: 64– 68.

Woodward G., Kroon P., Cassidy A., Kay C. (2009). *Anthocyanin stability and recovery: implications for the analysis of clinical and experimental samples.* Journal of agricultural and food chemistry, 57: 5271-5278.

Wolter A., Hager A.S., Zannini E., Arendt E. K. (2013). *In vitro/starch digestibility and predicted glycaemic indexes of buckwheat, oat, quinoa, sorghum, teff and commercial gluten-free bread.* Journal of Cereal Science. 58: 431-436.

Wong H.J., Lau T., Cai N., Singh J., Pedersen J.F., Vensel W.H., Hurkman W.J., Wilson J.D., Lemaux P.G., Buchanan B.B. (2009). *Digestibility of protein and starch from sorghum (Sorghum bicolor) is linked to biochemical and structural features of grain endosperm*. Journal of Cereal Science, 49: 73-82.

Yetneberk S., de Kock H.L., Rooney L.W., Taylor J.R.N. (2004). *Effects of sorghum cultivar on injera quality.* Cereal Chemistry. 81: 314-321.

Yetneberk S., Rooney L.W., Taylor J. (2005). I*mproving the quality of sorghum injera by decortication and compositing with teff.* Journal of the Science of Food and Agriculture. 85: 1252-1258.

Young R., Haidara M., Rooney L.W., Waniska R.D. (1990). *Parboiled sorghum: development of a novel decorticated product.* Journal of Cereal Science, 11: 277–289.

Elena Galassi

Yousif A., Nhepera D., Johnson S. (2012). *Influence of sorghum flour addition on flat bread in vitro starch digestibility, antioxidant capacity and consumer acceptability.* Food Chemistry, 134: 880–887.

Zhu F. (2018). *Chemical composition and food uses of teff (Eragrostistef).* Food Chemistry, 239: 402–415.

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CHAPTER 8: ABSTRACT

The objective of this study was to evaluate five sorghum genotypes, one for zootechnical use and four food-grade hybrids, and two commercial teff genotypes (brown and white grain), for their technological, biochemical and nutritional traits, comparing them with a durum wheat cultivar as control, in order to identify the most suitable for the formulation of innovative wholegrain, gluten free products of high qualitative value to develop a sustainable agri-food chain.

The data concerning kernel and flours properties of the sorghum food-grade, on average, reported lower values than durum wheat control for kernel weight (-56%), test weight (-14%), sedimentation test (-56%), falling number (-16%) and yellow index (-19%), whereas brown index was higher (+38%) and kernel hardness (+0.5%) comparable to control. Furthermore, teff genotypes showed lower values than durum wheat control for kernel weight (-99%), sedimentation test (-50%), falling number (-18%), yellow index (-11%), whereas brown index was higher (+90%) and test weight (+2%) was comparable to control.

The results showed that, on average, sorghum food-grade hybrids had higher contents of resistant starch (RS) and RS/TS (Total Starch) ratio (about 6 times higher), yellow pigments, free polyphenol and anthocyanin contents (+600%, +400% and +100% than the control, respectively). Teff genotypes, on average, were comparable to durum wheat for resistant starch content, RS/TS ratio, yellow pigments and anthocyanin contents, whereas total starch and folate contents were higher (+6 % and +40%) than in wheat.

Furthermore, the beverage transformations of these two gluten free cereals revealed malts with low amylase activity, typical of low-alcohol drinks, and useful for light and gluten-free beer market. Otherwise, the malts produced could be used in combination with other malts for the production of innovative fermented beverages, not yet available on the market.

Finally, the teff-based "Tigelle", typical Italian unleavened focaccias from Emilia Romagna, presented good overall acceptance scores from the hedonic analysis carried out by not-expert people, with higher scores for crumb and crust color in white teff bread, whereas the sample with malt was preferred for its aroma and taste.

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In conclusion, the data of this study showed that the four food-grade sorghum hybrids and the two teff genotypes examined have technological and nutritional characteristics comparable with the durum wheat, used as control, for the most of the parameters examined. When the analyzed lines showed lower values than wheat, they are still acceptable for the transformation processes.

The data obtained in this study, highlighted as sorghum and teff can constitute promising alternative ingredients in the gluten free market. Furthermore, given their resilience to both drought and heat stress, teff and sorghum could be one of the answer to global sustainability on food and nutrition security.

Elena Galassi

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