TEFF

"Survey on the nutritional and health aspects of teff (Eragrostis Tef)"

Client
Dr. Arnold Dijkstra
Hogeschool van Hall-Larenstein
Lector Food safety
T: 058 2846160
E: arnold.dijkstra@wur.nl

Teachers
Ing. Joyce Polman
Hogeschool van Hall-Larenstein
Teacher foodmicrobiology, chemistry and biochemistry
T: 0317 486284
E: joyce.polman@wur.nl

Dr. Arnold van Wulfften-Palthe
Hogeschool van Hall-Larenstein
Teacher
E: arnold.vanwulfftenpalthe@wur.nl

Authors
Dr. Patricia Arguedas Gamboa
Instituto Tecnológico de Costa Rica, Sede Central
Apdo. 159-7050 Cartago. Costa Rica
T: 00(506) 2550-2695
E: parguedas@itcr.ac.cr

Lisette van Ekris
Hogeschool van Hall-Larenstein
Student Food science and technology
E: lisettevanekris@wur.nl

Summary

Teff is an interesting grain used for centuries as the principal ingredient of the Ethiopian population diet. The principal meal in which teff is used is called enjera: a big flat bread or pancake, than is eaten alone or with any kind of meats, vegetables and sauces. Teff is the smaller grain ever known, and even that it has been demonstrated that it was used by Egyptian Pharaohs; it is until two decades ago that it became the issue of agronomic, nutritional, food technological, microbiological, chemical and physical research. Teff can be used too in all kind of bakery products, beverages, sauces ingredient and porridges. This grain is used too as a livestock.

The potential of this grain as an interesting raw material to new food products development is due principally to its protein composition: it is gluten free and it has a very high quality of amino acid composition. It is compared with egg protein and with an ideal protein for children between two and five years old. A lot of scientific people have been demonstrated that teff starch has a low glycemic acid, and that it has a mineral composition better than this or other cereals.

That's why, this microscopic grain is beginning a big war between different grain producers and processors. Some companies want to demonstrate that human been needs to include teff as an important component of his diet. This is against the economical interests of other big companies and associations, as this that grow, harvest, mill, process and storage wheat flour.

This work pretend to collect the different information generated about teff grain, in an objective way, and that can help governments, nutritional, agronomic, and food processing institutions to better target their research about this topic.

The authors made a brief description of the grain from an agronomical and genetically point of view. They go deeply in the chemical, physical and microbiological characterisation.

In the macro-chemical composition, this grain offers big possibilities to their processing. The starch can be modified with chemical procedures, in order to change its physical characteristics, which made this raw material useful to different technological applications.

In the micro-chemical composition, that is very important to define the nutritional value of a food, the authors of this work found a lot of contradictions between different articles and different writers. This made of teff a polemic grain.

The microbiological composition of teff, enjera, and ersho is widely different, according with the region, the fermentation step and the teff variety used. However, it is demonstrated than the fermentation step is very important from a nutritional point of view: The relationships phytate:iron, Tannin:iron, phytate:zinc and tannin:zinc decrease. This increase the availability of minerals, to be used by human enzymes.

At the last chapter; Medical aspects and teff benefits, the reader can find a little description of diseases in which teff can help to decrease their incidence or their symptoms (celiac, annemia, osteoporosis, diabetes and obesity). In almost all the cases, the information found at the literature it´s not enough to obtain trusty conclusions about the benefits obtained by patients if they include teff as a normal ingredient of their diet.

Finally, the authors finish with the proposal to elaborate and interdisciplinary and intersectional big project, in order to know the true, and to define standardized methods to produce, harvest, processing and storage teff, enjera and other teff products.

1 Teff

1.1 Introduction

Teff (Eragrostis Tef) is an intriguing grain, ancient, minute in size, and packed with nutrition. Teff is believed to have originated in Ethiopia between 4000 and 1000 before Christ (BC). Nowadays, teff represents the re-discovery of a crop used by ancient civilizations (Stallknecht, 1993).

It is possible to speak of a re-discovery, because nowadays, there are new techniques to analyze and well know the chemistry and physical characteristics of crops. In addition to that, new methods to collect and analyze these data have been developed, leading us to understand that our ancestor had valuable information about their crop benefits, meanly about teff.

Recently, a lot of scientists of developing countries, trying to offer new products for consumers, and trying to satisfy their nutritional needs, they were wondered about the lack of anemia, osteoporosis, celiac disease and diabetes in the Ethiopia population. It is also well known, in a worldwide level, that the resistance and general good fitness of Ethiopian sport people is very good. That's why new scientists are interested to know all about the teff composition, the nutritional properties, and the changes that happens at the moment of grain fermentation, during the preparation of enjera, a flat bread that is responsible for about 70 % of the Ethiopian population. This research has been done by universities from Ethiopia and other countries, and also privates companies are working with this crop to make it a ¨golden grain¨.

Teff seeds were discovered in a pyramid thought to date back to 3359 BC. In contrast to amaranth, another little grain which was utilized by early civilizations throughout the world, the grain has been widely cultivated and used only in Ethiopia, India and it's colonies and Australia (Railey,). Teff is grown primarily as a cereal crop in Ethiopia.

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. It is the smallest grain in the world, ranging from 1–1,7mm long and 0,6–1mm diameter with l000 seed weight averaging 0,3–0,4 grams and taking 150 grains to weigh as much as one grain of wheat. The common English names for teff are teff, love grass, and annual bunch grass. It is intermediate between a tropical and temperate grass.

The colour of the Teff grains can be ivory, light tan to deep brown or dark reddish brown purple, depending on the variety. Teff has a mild, nutty, and a slight molasses like sweetness. The white teff has a chestnut-like flavor and the darker varieties are earthier and taste more like hazelnuts. The grain is somewhat mucilaginous. It is interesting that documents dated from the late 1800's indicate the upper class consumed the lighter grains, the dark grain was the food of soldiers and servants. The cattle consumed hay made from teff (Railey).
It is then, an interesting activity for scientific people like nutritionists, chemists, food engineers and for trade people, to re-discover this attractive little grain that is really ancient but new for developing countries. It is of a very small size but with a giant nutritional content.

Figure 1.1, Teff. From
http://images.google.nl/images?hl=en&q=teff&btnG=Search+Images&gbv=2

1.2 Agronomy and taxonomy aspects

Eragrostis is a member of the tribe Eragrosteae, sub-family Eragrostoidae, of the Poaceae (Gramineae). Teff is a tetraploid plant 2n = 40. (Stallknecht, 1997).

There are approximately 350 species in the genus Eragrostis consisting of both annuals and perennials which are found over a wide geographic range. Eragrostis teff is one of those species. The closest relative of teff is E.Pilosa (Yu, 2006). Eragrostis species are classified based on characteristics of culms, spike lets, lateral veins, pedicels, panicle, flowering scales, and flower scale colours. Recently, the taxonomy of teff has been clarified by numerical taxonomy techniques, cytology and biochemistry, including leaf flavanoids and seed protein electrophoretic patterns (Jones *et al*. 1979; Costanza *et al*. 1980; Bekele and Lester 1981).

Teff is a fine stemmed, tufted annual grass characterized by a large crown, many shoots, and a shallow fibrous diverse root system. The plants germinate quickly and are adapted to environments ranging from drought stress to water logged soil conditions. The inflorescence is an open panicle and produces small seeds (1.000 weigh 0,3 to 0,4 g). The florets consist of a lemma, 3 stamens, two stigma and two lodicules. Floret colours vary from white to dark brown. Plant height of teff varies from 25–135 cm which dependents on cultivar type and growing environments. Panicle length 11–63 cm, with spike lets numbers per panicle varying from 190–1410. Panicle types vary from loose, lax, compact, multiple branching multi-lateral and unilateral loose to compact forms. Maturity varies from 93–130 days (Stallknect, 1999).

Teff is an annual warm season grass crop (Stallknecht, 1997). Teff is a self pollinating chasmogamous plant. It is a reliable low risk crop, and can be planted in late May similar to millets. Late plantings have the advantage to control emerged weeds by tillage prior to planting, which can be significant since teff is a poor competitor with weeds during the early growth stages. Planting of teff requires a firm moist seed bed, similar to planting for alfalfa. To affect good soil moisture-seed contact due to the extremely small seed size. Seeding rates varies from 2.3 to 9 kg/ha, with 5 to 8 kg/ha generally recommended (Twidwell *et al*, 1991). Teff should be seeded 12–15 mm deep either broadcast or in narrow rows (Stallknecht, 1997). Control of broadleaf weeds should be considered, particularly Amaranthus retroflexus redroot pigweed, which produces seed that cannot be separated from teff. Moderate rates of nitrogen and phosphorus fertilizer are suggested to prevent

lodging. Several special considerations must be given to teff harvested for grain. Due to the small seed size, combine seed delivery systems must be checked for gaps and areas through which the small teff seed can be lost. Soil particles must be prevented from going through the combine and into the grain hopper, since it is very difficult if not impossible to separate fine soil particles from the teff grain. Teff germinates rapidly, and the broadcast and narrow row seeding allow for stronger weed competition (Stallknecht, 1997). Teff is adapted to environments ranging from drought stress to water logged soil conditions. Maximum teff production occurs at altitudes of 1800–2100 m, growing season rainfall of 450–550 mm, with a temperature range of 10–27°C. Teff grain yields in the U.S. average from 700 kg/ha dry land to 1400 kg/ha irrigated in Montana (Eckhoff *et al*. 1993; Stallknecht *et al*. 1993). Forage yields vary from 9.0 to 13.5 Mg/ha, dependent upon moisture levels during the growing season (Boe *et al*. 1986; Eckhoff *et al*. 1993). Teff is day length sensitive and flowers best during 12 hours of daylight.

While teff grain still provides over two-thirds of the human nutrition in Ethiopia, occupying two million hectares in 2003–2004 (Yu, 2006), representing 20% (2 8 106 t) of the total cereal production of the total cereal production of the country (CSA, 1997). It is relatively unknown as a food crop elsewhere. Teff has adaptive characteristics similar to other crops grown by early civilizations. Teff can be cultivated under a wide range of environmental conditions even on marginal soils under water logged to drought conditions. Teff can produce a crop in a relative short growing season and will produce both grain for human food and fodder for cattle (Stallknecht, 1993).

Planting can be accomplished using a Brillion grass seeder and cultipacker combination, or by a spinner type grass seeder. Teff germinates rapidly when planted an average depth of 1,2 cm, however, the initial growth is slow until a good root system has been established. Forage yields of teff in South Dakota have ranged from 4 to 11 t/ha depending upon planting date and number of cuttings (Boe *et al.* 1986). In Montana, forage yields cut from dry land and irrigated cropping ranged from 2.2 to 15 t/ha. Teff seed yields in Montana ranged from 0.2 to 1.5 t/ha. The low seed yields were obtained at the MSU Southern Agr. Res. Center, Huntley, when planted as a non-irrigated dry land crop, due to poor stands and drought conditions. Harvesting teff for either forage or seed production is easily accomplished, as long as the combine is seed tight. Teff seed can shatter if harvest is delayed. Broad leafed weeds in teff can be easily controlled by use of broad leaf herbicides, however grass weeds if present, can out compete the teff during the early stages of plant growth. Now specific fertility studies have been conducted, but rates similar to those suggested for millets or sorghums are recommended. Green house studies on nutritional requirements of teff have been conducted at Oklahoma State University Department of Agronomy, Stillwater (pers. commun.).

Inadequate nutrient and organic-matter supply constitutes the principal cause for declining soil fertility and productivity in much of sub-Saharan Africa (SSA). Increasing food production and sustaining soil fertility on the smallholder farms is an enormous challenge in the SSA. Soil nutrient status is widely constrained by the limited use of inorganic and organic fertilizers and by nutrient loss mainly due to erosion and leaching (Tulema *et al*, 2005). Many small holder farmers do not have access to synthetic fertilizer because of high price of fertilizers, lack of credit facilities, poor distribution, and other socio-economic factors. Consequently, crop yields are low, in fact decreasing in many areas, and the sustainability of the current farming system is at risk (Stangel, 1995; UNDP, 1992). Ethiopia is one of the 14 sub-Saharan countries with highest rates of nutrient depletion (Stoorvogel and Smaling, 1990) due to lack of adequate synthetic-fertilizer input, limited return of organic residues and manure, and high biomass removal, erosion, and leaching rates. The annual nutrient deficit is estimated at 41 kg N, 6 kg P, and 26 kg K per ha per year (Stoorvogel and Smaling, 1990). There is an urgent need to improve nutrient management.

In the other hand, in a survey in Gare Arera, the central Ethiopian highland, farm yard manure (FYM) and compost, enriched with ash, were identified as underutilized organic nutrient sources. Mustard meal, a by product of mustard-seed oil production, is also locally available. That's why the Ethiopian researchers, as Tulema *et al*, are working with the purpose to find the way to increase, with local and not expensive resources, the quality of their country lands. FYM is a potential source of organic fertilizer as the country has the highest livestock number in Africa. The annual dry-manure production is estimated at 22,7 million Mg. Annual crop-residue production is estimated at 12,7 million Mg. Moreover, there are various other unexploited organic by-products or wastes from processing animal and plant products such as mustard meal, coffee husk, sugar cane straw, and abattoir by-products. Use of FYM and other locally available organic materials is important for improving soil quality.

Tulema *et al*, they found that farmers did not utilize FYM efficiently due to lack of confidence in the effect of FYM, labour shortage for FYM management, and weed problems. The distance between croplands and homesteads (kraal location) was another important constraint as the fields were scattered over a large area in the watershed. Previous studies have also suggested the distance of fields from kraal as a major problem in FYM utilization (Hailu *et al*, 1992; Dereje *et al*, 2001). The writers appointed too, that compost could be an important organic fertilizer that all farmers produce and use, as materials such as household wastes, ash from burnt biomass, and water were available for composting.

Tulema *et al* concluded that the effects of organic fertilizers on teff partly exceeded the effects of equivalent amounts of N given as urea together with triple super phosphate. The investigation showed that available organic resources in Gare Arera are underutilized for lowing down soil nutrient depletion and for improving the productivity. However, continued on-farm experimentation with farmers' participation is important in order to evaluate the technical and economic efficiencies under different ecological conditions.

Mixed cropping is often superior to sole cropping in terms of insurance against risk, efficient use of resources and higher net returns. Most successful mixtures have been of a legume and non-legume, and there are few reports of yield increases from non-legume mixtures. Bayu *et al*. mixed cropping of teff and sunflower under the semi-arid conditions of north-eastern Ethiopia. They found that Intercropping teff and sunflower in semi-arid areas of Welo, Ethiopia has yield advantages over monoculture yields as the resource utilization is complementary. In intercrops the component crops can exploit different soil horizons, whereas a sole crop has its own specific rooting horizon.

Diseases and Pests

Teff is relatively free of plant diseases when compared to other cereal crops. In Ethiopia, in locales where humidity's are high, rusts and head smuts are important diseases. In Ethiopia, 22 fungi and 3 pathogenic nematodes have been identified on teff (Bekele 1985). Teff seedlings are also susceptible to Damping-off caused by Drechslera poae and Helminthosporium poae (Baudys) Shoemaker, when sown too early (Ketema 1987). Insect pests of teff in Ethiopia are Wello-bush cricket, Decticoides brevipennis, red teffworm, Mentaxya ignicollis, teff epilachna, and teff black beetle. Since teff has been limited to small areas in the United States a few disease and insect problems have been observed. However, a serious problem was observed in South Dakota where the stem boring wasp, Eurytomocharis eragrostidis (Howard) reduced forage yields by over 70% (McDaniel and Boe 1990). Although the insect problem was observed in only one out of the five years in research trials, the significant losses obtained could be a deterrent to commercial expansion of teff production.

Cultivars

Develop methods to improve the breeding of teff cultivars (which are self-pollinating) there have only met limited success (Mengesha *et al*. 1965; Berhe and Miller 1978; Berhe *et al*. 1989). While teff production in Ethiopia occupies large areas and is the most important staple of the country, most cultivars are selections that have been grown for thousands of years. Although cultivar development has been given a high research priority most on going studies have focused on agronomic practices.

Today, Ethiopia is assumed to refuge about 6500 to 7000 higher plant species of which 12% are endemic (exist only in Ethiopia) and over 1400 wild vertebrate (not considering domesticated ones) species which 85 of them are reported to be endemic. Yet the country is believed to be highly endowed with smaller flora and fauna including microbial species. Ethiopia does not only have high species diversity but also high genetic diversity with in a species which obviously is attributable to its diverse ecosystems and historical intervention of its people (Sertse, 2008).

Figure 1.2, Growing teff. From
http://images.google.nl/images?hl=en&q=teff&btnG=Search+Images&gbv=2

1.3 Genetic characterisation

Teff belongs to the family Poaceae, sub-family *Eragrostidae* and genus *Eragrostis*. This genus has 350 species and it is the only cultivated cereal species. The teff center of origin and diversity is Ethiopia (Bay *et al*, 2000, Yu *et al* 2006, Yu *et al*, 2007).

Some cultivars have been identified using phenotypic characteristics, such as: plant size, maturity, seed colour, panicle form. As a result of using these characters for cultivar identification, a large variation has been observed. This variation can be explained because morphological characters are susceptible to environment. New biotechnology tools have been developed for cultivar identification. Molecular markers have been used to study genetic variation and the relationship within and amount species. These new techniques are not affected by phenotypic nor by the environment (Bay *et al*, 2000).

Bay *et al,* 2000 set up a study to evaluate genetic diversity of teff and its relatives. Forty seven accessions of *E. teff*, some of them were describe according to agronomic and morphological traits, three accessions from *E. pilosa* and six accessions from *E, curvula*. Two accessions from *E. curvula* came from United States and another two from Argentina. The molecular marker used in this work was Random Amplified Polymorphic DNA (RAPD). This molecular marker has been used in many other crops to study the genetic relationships among species and cultivars.

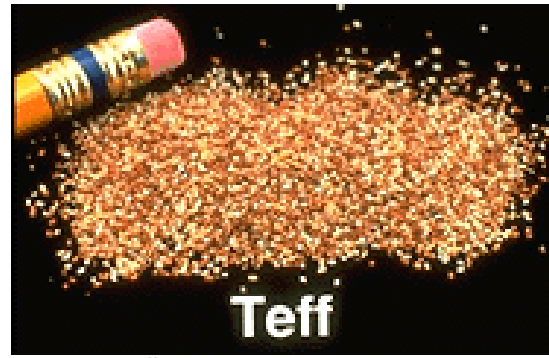

Figure 1.3, Teff seeds. From www.hort.purdue.edu/.../eragrostis_tef_nex.html

According to the results, the genetic distance between teff accessions was between 0,84 and 0,96, indicating high similarity or low polymorphism at the DNA level (Figure 1.4). Therefore, teff has a narrow genetic base and a few genes could control the morphological variations showed by teff germoplasm. When they studied the genetic relationship between teff and wild species, a higher polymorphism was found between the three species. The genetic distance between them was from 0,18 to 0,88 (Fig. 1.5), which indicates the high degree of genetic diversity. As a result, the three species were classified into two main groups. *E. teff* and *E pilosa* were in one group because they had a higher polymorphism, and *E. curvula* was in another group. Also, other researchers had concluded that the closest relative to *E. teff* was *E. pilosa* (Yu *et al*, 2006 and Yu *et al,* 2007). These two species could cross and new genes from the wild relative could be transferred to *E. teff*, providing new genetic resources for teff improvement (Bay *et al* 2000 and Yu *et al* 2006). Another result from Bay *et al,* 2000´s study was the higher genetic distance between accessions from *E. curvcula*. Those accessions collected from Ethiopia were closed related (86%), but they were distant from those from United States and Argentina (Bay *et al*, 2000). Even though, *E. teff* has a very narrow genetic variation, it could be increased by wild relatives, mainly *E pilosa*, which *E. teff* could be crossed. However, *E. curvula* has the higher genetic distance and it could be a source of genes that could be introduce to *E. teff* by biotechnology tools.

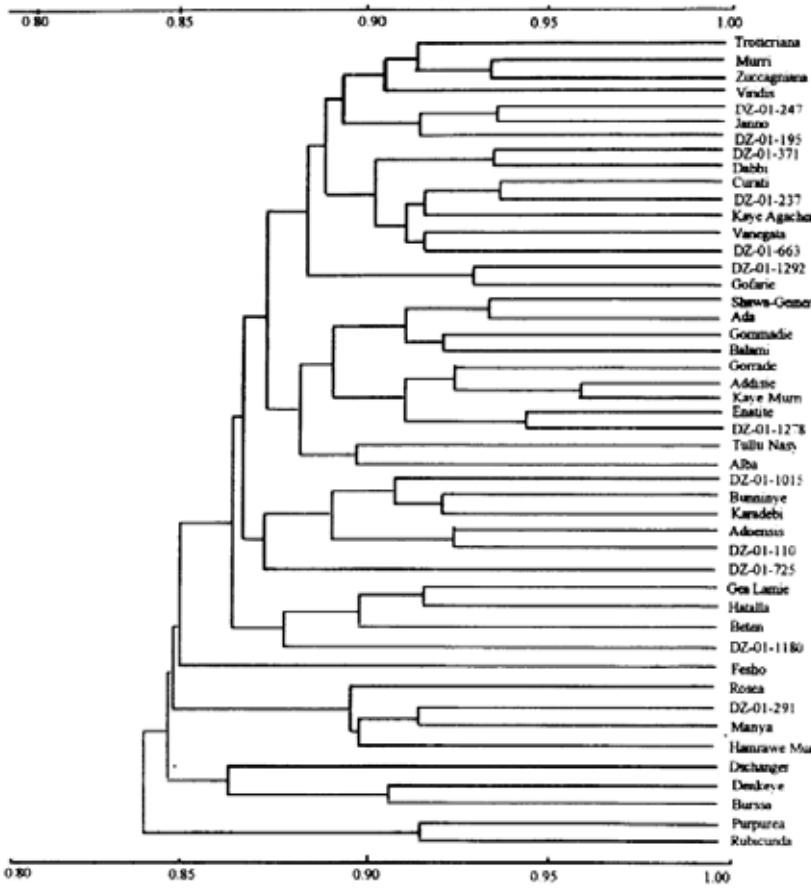

Figure 1.4, Diagram of teff accessions (accessions name given next to its corresponding branch (Bay *et al*, 2000))

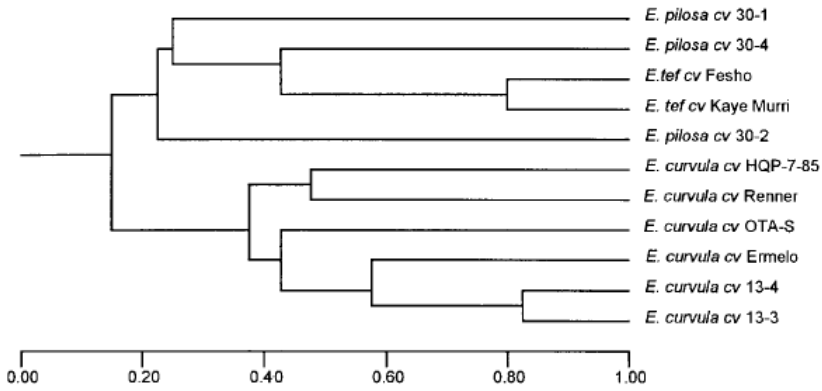

Figure 1.5, Diagram of *E. teff* and other *Eragrostis* species (Bay *et al*, 2000)

2 Products

2.1 The uses of teff

The principal use of teff grain for human food is the Ethiopian bread (enjera). It is used to wrap all kind of foods. This is an easy way to eat them, without fork or spoon, and the nutritional level of the meal increases (www.globalnomad.net/pages/enjera.jpg). Teff is ground into flour, fermented for three days then made into enjera. Enjera is a sourdough type flat bread. It is described as a soft, porous, thin pancake, which has a sour taste. Teff is free in gluten and therefore, the bread remains quite flat. When eaten in Ethiopia, teff flour is often mixed with other cereal flours, but the flavour and quality of enjera made from mixtures is considered less tasty. Enjera made entirely from barley, wheat, maize or millet flours is said to have a bitter taste. The degree of sour taste is imparted by the length of the fermentation process. If the dough is fermented for only a short period of time (no more than ten days),

Figure 2.1, Enjera with vegetables sauce. From fooditudeblog.blogspot.com/

enjera has a tasty sweet flavour. Research studies on the techniques used to make enjera have indicated that the yeast, Candida guilliermondii (Cast.), is the micro-organism primarily responsible for the fermentation process (Stewart and Getachew 1962). Enjera is a major food staple, and provides approximately two-thirds of the diet in Ethiopia (Stewart and Getachew 1962). It is also eaten as porridge and used as an ingredient of home-brewed alcoholic drinks. Teff is a very versatile grain. Teff flour can be used as a substitute for part of the flour in baked goods, or the grains added uncooked or substituted for part of the seeds, nuts, or other small grains.. It is a good thickener for soups, stews, gravies, and puddings and can also be used in stir-fry dishes, and casserole dishes. Teff may be added to soups or stews in either of two ways:
1) Add them, uncooked to the pot a half-hour before serving time.
2) Add them cooked to the pot 10 minutes before serving.

Cooked teff can be mixed with herbs, seeds, beans or tofu, garlic, and onions to make grain burgers. The seeds can also be sprouted and the sprouts used in salads and on sandwiches.

Teff flour is also used for making traditional alcoholic drinks like tella (local opaque beer) and katikalla (local spirit), kitta (sweet dry unleavened bread), muk (gruel)(Bultosa *et al,* 2002).

Teff has been used by Yigzaw *et al,* to be mixture with Grass pea (Lathyrus sativus). This is one of the important food legumes in countries like Bangladesh, India and Ethiopia. It has desirable agronomic in intercrops the component crops can exploit different soil horizons, whereas a sole crop has its own specific rooting horizon. Another example of mixed cropping with teff, is the case of sunflower, that we talked about in the Agronomy section. Putnam et Allan, 1992, indicate that differences of maturity between both crops, to make better use of light.

Figure 2.2, Enjera with vegetables and meat. From www.dkimages.com/.../Ethiopia/Ethiopia-19.html

Fermentation of cereals or their blend with legumes is a potentially important processing method that can be expected to improve the nutritive value such as availability of proteins and amino acid profile. It could also decrease certain anti nutritional factors like phytates, protease inhibitors and flatulence factors.

Although mixing teff with grass pea has not been part of the traditional practice for food preparation in Ethiopia, exploring the potential of fermentation of their blend may be beneficial. One obvious reason is developing an affordable nourishing crop for the poorer section of the population. Yigzaw *et al*. did not want to go higher than 8:2 (teff: grass pea) ratio as a compromise between nutritional adequacy and sensory value.

Teff is also produced in other countries. Countries such as USA, Canada, Australia, South Africa, and Kenya have produced teff for different purposes such as a forage crop and a thickener for soups, stews, and gravies (Zedwu, 2007).

Teff has also a lot of fanatic consumers, like the top Ethiopians sportsmen Haile Gebrelassie and Kenisse Bekele (Turkensteen, 2008), they say that the teff products are not only gluten free but might help consumers to control their weight. Different then the modern grains teff helps the body to be fit for life. They think that products made out of teff, including enjera, helps them break international records over and over again.

This is possible because teff has a high content of iron. This made that the haemoglobin in the blood is higher, so more oxygen can be transmitted, and the sportsmen can reach better sport results.

In the first page of the Soil & Crop Company Website, there can be found the following phrases that enhance people to consume teff. **"A grain as healthy as nature itself. Loaded with all compounds which are necessary for our body. Healthy food for everyone. Is teff made for our body or is our body made for teff?"**

Even Soil & Crop say: "Eragrain teff (they call teff Eragrain teff) is a wholegrain cereal. A valuable grain, loaded with good food compounds (http://www.soilandcrop.com /index.php?lang=eng). A grain so valuable that 55 centuries ago, teff was placed in the pyramids together with pharao's as food for their last journey. People in Ethiopia have always been loyal to their teff. They eat teff at every meal. Because teff is so nutritious, people in Ethiopia hardly suffer from such diseases as anemia, osteoporosis and diabetes. Scientist does connect this to the consumption of teff. Compared to wheat, barley and oats, Eragrain teff has a high content of minerals such as: iron, calcium, zinc and magnesium".

Teff has been used to increase the quality of enjera made with tannin-containing sorghums. In a research made by Yetneberk *et al*, 2005, enjera was produced with a 50:50 (w/w) composite of whole tannin-containing sorghum and teff. This process reduced the tannin

content of the flours, which appeared to relieve the inhibiting effects of tannins on the fermentation (Yetneberk *et al*, 2005).

Teff is used a lot for livestock feeding in Ethiopia and other countries. It is complemented with other crops, in order to increase nutritive value. Mengistu *et al*. studied supplementing the teff (Eragrostis teff) straw feed of Arsi oxen (Bos indicus) with noug (Guizotia abyssinica) meal. Supplementation significantly increased feed intake, average daily gain, and the weight of dressed carcass and lean meat. Supplementation with 1 kg of noug meal was the most profit table, giving a net return per animal of US$17.10, whereas a sole diet of teff straw gave a loss of US$18.66 per animal (Mengistu *et al*, 2007)

As said before teff has been used by Yigzaw *et al*, to be mixtured with Grass pea (Lathyrus sativus). In their experiments, they found that the fungal fermentation improved the amino acid profile for the essential amino acids in all the mixtures grass pea:teff. Fermentation of teff:grass pea (8:2) , in particular has been found to be quite comparable in essential amino acid profile to an ideal reference protein recommended for children of 2 – 5 years old.

Finally, teff is used as a component in adobe construction in Ethiopia.

Figure 2.3, Baking of enjera. From www.fao.org/.../compend/img/ch16/ph02509.htm

2.2 Teff products

As teff is a cereal, it is possible to develop with it, the same products than with other cereals. It is not only for human consumption, but also as feed for animals. In a first step, the seed is milled to obtain flour. As it will be exposed in the Chemical Characterisation chapter, teff has no gluten proteins. This made this flour very interesting to be used in gluten-free-diets. But, in the other hand, this decrease the sensory quality of baked products made from teff.
The food alternatives for Celiac Disease patients are mostly based on maize, rice, and soy. Teff appears to be another interesting possibility. In wheat bread dough the gluten proteins naturally form a viscoelastic network required for the desired functional properties of bread products (Hooseney, 1986). Because these kinds of proteins are lacking (or in insufficient quantity) in maize and rice, the gluten-free breads lack good quality properties. Therefore, to improve the functional properties of gluten-free bread, the dough could be treated with microbial transglutaminase (mTG), which cross-links proteins and improves functionality (Moore *et al*, 2006). In general, gluten-free breads treated with mTG tend to have a better overall quality due to the formation of a stable protein network. The authors of this work think that the same can be applied to teff, and in this manner, it will be possible to obtain high quality baked products.
However, teff can be used to elaborate the following products, and it is possible to find any kind of recipes. (http://www.bobsredmill.com/recipe/ingredient.php?pid=386)

Appetizers
Baked goods
Biscuits and scones
Breads
Breakfast and desserts bars
Breakfast dishes (to be eaten with fruits and milk, hot or cold)
Brownies
Cakes and cupcakes
Casserole dishes
Cookies
Crackers
Desserts
Dips, sauces and gravy
Granolas (muesli)
Muffins
Pancakes & waffles
Pastas
Pie crusts
Pizza crusts
Rolls & buns
Soups & stews
Tortillas and flat breads

Figure 2.4, Teff bread mix. From Railey.

There can be conclude that with the nowadays technology every product that normally is made from wheat can be made with teff. There is not found a product that can be made from wheat and not from teff.

3 Characterisation of teff

Even if some companies and writers are enhancing the composition of teff as a way to accomplish its re-discovery, teff chemical composition is not far of this of other cereals, even from a macro component as from a micro components point of view. Bultosa *et al*, 2002, they say about that: The micro- and macronutrients level of grain teff is apparently higher than that of barley, wheat and sorghum. The nutrient composition of grain teff indicates that it has good potential to be used in foods and beverages worldwide (Bultosa *el al*, 2002). The amino acid composition of grain teff is reported to be comparable to that of egg protein, except for its lower lysine content.

To made a difference between chemical and physical characterisation begin sometimes a hard work because they are extremely related. The relation between amylose and amylopectin, define some physical characteristics, as temperature gelatinization, gelation characteristics, solubility and starch resistance. Thus, the small variations in the amylose content among teff varieties may influence also the starches to have slightly different properties.

Using different chemical substances as NaCl, EtOH, $NaHCO_3$ and $CaCO_3$ it is possible to change the medium and as a consequence, physical teff starch properties as foaming capacity and protein solubility will change.

3.1 Chemical

3.1.1. Macro components

The concentration, relationship and rates between the different macro-components are essential to determine the texture, appearance and physical characteristics of a food. In relation with this composition, Food Engineers we have to design food products, and to select machinery, additives, package materials, etc. The shelf life and then, the storage systems are defined in function of the food macro composition; Protein, fat, ash and carbohydrate content are given as 9,6%, 2,0%, 2,9% and 73,0%, respectively.

Lipids: teff starches had slightly lower hydrolyzed lipids (mean 8,9 mg/g) than maize starch (9,9 mg/g). The crude fat (ether extract) content of the teff starches (mean 0,29%) was relatively low as compared to that of maize starch (0,34%). The crude fat of grain maize is around 4,45%, higher than that of grain teff which is around 2% (db) (National Research Council, 1996). Crude fat (petroleum ether extract) consist mostly of non-starch lipids i.e., it is not endogenous to the starch [20]. The low crude fat content in teff starch is most probably related to the low crude fat content of the grain. Bultosa found that the teff total starch lipid was higher than that of pearl millet (5,0 mg/g) and slightly higher than that of rice (7,6 mg/g).

Starch: In the carbohydrate fraction of grain teff, starch is is the largest proportion (Umeta and Faulks, 1988). After Bultosa, 2002, the mean amylose content of the teff starches varies from 28,2 to 28,4, depending of the method used for the determination, and on the teff variety analysed. Belta *et al*, 2002, they found an amylose content ranges 24,9-31,7 %. The authors of this work we think that this differences are not important to determine the potential of teff as an important component of healthy diets. Amylose determination was made with two different methods. Both methods showed that the amylose content of the teff varieties studied is typical of normal native cereal starches like maize, sorghum and wheat (BETTA *et al*, 2000) with no waxy- or amylo-type starches.

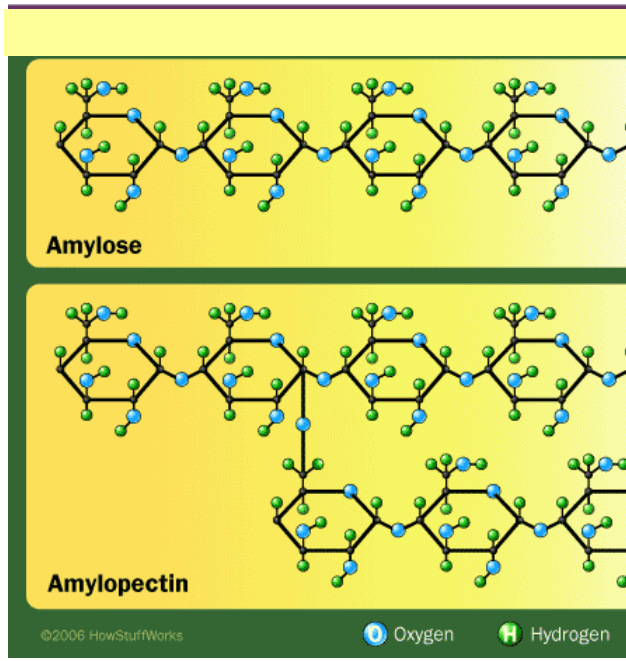

Figure 3.1, Starch components, amylase and amilopectin

During the teff products processing, and depending on the degree of enzyme (Whistler and BeMiller, 1997) or acid treatment, starch can be depolymerised to different types of oligo and mono saccharides (maltodextrins and glucose).

Table 3.1, Composition of starches from teff and maize.

	Teff (five varieties mean)	Maize
Ash (%) (db)	0,16 ± 0,04	0,12 ± 0,03
Protein (%) (N *6.25) (db)	0,19 ± 0,13	0,07 ± 0,01
Crude fat (%) (db)	0,29 ± 0,03	0,34 ± 0,01
Amylose (%)	28,4 ± 2,8	29,5 ± 2,1

Protein: Teff starches had protein contents in the range of 0,16 – 0,23%. Watson, 1998, he found a mean protein content of the teff starches of 0,19%. This is higher than that of maize starch (0,07%). Watson think that this is probably because in commercial maize starch extraction, SO2 is used, which breaks disulphide bonds solubilising protein. The protein content among the teff varieties probably varied depending upon the degree of contamination of the starch by the proteins of the endosperm.

3.1.2. Micro components

As these components are present in very small quantities in the foods, they don't determine their texture or their appearance, but their nutritional value, and its function to help human

body to accomplish it different functions. Micro-components are enzyme cofactors and then, they play an important job in the development of metabolism reactions.

The grain of teff has a very big nutritive value, with a grain protein content (10-12%) similar to other cereals. Besides providing protein and calories, teff is a good source of minerals, particularly iron. It has a very high calcium content and contains high levels of phosphorus, copper, aluminium, barium and thiamine (Yigzaw *et al*, 2001). But the bigger nutritional importance that teff has, is the lack of gluten in the grain. This made it useful for patients with the celiac disease.

The teff starches had ash contents in the range of 0,13 – 0,23%. This is a value comparable to typical cereal starch ash (0,1 – 0,2%) reported by Swinkels, 1985. Phosphorus content is similar to that of rice starch.

In the nutritional point of view, it is interesting to know the opinion of different groups. We exposed what a group of researchers from a USA university found, and what Soil & Crop researchers they exposed.

The nutritive value of teff for livestock fodder is similar to other grasses utilized as hay or ensiled feeds (Boe *et al*. 1986; Twidwell *et al*. 1991). Digestability studies of cell wall contents suggest that teff has tropical grass characteristics (Morris 1980), protein and digestability as forage decreases with increased maturity. Protein content of teff forage produced in South Dakota ranged from a high of 19,5 to a low of 12% as the plant matured. In Montana, teff hay protein content ranged from 13,7 to 9,6%. Protein level (10 to 12%) of teff grain is similar to other cereal grains.

Another point of view that is not favourable to teff, is expressed boy Whistler and BeMiller. The say: teff is used to elaborate a big quantity of foods and beverages. The nutritional value of these foods can be also negatively affected because of starch staling and the possible formation of resistant starch type III on starch retrogradation (Whistler and BeMiller, 1997).

In the other hand, in a study made with the fermentation of a grass pea:teff mixture (8:2), to be used in cattle feed Yigzaw *et al*, 2001, has been found that de aminoacidic composition of he result is comparable to an ideal reference protein recommended for children of 2 – 5 years old.

In the following tables, it appears the teff flour (Eragrain is the flour obtained by Soil & Crop company) vitamin, metal and aminoacid content, and the comparison with the advised daily intake for a 75 kg human.

Table 3.2, A selection of the vitamins in Eragrain® flour.

VITAMIN	Eragrain content (mg/100g)	Advised daily amount for a 75 kg human (mg)	Available in 150 g of Eragrain (%)
(B1) Thiamin	0,51	1,0	76%
(B2) Riboflavin	<0,1	1,5	10%
(B3) Niacin	0,80	16	8%
(B6) Pyridoxin	<0,1	3	10%
(C) Ascorbic acid	0,25	70	1%
(M) Folic acid	<0,02	0,4	8%
Source: WHO (1991), Energy & Protein requirements			

Table 3.3, Amino Acids (mg per 100g) in Eragrain® flour

Amino Acids	Wheat (whole grain)	Eragrain flour	Advised daily amount for a	Available in 150 g Eragrain flour
	www.nutritiondata.com	S&C Research	human 75 kg (mg)	
Isoleucine	508	441	750	88 %
Leucine	926	924	1050	132 %
Lysine	378	327	900	55 %
Methionine (S)	212	433	975	100%
Cystine	317	217		
Phenylalanine	646	601	1050 (incl. tyrosine)	156% (incl. tyrosine)
Threonine	395	449	525	128%
Tryptophane	212	126	263	72%
Valine	618	601	750	120%
Source: WHO (1991), Energy & Protein requirements				

Table 3.4, Nutritional content in Eragrain® flour (mg/100g)

Component	Wheat (whole grain)	Eragrain® flour	Advised daily amount	Available in 150 gram
	www.nutritiondata.com	S&C Research	for a human 75kg (mg)	Eragrain® flour
Water (g)	10,3	10,0		
Energy (kJ)	1419	1468		
Protein (g)	13,7	12,3	75	25%
Fat (g)	1,9	2,1		
Starch (g)	60,0	59,8		
Fibers (g)	12,2	7,9	30	40%
Calcium (mg)	34	167	900	28%
Iron (mg)	3,9	5,7	12	71%
Magnesium (mg)	138	194	420	69%
Potassium (mg)	405	477	3500	20%
Zinc (mg)	2,9	4,6	15	46%
Copper (mg)	0,4	0,8	1,1	104%
Vitamin C (mg)	0	0,3	70	1%
Phytic acid (mg)	800	393		
Source: WHO (1991), Energy & Protein requirements				

Additionally, Soil & Crop staff says: Eragrain® teff contains protein with a good ratio of essential amino acids, but no gluten. People do not need gluten. On the contrary, gluten, present in nearly all food products on the market nowadays, can trigger certain allergies or resistance reactions in our body. Eragrain® teff contains vitamin C and a relative low content of phytic acid, which is the reason why the body can absorb the minerals to a much larger extent. This is useful to prevent diseases like anemia. Scientific research in Ethiopia has shown these results. Uptake of calcium is useful to prevent osteoporosis. Other grains than teff show all this to a much lesser extent.

3.2 Physical

Physical characteristics of a food are the result of the macro components concentration, their relationship and their behaviour under different environment conditions.

The teff starch granule is a compound type from which many simpler (2–6 µm in diameter) polygonal shaped granules are released on milling (Umeta and Parker, 1996). The compound granule surface is smooth, with no evidence of pores. The small granule size of teff starch, when compared with maize starch, was considered as one factor responsible for the considerably lower paste viscosity (peak, breakdown and setback), higher water absorption index and lower water solubility index than maize starch (Bultosa, 2002).

An anatomical study of teff grain has revealed that it contains compound starch granules (Umeta and Parker, 1996), similar to those of rice (Juliano, 1984) and amaranthus. The pericarp of the grain also contains starch granules like in the case of sorghum.

The granule size is thus slightly larger than that of individual amaranthus starch granules, which are 1–2 µm in diameter and comparable in size to individual rice starch granules, which are 3–5 µm in diameter. The starch granules in the different teff varieties appeared morphologically similar to one another. Most of the granules had a number of sides while a few of them had essentially cubic shape. The sides of the starch granules where other starch granules packed were well formed. Most protein bodies are located outside of the compound starch granules (Bultosa *et al*, 2002). The X-ray analysis of teff starch granule gives an A type starch diffraction pattern, apparently more amorphous than maize starch but similar to rice and sorghum starches in crystallinity level. The X-ray diffraction trace of native teff starch granules indicates some of the amylose forms an inclusion complex with the endogenous lipids (LPL or FFA).

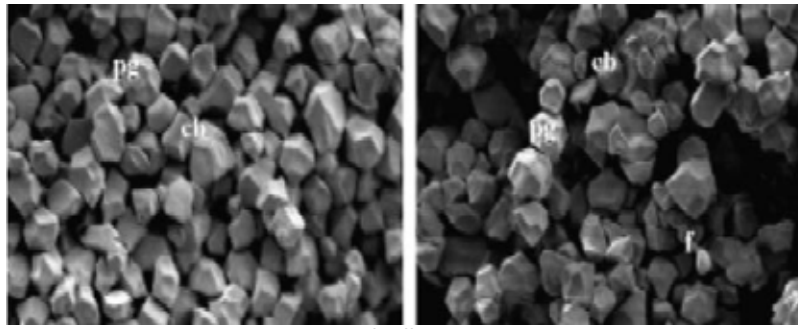

Figure 3.2, Individual starch granules of teff. Where: pg: polygonal shape starch granules. Pb: protein bodies and f: fibre. (From Bultosa *et al*, 2002).

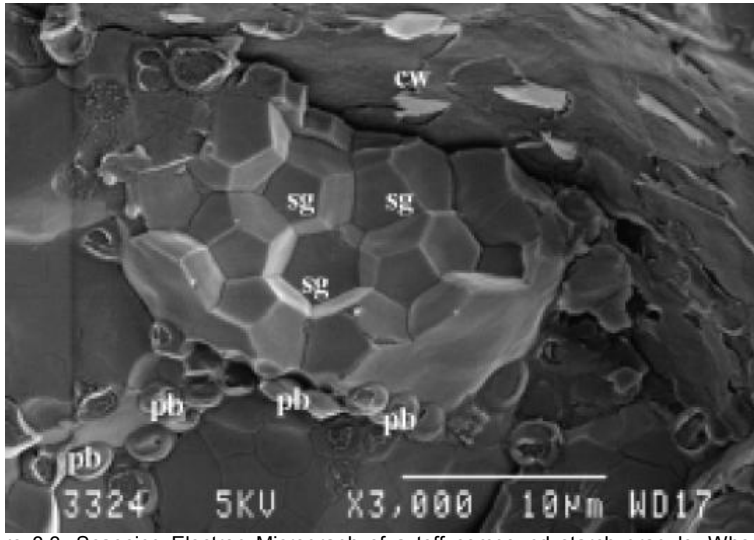

Figure 3.3, Scanning Electron Micrograph of a teff compound starch granule. Where: sg: individual starch granule, pb: protein bodies and cw: cellular wall. (From Bultosa *et al*, 2002).

Pasting properties

This property is very important to know the flour or starch characteristics of a cereal or starchy product. They are useful to predict the behaviour of the flour in baking and brewing process, for example. To determine these characteristics, it is necessary to use a Rapid Visco Analyser, and the main characteristics are:

Ti: Initial swelling temperature. It indicates the minimum temperature required to cook a starch (Newport Scientific, 1995).
PV: Peak viscosity. It indicates water-holding capacity of the starch. The units used are RVU, Rapid Viscosity Unitis, equal to cp*10 Peak viscosity can be affected by granule size (Fortuna *et al*, 2000), molecular structure of amylopectin (Shibanuma *et al*, 1996), cross-linking, starch water concentration, lipids, residual proteins (Li and Yeh, 2001) and RVA operating conditions (Batey and Curtin, 2000).
BV: Breakdown viscosity. The units are the RVU.
Rst: rate of shear thinning.
HPV: Hot paste viscosity.
CPV: Cold paste viscosity. Cold paste viscosity is related to the ability of the starch paste to form a gel after cooling. Gelation occurs with junction zone formation (mostly through hydrogen bonding), re-associating the hydrated and dispersed starch molecules, and can vary with the botanical sources of the starch, amylose content and formation of amylose-lipid complexes, amount of water, other ingredients like proteins and temperature of cooling (Bultosa *et al*, 2002). High-amylose (linear) starches re-associate more readily than high-amylopectin (branched) starches.
SBV: Set back viscosity.
WAI: Water absorption index. WAI is related to the amount and swelling degree of this gel phase. It reflects the extent of association of the molecules within the starch granule (French, 1994).

WSI: Water solubility index. Water solubility index reflects the strength of the micellar network within the starch granules (Qian *et al*, 1998). The leaching of small molecular weight polysaccharides will increase as the micellar network of the starch granules become weak.

The RVA (Rapid Visco Analyser) pasting curves of teff starches and maize starch are given in Fig. . The viscosity parameters evaluated are shown in Tab. 3.5
The mean initial swelling temperature (Ti) for teff starches (74,0 °C) was virtually identical to that of maize starch (74,1 °C) (Tab.3.5), but apparently higher than that of sorghum starch.
The mean peak viscosity (PV) (269 RVU) of the teff starches was considerably lower than for maize starch (313 RVU). Small granule size was positively correlated with resistance to swelling, less swelling and less peak viscosity in wheat, potato and maize native starches (Fortuna *et al*, 2000) and this may apply to the case of teff starch. Teff starches took longer time (mean Pt 4,19 min) to reach PV than the maize starch (mean 2,90 min).

Figure 3.4, Rapid Visco Analyzer.
From:http://images.google.nl/images?hl=en&q=Rapid+Visco+Analyzer&btnG=Search+Images&gbv=2

Table 3.5, Pasting properties of starch from teff and maize

Parameters	Mean teff varieties	Maize
Ti	74,0 ± 1,1	74,1a ± 0,1
PV (PVU)	269 ± 13	313d ± 2
Pt (min)	4,19 ± 0,62	2,90a ± 0,04
HPV (RVU)	190 ± 13	184b ± 2
BV (RVU)	79 ± 17	129e ± 3
Rst (RVU/MIN)	8,4 ± 1,8	12,2c ± 0,3
CPV (RVU)	292 ± 14	344c ± 4
SBV (RVU)	101 ± 11	161c ± 6

The mean breakdown viscosity (BV) for teff starch pastes (79 RVU) was considerably lower than that of the maize starch paste (129 RVU). At BV, the swollen granules disrupt further and amylose molecules will generally leach out into the solution and align in the direction of the shear (Whistler and BeMiller, 1997).

The rate of shear thinning (Rst) for all the teff starches (mean 8,4 RVU/min) was lower than that for maize starch (12,2 RVU/min). The degree of Rst is reported to be influenced by the structural network of starch molecules, morphology and rigidity of the swollen starch granules (Subramania *et al*, 1994), and starch granule associated proteins (Han *et al*, 2001). Higher resistance of teff starch to Rst compared to maize starch is an indication of inherent lower granule deformability and swelling, since these were positively correlated to Rst resistance in other native starches (Whistler and BeMiller, 1997).

The cold paste viscosity (CPV) of all the teff starches (mean 292 RVU) was considerably lower than that of the maize starch paste (344 RVU). However, amylose-lipid complexing reduces re-association to some extent (Whistler and BeMiller, 1997). Teff starches showed a slight trend in their CPV, vrs. higher amylose contents giving higher CPV.

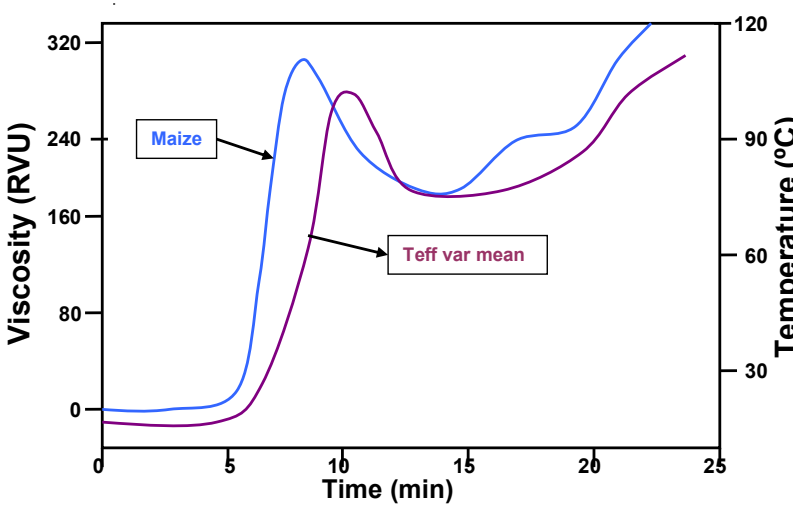

Figure 3.5, RVA pasting curves of starches from teff and maize. (Adapted by the writers, from Bultosa *et al*, 2002)

The setback viscosity (SBV) of all the teff starches (mean 101 RVU) was considerably lower than that of maize starch (161 RVU). The higher the SBV, the more syneresis is likely to take place (Whistler and BeMiller, 1997). A preliminary observation on gel syneresis indicated that teff starch showed slower syneresis than maize starch.

The water absorption index (WAI) of all the teff starches (mean 108%) was considerably higher than that of maize starch (86%) (Table 3.6). The higher WAI of teff starch is probably also related to its smaller granule size. The smaller the granular size of starch, the larger the bulk surface area and the higher the water absorption. The high WAI of teff starch possibly also contributes to the high volume of enjera made from teff flour, since from the same weight of teff, maize, sorghum, wheat and barley flours, more enjera is obtained from teff flour than from the other flours (personal communication of Bultosa *et al* from teff improvement program of the Debre Zeit Agricultural Research Centre, Ethiopia)

Table 3.6, Gelatinisation temperature, Water Absorption Index (WAI) and Water Solubility Index (WSI) of starches from teff and maize.

	Teff (five varieties mean)	Maize
Granule size (µm)	2-6	5-30
Gelatinisation temp. (ºC) (To-Tp-Tc)	68,0-74,0-80,0	65,0-73,0-80,0
WAI (%) (db)	108 ± 4	86 ± 2
WSI (%) (db)	0,34 ± 0,08	0,98 ± 0,06

To be onset, Tp is peak and Tc is conclusion gelatinisation temperatures; amylose [%]by the Con A method of Gibson *et al*. and amylose [%]by the iodine binding of Chrastil.

Abstract made by the writers of this work, from Bultosa *et al*, 2002

Mean onset (To), peak (Tp) and conclusion (Tc) gelatinization temperatures of teff starches were 68,0, 74,0 and 80,0 °C, respectively (Table 3.6). For the maize starch the values were 65,0, 73,0 and 80,0 °C, respectively. The teff starch gelatinisation temperature is thus similar to that of tropical cereal starches and resembling most closely that of rice starch (68,0, 74,5, 78,0 °C). The range is somewhat narrower when compared to that for maize starch. Starch gelatinisation is an irreversible process and includes granule swelling, native crystallite melting, loss of birefringence and starch solubilisation.

The WSI of all teff starches (mean 0,34%) was considerably lower than that of maize starch (mean 0,96%) (table 3.6).

Aerodynamic properties

Teff threshing is carried out in Ethiopia by trampling over the cut crop collected on a flat surface with oxen. Separation of teff grain is carried out by throwing the grain and material out of grain mix in air using the difference in aerodynamic properties. Cleaning is performed by manually wafting air over the grain chaff mix with a dried hard leather strap (Zedwu, 2007).

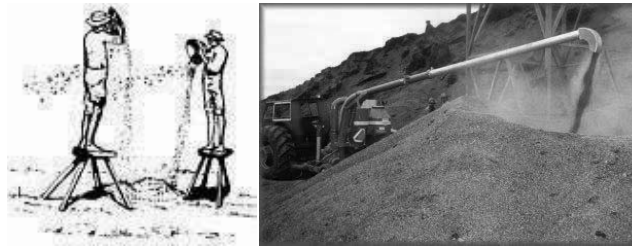

Figure 3.6. Small and medium level grain cleaning. From www.cd3wd.com/.../X0027S/ES/X0027S04.HTM

Bultosa and Taylor commented on the possibility of using a combine harvester for harvesting of teff, but they added that teff grain losses can be high due to the very small size and light mass of the grain. The equivalent diameter of teff grain was reported to vary between 0,71 and 0,87 mm and thousand grain mass 0,257–0,421 g (Zewdu & Solomon, 2007).

By defining the terminal velocity of different threshed materials, it is possible to determine and set the maximum possible air velocity in which material out of grain (MOG) can be

removed without loss of grain (Zewdu, 2004; Freye, 1980) or the principle can be applied to classify grain into different size groups (Wu *et al*, 1999).

It is necessary then, to determine the aerodynamic properties of teff and straw in order to apply this information in the design and construction of equipment to harvest, clean, transport and store this grain. The two important aerodynamic characteristics of a body are its terminal velocity and aerodynamic drag.

During the separation of threshed materials the fundamental forces involved are the weight of the particle and the aerodynamic drag. Aerodynamic drag force is a function of the relative velocity of the particle with air (vr), the density of air (ra), and the size of the particle as expressed by its frontal area (Af). The drag force is related to properties of the particles and of the fluid through the following relationship (Mohsenin, 1986):

$$F_D = \frac{1}{2} C_D \rho_A A_f v_r^2.$$

Grain crop materials are usually small and irregular in shape, which makes direct drag force measurements difficult. In order to predict drag coefficients the usual approach is to define the shape of the material rough sphericity (Gorial & O'Callaghan, 1990). This is the ratio of the surface area of a sphere equal in volume to that of the true surface area of the grain. Once sphericity is established the drag coefficient can be established using relationships proposed by different researchers.

Zedwu found that the terminal velocity of teff grain increased linearly from 3,08 to 3,96m/s as shown in Fig. with increase in moisture content from 6,5% to 30,1% w.b. The resulting drag coefficient of teff grain decreased from 0,83 to 0,65 with an increase in moisture content from 6,5% to 30,1% (Fig.). Both terminal velocity and drag coefficient were linearly related to moisture content as shown in:

vt = 0,0363μ + 2,8858. (7)
CD =0,0074μ + 0,8627 (8)

with R2 values of 0,98 and 0,96, respectively. The decrease in drag coefficient is related to the increase in mass and as well increased frontal area. The range of drag coefficients showed that the teff grains behaved more like spheres when they had higher moisture content. A linear increase in terminal velocity against moisture content is reported with most terminal velocity measurements of seeds.

As a reason for the increase in terminal velocity, with moisture content, different authors cited the increase in mass per unit frontal area.

Terminal velocity and drag coefficient of teff straw

Short straws are the major contaminant of threshed materials. They are also very difficult to separate from the grain (Freye, 1980). Analising Zedwu results, it is possible to observe an overlap in terminal velocities between grain and straw materials of teff. As a result, complete pneumatic separation is not possible. In most cases the terminal velocities of end node straws were greater than that of teff grain. This is likely due to the small size and weight of teff grain.

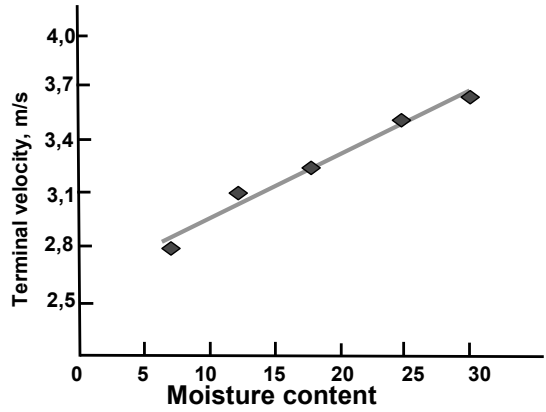

Figure 3.7, Effect of moisture content on terminal velocity of teff grain

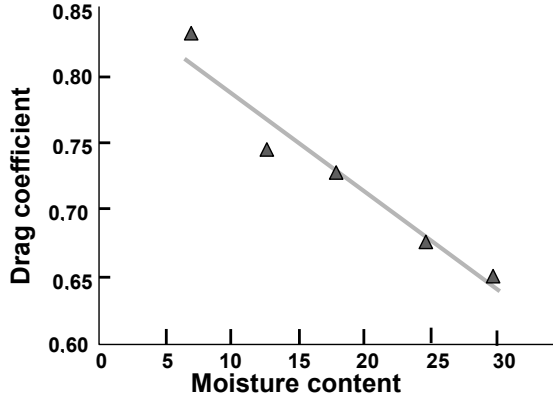

Figure 3.8, Effect of moisture content on drag coefficient of teff grain

Processing modification of physical properties.

In all the food and beverage products in which teff are used, the starch granules are structurally transformed (Bultosa and Taylor, 2004).
During the baking of enjera, starch is completely gelatinised to form a steam-leavened, spongy textured matrix, in which fragments of bran, embryo, micro-organisms and organelles are embedded. Gelatinised starches have a tendency to retrograde, which can affect the texture and shelf-life acceptability of foods (Whistler and BeMiller, 1997). The smaller setback and low cold paste viscosity of teff starch compared to maize starch is an indicator of slow retro gradation tendency which might have a positive role in respect of storage stability of food products made with teff starch.

The differential scanning calorimetry (DSC) gelatinisation temperature is similar to that of other tropical cereals. The lower swelling power, apparently lower percentage crystallinity and lower DSC gelatinisation endotherms compared to maize starch suggest the degree of crystallinity in the teff starch is less and the proportion of long amylopectin, a chain is probably smaller (Bultosa, 2003).

Gelatinisation temperature range was 68,0–74,0–80,0 °C, typical of tropical cereal starches, and resembling the temperature range of rice starch.

Figure 3.9, Differential scanning calorimeter.
From:http://content.answers.com/main/content/wp/en-commons/thumb/c/c8/288px-Differential_scanning_calorimeter.jpg

The mean intrinsic peak viscosity (269 RVU), breakdown viscosity (79 RVU), cold paste viscosity (292 RVU) and setback viscosity (101 RVU) determined were considerably lower than that of maize starch. Teff starch has higher water absorption index (WAI) (mean 108%) and lower water solubility index (WSI) (mean 0,34%) than maize starch.

Matrix change of starch was reported to be a major contributor to the texture of enjera (Parker et al, 1989). During the baking of enjera, starch is completely gelatinised to form a steam-leavened, spongy matrix, in which fragments of bran, embryo, micro-organisms and organelles are embedded.

Bultosa *et al*, they think that the observed narrow gelatinisation temperature range for teff starch is probably related in part to its relatively more uniform granule size distribution (2–6 µm in diameter) as compared with maize (5–30 µm in diameter) (Whistler and BeMiller, 1997), because in granules of wide size range like wheat (2–55 µm in diameter, 52 – 85 °C) and barely (0,9–44,9 µm in diameter, 52,0 – 69,7 °C) (Tang *et al*, 2001) the range is broader.

Effect of chemical environment on teff physical properties.

An effective utilization of a new plant protein in food product formulation demands that its food properties be investigated in order to find out whether such supplement possesses the appropriate functional properties for acceptable food application (Akintayo *et al*, 1999; Chavan *et al*, 2001). In addition to acceptable functionality, the development in food

industries in the use of certain acids, alkali and salts in food formulation should be given a due consideration since among their factors, physiochemical environment (pH, ionic strength, presence or absence of surfactants), affect structural composition, interfacial tension and energy of binding of food constituents, and in turn their functional performances (Chavan *et al*, 2001; Philips *et al*, 1991; Yadav, 2002). These acids, alkalis and salts are added to foods for a number of reasons: to improve nutritive values; control of acidity and alkalinity; production of entrapped gas to initiate dough expansion; as leaving, preservative and flavouring agents (Gimono, Astiasaran & Bello, 1999; Arongudade, 2005). Even though, Arongudade made a study on this teff functional properties, and he determines these functional properties and the effect of CH_3COOH, $NaHCO_3$, $CaCO_3$ and NaCl commonly employed in food processing on such properties.

Least Gelation Concentration (LGC) of Eragrostis teff protein concentrate (ETPC) in distilled water (control) was 6%, which is the same as that of bovine plasma protein concentrate, but lower than soy flour and soy protein. The change in chemical environment had different effect on ETPC gelation. Increase in the concentration of NaCl from 0,05 to 1,5 M gradually improved the LGC from 6 to 2%. Saturated solution of $NaHCO_3$ also improved the LGC to 4%. But 0,05–1,5 MCH_3- COOH and saturated solutions of $CaCO_3$ had no effect on the LGC (Arongudade, 2005). The value LGC is taken as an index of gelation capacity (http://images.google.nl/imgres?imgurl=http://content.answers.com/main/content/wp/en-commons/thumb/c/c8/288px)

If the chemical environment is changed, the foaming capacity (FC) of ETPC is affected. About two and half fold increase was observed in FC as the concentration of NaCl increased from 0,0 to 0,5 M and later decreased at higher concentration (1,5 M) to 9,7% (this was however still better than what was obtainable in distilled water—6,5%) (Arongudade, 2005). A foam depression of 5,0 to 4,2% was observed in CH_3COOH as concentration increased from 0,05 to 1,0 M, respectively. This was later increased to 6,3% in 1,5 M CH_3COOH. Saturated solutions of $NaHCO_3$ and $CaCO_3$ gave an FC of 15,5 and 9,0%, respectively. The results are displayed in fig.

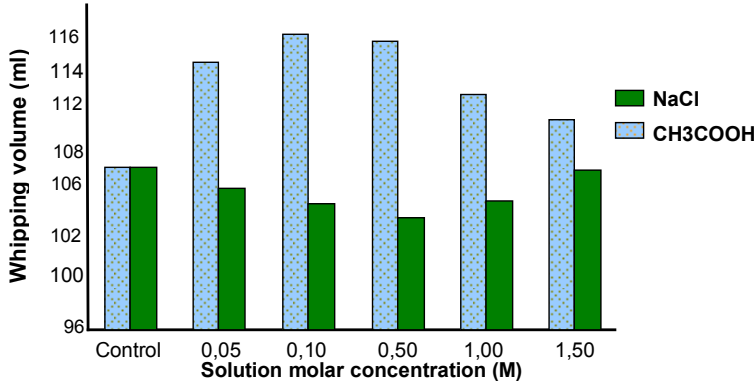

Figure 3.10, Foaming capacity of ETPC in different concentration of NaCl and CH_3COOH. From Arongudade.

Since chemical environment that are able to aid protein solubility and diffusion to the interface do improve FC (Oshodi & Ojokan, 1997), Arongudade concluded that the ability of CH_3COOH, $NaHCO_3$, NaCl and $CaCO_3$ to initiate protein solubility and diffusion in foam formation are in the order of $NaCl \approx NaHCO_3 > CaCO_3 > CH_3COOH$.

3.3 Microbiology

The principal use of teff, is in enjera production, a flat bread, big and thin, that constitutes the 70% of Ethiopians diet. The preparation includes a fermentation step, that gives enjera their sensorial characteristics, as flavour, aroma and colour. But the more important effect of teff fermentation is an increase in the nutritional content, because the decreasing of the relationships Iron:Phytates and Iron:Tannins.

Ersho is a clear, yellow liquid that accumulates on the surface of fermenting teff-flour batter and is collected to serve as an inoculum for the next fermentation. The pH of ersho samples was about 3,5 and titratable acidity ranged between 3,1% and 5,7%. Mean yeast counts ranged between 5,2x105 and 1,8x10^6 cfu/ml and comprised, in order of abundance, Candida milleri, Rhodotorula mucilaginosa, Kluyveromyces marxianus, Pichia naganishii and Debaromyces hansenii. Candida milleri was the most dominant isolate in all samples. Other authors, they found as the principal responsible of teff fermentation Candida guillermondi. About 90% of the teff flour samples had aerobic mesophilic counts ≥ 105 cfu/g and Gram-positive bacteria constituted about 71% of the total isolates. About 80% of samples had Enterobacteriaceae counts of 10^4 cfu/g. Before of the Ashenafi work, Gifawossen and Bisrat (1982) were able to isolated Candida and Pichia from ersho.

Figure 3.11, Injera, with a vegetables sauce. From www.globalnomad.net/.../print_quick%20facts.htm

The preparation of teff enjera consists of two stages of natural fermentation, which last for about 24 to 72 h, depending on ambient temperatures. The only required ingredients are the teff flour and water. Inoculation is accomplished by consistently using a partially-cleaned fermentation container and by adding some ersho. This ersho contains 96,4% moisture, 0,05 mg riboflavin/100 g, and 0,4 rag niacin/ 100 g (Steinkraus 1983). About 480 g ersho is added to 3 kg teff flour and 6 1 water. The various traditional teff threshing processes mean that teff flour probably contains a very wide variety of soil and faecal micro organisms.

Ashenafi concluded that the very low pH values of the ersho (about 3,5), consistently recorded for all samples, and would be inhibitory for most kinds of micro organisms. The absence of members of Enterobacteriaceae or lactic acid bacteria, which were reported to be important in teff fermentation (Gashe *et al*. 1982), indicated that the ersho could not serve as a source of these bacteria (Ashenafi, 1994).

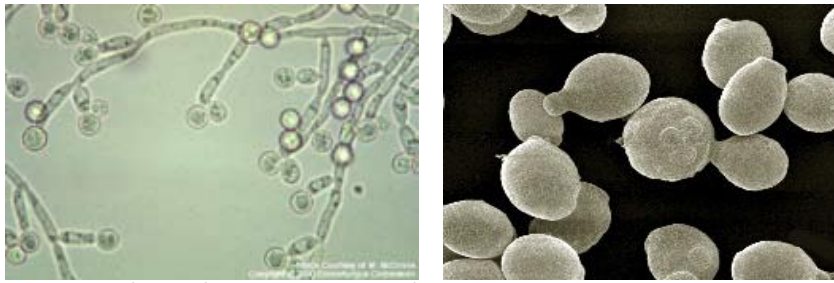

Figure 3.12, Some of the microorganisms found in ersho, and that participate in the teff fermentation process. *Candida* and *Pichia*.
From: http://www.jgi.doe.gov/education/bioenergy/Pichia_stipitis_JGI.jpg

Althout Candida milleri is the most abundant yeast found by Asehenafi, in the different households analysed, he could found different Candida species. This variation may be one of the reasons why enjera produced at one household at different times or in different households could have different flavours (Ashenafi, 1994).

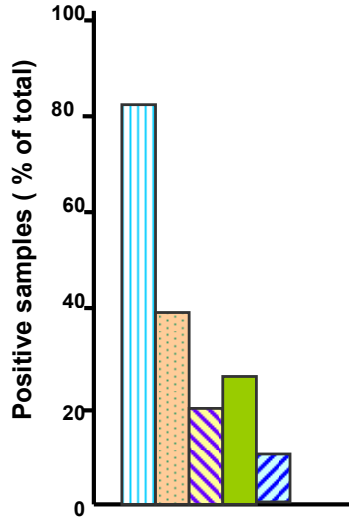

Fig. Distribution of *C. milleri* ( ), *R. mucilaginosa* ( ), *K. marxianus* ( ), *P. naganishi* ( ) and *D. hansenti* ( ) in ersho samples, collected from four households.

Figure 3.13, Microbiological ersho composition, adapted by the authors from Ashenafi, 1994.

The physiological properties of the yeast isolates in this study indicated that the Candida and Kluyveromyces species were active gas producers from glucose, sucrose and a variety of

other sugars. Pichia naganishii and Debaromyces hansenii produced a certain amount of gas from glucose only. The starch is not utilized for fermentation. That means that these micro organisms are not able to hydrolyse the starch molecule. These species could only be important in the fermentation of teff then fermentable sugars are available after the degradation of teff starch (Ashenafi, 1994).

The second most frequently isolated species, R. mucilaginosa, was fermentative inactive and may not therefore be important in leavening the batter of teff.

Since ersho is the clear yellow liquid decanted from the batter at the end of the primary fermentation the yeast flora isolated from ersho, even those which were fermentative very active, may not be important as starters in the initiation of teff fermentation.

Another source of inoculums for teff fermentation could be the teff flour itself. The traditional threshing processes of teff result in the contamination of the teff seeds with a very wide variety of soil and faecal material (Gashe *et al*, 1982; Steinkraus 1983). Gram-positive bacteria dominated the aerobic flora of teff in Asehnafi study and among these, micrococci and Bacillus spp. could be important in the initiation of the fermentation until members of Enterobacleriaceae could reach a large enough number to make any marked contribution to the fermentation, i.e. about 12 h (Gashe 1985). The high counts of Bacillus spores in ersho and Bacillus and Micrococcus spp. in teff flour may contribute markedly to the fermentation of teff flour. In the absence of acid-forming lactic acid bacteria, micrococci may acidify the flour-and-water paste or Bacillus may grow, producing lactic acid, gas, alcohol, acetoin and small amounts of esters and aromatic compounds (Anon. 1980). Controlled experiments are, however, needed in order to determine the actual role of Micrococcus and Bacillus spp. in the initiation of teff fermentation. The low numbers of moulds in the teff examined in this study (103 to 104/ml) are in agreement with the reports of Hobbs & Greene (1976). Moulds, however, may not be important in such acidic fermentations.

Teff contains 2,7 g/ lOO g DM of free sugars, predominantly sucrose (95%). Fermentation initially increased the amounts of free sugars; thereafter the total fell. The changing pattern of free sugars during fermentation was due to changes in the microbial population dynamics resulting from changes in dough pH. Fructose was found to be the principal free sugar in the fermenting dough and cooked product. After 72 h fermentation, the microbial population had utilised 9% of the starch. The non-starch polysaccharides (NSP) (dietary fibre) were inaffected (Umeta and Faulks, 1987).

All the cereals produced in Ethiopia present big quantities of mycotoxins, and teff is not the exception. Ayalew *et al*, 2006 detected Aflatoxin B1 (AFB1) at concentrations ranging from trace to 26 µg/kg. Ocharatoxin A was detected at a mean concentration of 54,1 µg/kg and a maximum of 2106 µg/kg (Ayalew, 2006).

The consumption of diets based on cereals and legumes but poor in animal products can lead to deficiencies of zinc and iron (Umeta et.al, 2007). However, since fermentation can decrease the phytate content by a factor of 3–4, traditional household practices such as fermentation need to be encouraged to address the problem of zinc deficiency, which is particularly prevalent in Ethiopia (Umeta et. al, 2007).

4 Medical aspects and teff benefits

Teff grain is a very small grain. This made that the flour consists the bran and the germ. This makes teff flour high in nutrient value, because the bran and germ are the most nutritious parts of any grain. Teff has a very high calcium content, and contains high levels of phosphorous, iron, copper, aluminium, barium, and thiamin. It is considered to have an excellent amino acid composition, with lysine levels higher than wheat or barley and slightly less than rice or oats (Stallknecht, 1997). Teff is high in protein, carbohydrates, and fibre. The protein composition offers an excellent balance among the essential amino acids (Yu, 2006). It contains no gluten so it is appropriate for people with gluten intolerance (Stallknecht, 1999).

While the reported high iron content of teff seed has been refuted, the lack of anemia in Ethiopia is considered to be due to the available iron from enjera (Mamo and Parsons 1987). Teff is the main staple in the northern, western and central parts of the country (Umeta, 2007). Some scientists think that the high results about teffs iron content are due to ferruginous soil ground into the outside surface of the grains. That's why Sukian and Pittwell, 1968, they decided to check the iron content of teff once more.

It is concluded from these results that the true iron content of the actual dirt-free teff grain is about 0,0033%. However Melaks has obtained higher values than Almgdrd using teff fresh from the plant, threshed in the laboratory (Sukian and Pitwell, 1968). This may mean either that the iron content is very variable, that Melak's sample was contaminated by wind-blown dust embedded in the grain wall, or that the outer seed wall as distinct from the husk is richer in iron than the central grain.

On the other hand, iron actually embedded in the grain walls must be considered to be a dietary source of iron along with the actual true iron content of the grain itself.
Zinc and iron are two of the micronutrients that are most often deficient in developing countries. Iron deficiency is the most important cause of nutritional anemia. This arises from the low bioavailability of non-haem iron (Hallberg and Hulthén, 2000) caused not only by phytate but also tannins in the diet. Phytic acid, which is present in significant amounts in the seed coat of cereals and legumes (Umeta *et al*, 2007) exerts its inhibitory effect on the absorption of zinc and iron by forming insoluble complexes in the gut under physiological condition (Wise, 1995). The formation of such chelates depends on the ratio of the content of zinc, iron or calcium relative to that of phytate in the food. Other minerals of nutritional importance that are chelated by phytate are copper and manganese (Wise, 1983; Hallberg *et al*, 1987).

Different authors have demonstrated than during the teff paste fermentation, the phytate:iron molar ratio decrease. That's why, Ethiopian scientists and nutritionist agree about the need to improve the practice to ferment teff before used in enjera production. Given the high iron content and the relatively favourable phytate:iron molar ratio, teff enjera was the best source of bioavailable iron of all foods analysed by Umeta *et al*, 2007, between the Ethiopian foods.

In the following table, Thompson, 2001, presents de metal concentrations in teff. The authors of this work calculated the phytate:iron (p/p) ratio.

Table 4.1, Metal concentrations in teff

Food type (n)	Zinc (mg/100 g)	Iron (mg/100 g)	Calcium (mg/100 g)	Phosphorus (mg/100 g)	Phytate (mg/100 g)	Tannin (mg/100 g)	PHYT/IRON (p/p)ratio (*)
Teff injera, unfermented (4)	1,41±0,30	30,3±3,0	62,7±0,4	179±9	389±10	60,1±6,2	12,84
Teff injera, fermented (5)	1,16±0,20	34,7±4,1	61,4±3,1	164±8	126±8	49,8±4,2	3,63
Maize injera, unfermented (5)	0,88±0,10	4,2±0,7	19,2±2,1	135±7	282±6	64,6±4,7	67,14
Sorghum injera, unfermented (6)	0,91±0,21	9,2±2,1	13,2±1,4	115±8	325±12	53,2±5,1	35,33
Sorghum injera, fermented (6)	0,74±0,21	8,1±1,7	11,2±1,9	102±9	75±2	49,8±4,1	6,15
Wheat injera, fermented (5)	1,50±0,32	3,5±0,8	23,1±2,1	188±7	137±9	21,2±2,3	39,14
Maize bread (4)	1,10±0,30	5,2±1,2	8,3±1,4	176±8	411±12	50,3±6,4	79,04
Sorghum bread (2)	0,69±0,20	6,8±0,2	13,1±1,9	109±4	296±7	83,0±2,0	43,53
Wheat bread (5)	1,60±0,24	5,4±1,2	23,1±3,1	182±9	542±11	23,3±3,2	100,37
Maize porridge (6)	0,60±0,20	3,6±1,2	10,2±1,3	149±5	205±9	23,9±3,7	56,94
Sorghum porridge (4)	0,69±0,13	6,3±1,3	9,2±1,2	101±6	237±7	111,5±2,5	25,76
Maize, boiled (6)	1,27±0,23	3,5±0,7	12,1±1,2	184±7	344±11	16,9±1,4	28,43
Sorghum, boiled (3)	0,63±0,05	3,6±0,8	11,2±1,0	94±3	272±8	121,7±2,6	75,56

(*) Calculated by the authors from the Thompson information.

Analysing this table, it is evident that teff enjera has a bigger iron content that other ethiopian foods, but it has a high phytic acid content too, even when the fermentation is applied.
It is evident too the big change caused by fermentation process on the phytate:iron p/p ratio, calculated by the authors of this work. In the particular case of teff, this value decrease 4 times, after the fermentation. In the sorghum case, the value decrease 5 times.
Enjera made from fermented paste, is the bread with the lower phytate:iron p/p ratio. It is easy to understand why, nutritionists in Ethiopia are promoting the practice to ferment teff, before to use it in enjera production.

Presently, there is general agreement that wheat, rye, and barley are harmful, and rice and corn harmless to people that has to follow a gluten-free diet. The acceptability of many other plant foods as amaranth, quinoa, buckwheat and teff continues to be debated (Thompson, 2001). The different institutions related with nutrition and health has thousands of contradictions. Some of the authors involved in this kind of discussion, they say that a lot of investigations were subject to significant methodological limitations: small study populations, short periods of investigation, and/or no available tests to measure the direct effect of oats and other cereals on the intestinal mucosa. There is not a matter to have doubts that humans need to continue with the research in this important topic.

After the Thompson point of view, many gluten-free cereal foods (eg. bread, pasta, cold cereal) are made from refined flour and/or starch and most are not enriched with iron and B vitamins. As a result, a gluten-free diet may contain inadequate amounts of fibre, iron, thiamin, riboflavin, niacin, and folate. Then, if we know that teff has a high iron content, a good amino acid composition and a lack of gluten protein it is evident that its addition in the products developed to patients with celiac disease, increase the nutritional value to this patient's gluten-free diet.

Presently, the only effective treatment for celiac disease is a life-long gluten-free diet. But Rizello *et al*, 2007, they used a new mixture of selected sourdough lactobacilli and fungal proteases to eliminate the toxicity of wheat flour during long-time fermentation. Albumins, globulins, and gliadins were completely hydrolyzed, while ca. 20% of glutenins persisted. The

kinetics of the hydrolysis dough made by lactobacilli were highly efficient (Rizello *et al*, 2007). People that are thinking to introduce cereals as teff, oats, buckwheat, rice, etc. in the diet of celiac disease patients, as the only possibility of this population, has to consider this wheat treatment alternative. This is a topic that can be considered, has to be included in a big "nutrition, health and food processing project". Obviously, the use of teff and other grains as buckwheat, amaranth, quinoa, and oats to elaborate breads, pancakes, cookies and other bakery products, is a practice that found a lot of adversaries: people that work in the wheat flour industry. They say the following: the riboflavin content of quinoa, niacin content of buckwheat flour, thiamin content of oats and iron content of teff compare favorably to that of enriched wheat flour. They add: this other grains might be harmful because no clinical feeding trials have been conducted to demonstrate their safety. These kinds of discussions are not possible to avoid when big groups or companies feel that their economic interests can be risked. But the authors of this work think that the more important issue is to offer to the different human groups, healthy and natural foods.

4.1 Celiac disease

Celiac disease (CD) is an inflammatory disorder of the upper small intestine triggered by the ingestion of wheat, rye, barley, and possibly oat products. The clinical feature of CD is characterized by the destruction of the microscopic finger-like projections of the small intestine that are called villi (Morón *et al*, 2008). Consequently, large proline and glutamine rich peptides are cumulated in the small intestine and reach the subepithelial lymphatic tissue, results in mal absorption of nutrients. These characteristics may vary from patient to patient, depending on the severity and extent of the disease (Wieser and Koehler, 2008).

Previously, CD was considered to be a rare childhood disorder but it is actually a frequent condition present in every age. CD is mostly prevalent in Europe and countries to which Europeans have emigrated, including North America, South America, and Australia.
However, CD is increasingly found in areas of the developing world such as North Africa, Middle East, and India (Wieser and Koehler, 2008). Then, nowadays it is recognized to be universally distributed, to include all races, with an estimated prevalence of 1–2% in the general population (Cabrera *et al*, 2008).

The principal toxic components of wheat gluten belong to a family of closely related proline and glutamine rich proteins called gliadins (Mendoza, and Mc Gough, 2005). The currently accepted theory of pathogenesis is based on a primary immune response to gluten proteins as antigens and to tissue transglutaminase (TG), which has been identified as an autoantigen (Wieser and Koehler, 2008). The high Pro content renders these proteins resistant to complete proteolytic digestion by gastrointestinal enzymes.

A peptide from α-2 gliadin has been identified as a principal contributor to gluten immunotoxicity. It was identified as the peptide LQLQPFPQPQLPYPQPQLPYPQPQLPYPQPQPF. Homologs of this peptide were found in all food grains (except oats) that are toxic to CD patients, but they were absent in all nontoxic food grains (Moron *et al*, 2008).

There are two major subclasses of flowering plants: monocots (one seed leaf) and dicots (two seed leaves). Wheat, rye, and barley (the grains generally regarded as harmful to persons with CD) are monocots. Buckwheat, amaranth, and quinoa are dicots a very distantly relate to grains in the monocot subclass. As a result, it is highly unlikely that buckwheat, amaranth, and quinoa would contain the harmful amino acid sequences found in wheat (Kasarda, 2001).

On the basis of plant taxonomy and amino acid sequencing, it has been suggested that millet, sorghum, Job's tears, teff, and ragi are more closely related to corn than to wheat (Thompson, 2001). Thus, if corn do not contain harmful aminoacid sequences—which is the general consensus—then other grains (eg,millet,sorghum, Job's tears, ragi, teff, wild rice) similar in chemical makeup to rice and corn are also unlikely ton contain harmful sequences (Kasarda, 2000).

In the search to found articles about teff in relation with CD there is found one interesting article. The article is written by E. Hopman *et al*, 2007. The title of the research is "Teff in the diet of celiac patients in The Netherlands".

The objective of this research is to investigate whether the naturally gluten-free cereal Eragrostis teff (teff) is associated with health problems when used by CD patients.

The results of this research showed that teff is used by 66% of the CD patient that participate the research. Also 76% of the healthy family members consumed teff. In this research population there are significantly more female CD patients.

61% of the CD patients with a gluten free diet (GFD) which do not consume teff reporting symptoms of CD. This percentage was comparable with the percentage of symptoms reported by teff users before teff was introduced into their GFD. However, a significant reduction of symptoms from 58% to 17% was reported after adding teff to the GFD. The patients who get CD symptoms after eating teff reported that the symptoms are fewer and significantly shorter in duration than before.

The patients who reported symptoms after eating teff were significantly older and they also reported significantly more symptoms before the teff was introduced in their GFD.

So their can be conclude that teff has a good influence on the healthiness of CD patients. The CD patients who are using teff reported a significant reduction in symptoms. This is possibly related to a reduction in gluten intake or to an increase in fiber intake. Hence, teff can be a valuable addition to the GFD of CD patients.

4.2 Iron

4.2.1. Anemia

Anemia, also called “iron poor blood”, is a condition in which a person’s blood has a low numbers of red blood cells (RBC), or the RBCs don’t have enough haemoglobin. Haemoglobin is an iron-rich protein that gives the red colour to blood and carries oxygen from the lungs to the rest of the body. The body needs iron to make the haemoglobin. In people with anemia, the blood does not carry enough oxygen to the rest of the body. As a result, people with anemia feel tired, along with other symptoms, because their bodies are not receiving enough oxygen. In severe or prolonged cases of anemia, the lack of oxygen in the blood can cause serious and sometimes fatal damage to the heart and other organs of the body.

A low Iron content is not the only cause of anemia. A shortage of folic acid or vitamin B12 can also be the cause of anemia.

Women and people with chronic diseases are at greater risk for anemia. Many types of anemia can be mild, short-lived, and easily treated. Some forms of anemia can be prevented with a healthy diet, and other forms can be treated with diet supplements.

Certain types of anemia may be severe, long-lasting, and life threatening if not diagnosed and treated. People who have symptoms of anemia should see their doctor to find out if they have the condition, its cause and severity, and how to treat it.

While Ethiopian people are eating a lot teff, which is rich of iron it is probable to think that Ethiopian people do not have iron deficient. Unfortunately this is not true.

It is estimate that 36% of the developing world’s population suffers from anemia. Preschool children in Africa, which includes Ethiopia, have some of the highest rates of anemia in the world— nearly 56%(United nations). In Ethiopia, the magnitude and importance of iron deficiency anemia as a public health problem is still disputed. Some studies reported iron deficiency anemia rates of less than 18% while others have reported rates of 25% and above.

In several developing countries the intake of iron from diet is more than adequate. For example, in parts of Ethiopia, the daily intake of iron is estimated to be between 180 and 500 mg day which is 10–20 times the suggested daily requirement. This presumed high intake is attributed to consumption of teff. In spite of this high intake of iron, some studies have reported a high prevalence of anemia, even in teff-consuming communities (Zein *et al*, 1987, & ministry of health Ethiopia, 1987). Therefore, the cause of iron deficiency in Ethiopia may not be the inadequate dietary intake of iron. Other factors, ultimately related to poverty and underdevelopment, might also play a role in iron deficiency anemia (Foy *et al*, 1960, Layrisse *et al*, 1964). In such communities with an already high intake of iron, the conventional supplementation of iron might not be effective or might even be harmful. Therefore, all important risk factors have to be identified and their role in causing anemia evaluated. The objective of this study is to identify these risk factors and assess their role in anemia.

The haematocrit results were available for 2.080 children. The mean haematocrit was 35,4 ± 4,8%. 42% of children were anemic, largely due to iron deficiendy. From those children 83,0% ate enjera in the 7 day’s before the specification of the blood values. Only 4% of the study children had an iron intake of less than their daily Recommended Nutrient Intake (RNI).

But however the children has a high iron intake, the intake of meat and other foods rich in ascorbic acid, which improve the absorption of non-haem iron, were rarely consumed (Mahiou *et al*, 1992).

There can be concluding that teff contains a high level of iron. This means that the most people in Ethiopia eat their daily recommended nutrient intake of iron. Unfortunately this does not mean that the people in Ethiopia does not have anemia, this is probably caused by a lack of foods who are rich in ascorbic acid, which improve the absorption of iron (Adish *et al*, 1998).

4.3 Osteoporosis

Osteoporosis, or porous bone, is a disease characterized by low bone mass and structural deterioration of bone tissue, leading to bone fragility and an increased risk of fractures of the hip, spine, and wrist. Men as well as women are affected by osteoporosis, a disease that can be prevented and treated.

Risk Factors
Certain risk factors are linked to the development of osteoporosis and contribute to an individual's likelihood of developing the disease. Many people with osteoporosis have several risk factors, but others who develop the disease have no known risk factors. There are some risk factors you cannot change, like; gender, age, ethnic and family history. Others risk factors that you can change, like; intake of calcium and vitamin D, lifestyle, cigarette smoking, intake of alcohol and the use of medication.

Prevention
To reach optimal peak bone mass and continue building new bone tissue as you age, there are several factors you should consider. Enough intakes of calcium and vitamin D are important. The use of cigarettes and alcohol should be minimized. Also doing exercises has a positive influence, because it works as fall prevention.
Also the use of medications that cause the bone loss and other preventive medications will prevent to osteoporosis.

Symptoms
Osteoporosis is often called the "silent disease" because bone loss occurs without symptoms. People that doesn't have a periodical control may not know that they have osteoporosis until their bones become so weak that a sudden strain, bump, or fall causes a hip to fracture or a vertebra to collapse. Collapsed vertebrae may initially be felt or seen in the form of severe back pain, loss of height, or spinal deformities such as kyphosis (severely stooped posture).

Detection
Following a comprehensive medical assessment, your doctor may recommend that you have your bone mass measured. A bone mineral density (BMD) test is the best way to determine your bone health. BMD tests can identify osteoporosis, determine your risk for fractures (broken bones), and measure your response to osteoporosis treatment.

Treatment
A comprehensive osteoporosis treatment program includes a focus on proper nutrition, exercise, and safety issues to prevent falls that may result in fractures. In addition, the physician may prescribe a medication to slow or stop bone loss, increase bone density, and reduce fracture risk.

Teff maybe have a positive influence on osteoporosis, because it is high in calcium content, which prevents osteoporosis. Calcium makes the bones stronger. There is done research to found information about osteoporosis in relation with teff, but there is not found any relevant information. (http://www.niams.nih.gov/Health_Info/Bone/Osteoporosis/overview.pdf)

4.4 Diabetes

Diabetes is a disorder of metabolism, the way our bodies use digested food for growth and energy. Most of the food we eat is broken down into glucose, the form of sugar in the blood. Glucose is the main source of fuel for the body. After digestion, glucose passes into the bloodstream, where it is used by cells for growth and energy. For glucose to get into cells, insulin must be present. Insulin is a hormone produced by the pancreas. When we eat, the pancreas automatically produces the right amount of insulin to move glucose from blood into our cells. In people with diabetes, however, the pancreas either produces little or no insulin, or the cells do not respond appropriately to the insulin that is produced. Glucose builds up in the blood, overflows into the urine, and passes out of the body in the urine. Thus, the body loses its main source of fuel even though the blood contains large amounts of glucose.

What are the types of diabetes?
The three main types of diabetes are

- Type 1 diabetes
- Type 2 diabetes
- Gestational diabetes

Type 1 Diabetes
Type 1 diabetes is an autoimmune disease. An autoimmune disease results when the body's system for fighting infection (the immune system) turns against a part of the body. In diabetes, the immune system attacks and destroys the insulin-producing beta cells in the pancreas. The pancreas then produces little or no insulin. A person who has type 1 diabetes must take insulin daily to live.

It develops most often in children and young adults but can appear at any age. Symptoms of type 1 diabetes usually develop over a short period, although beta cell destruction can begin years earlier. Symptoms may include increased thirst and urination, constant hunger, weight loss, blurred vision, and extreme fatigue. If not diagnosed and treated with insulin, a person with type 1 diabetes can lapse into a life-threatening diabetic coma, also known as diabetic ketoacidosis.

Type 2 Diabetes
The most common form of diabetes is type 2 diabetes. About 90 to 95 percent of people with diabetes have type 2. This form of diabetes is most often associated with older age, obesity, family history of diabetes, previous history of gestational diabetes, physical inactivity, and certain ethnicities. About 80 percent of people with type 2 diabetes are overweight. Type 2 diabetes is increasingly being diagnosed in children and adolescents.
When type 2 diabetes is diagnosed, the pancreas is usually producing enough insulin, but for unknown reasons the body cannot use the insulin effectively, a condition called insulin resistance. After several years, insulin production decreases. The result is the same as for type 1 diabetes. Glucose builds up in the blood and the body cannot make efficient use of its main source of fuel. The symptoms of type 2 diabetes develop gradually. Their onset is not as sudden as in type 1 diabetes. Symptoms may include fatigue, frequent urination, increased thirst and hunger, weight loss, blurred vision, and slow healing of wounds or sores. Some people have no symptoms.

Gestational Diabetes
Some women develop gestational diabetes late in pregnancy. Although this form of diabetes usually disappears after the birth of the baby, women who have had gestational diabetes have a 20 to 50 percent chance of developing type 2 diabetes within 5 to 10 years.

Maintaining a reasonable body weight and being physically active may help prevent development of type 2 diabetes

What is the impact of diabetes?
Diabetes is associated with long-term complications that affect almost every part of the body. The disease often leads to blindness, heart and blood vessel disease, stroke, kidney failure, amputations, and nerve damage. Uncontrolled diabetes can complicate pregnancy, and birth defects are more common in babies born to women with diabetes.

Who gets diabetes?
Diabetes is not contagious.
Type 1 diabetes occurs equally among males and females but is more common in whites than in non-whites. Data from the World Health Organization's Multinational Project for Childhood Diabetes indicate that type 1 diabetes is rare in most African, American Indian, and Asian populations. However, some northern European countries, including Finland and Sweden, have high rates of type 1 diabetes. The reasons for these differences are unknown.

How is diabetes managed?
Before the discovery of insulin in 1921, everyone with type 1 diabetes died within a few years after diagnosis. Today, healthy eating, physical activity, and taking insulin are the basic therapies for type 1 diabetes. The amount of insulin must be balanced with food intake and daily activities. Blood glucose levels must be closely monitored through frequent blood glucose checking. People with diabetes also monitor blood glucose levels several times a year with a laboratory test called the A1C. Results of the A1C test reflect average blood glucose over a 2- to 3-month period. Healthy eating, physical activity, and blood glucose testing are the basic management tools for type 2 diabetes. In addition, many people with type 2 diabetes require oral medication, insulin, or both to control their blood glucose levels. Adults with diabetes are at high risk for cardiovascular disease (CVD). In fact, at least 65 percent of those with diabetes die from heart disease or stroke. Managing diabetes is more than keeping blood glucose levels under control. It is also important to manage blood pressure and cholesterol levels through healthy eating, physical activity, and use of medications. People with diabetes must take responsibility for their day-to-day care. Much of the daily care involves keeping blood glucose levels from going too low or too high. When blood glucose levels drop too low— a condition known as hypoglycaemia—a person can become nervous, shaky, and confused. Judgment can be impaired, and if blood glucose falls too low, fainting can occur. A person can also become ill if blood glucose levels raise too high, a condition known as hyperglycemias. People with diabetes should see a health care provider who will help them learn to manage their diabetes and who will monitor their diabetes control. Most people with diabetes get care from primary care physicians from internists, family practice doctors, or paediatricians.
(http://diabetes.niddk.nih.gov/dm/pubs/overview/DiabetesOverview.pdf)

4.4.1. Glycemic index

The glycemic index (GI) is possibly a tool for diabetes patients to manage their blood sugar.
The GI is a ranking of carbohydrates on a scale from 0 to 100 according to the extent to which they raise blood sugar levels after eating. Foods with a high GI are those which are rapidly digested and absorbed and result in marked fluctuations in blood sugar levels. Low-GI foods, by virtue of their slow digestion and absorption, produce gradual rises in blood sugar and insulin levels, and have proven benefits for health. Low GI diets have been shown to improve both glucose and lipid levels in people with diabetes (type 1 and type 2). They have benefits for weight control because they help control appetite and delay hunger. Low GI diets also reduce insulin levels and insulin resistance.

To determine a food's GI rating, measured portions of the food containing 10 - 50 grams of carbohydrate are fed to 10 healthy people after an overnight fast. Finger-prick blood samples are taken at 15-30 minute intervals over the next two hours. These blood samples are used to construct a blood sugar response curve for the two hour period. See figure 1 for a graph with a low and high GI.

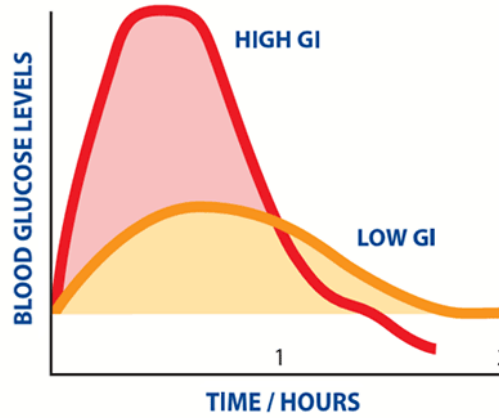

Figure 4.1, Glycemic index

Glycemic Index Reference Range

- High Glycemic Index 70-100
- Moderate Glycemic Index 5
- 0-70
- Low Glycemic Index <50

The higher the GI, the higher the rise in glucose in the blood stream, the more insulin is produced to store it. Over time this can lead to higher insulin levels that can result in inflammation, weight gain and insulin resistance. The end result can be the progression to type 2 diabetes. (http://www.glycemicindex.com/)

Teff has a low GI because it has a lot of slow carbohydrates. This may be positive for diabetes patients, because when they eat slow carbohydrates the blood sugar is more constant. But, the Dutch Diabetic Fund says GI is not useful for diabetic patients, because it depends extremely on the combination, quantity and the preparation of the food to make it trustworthy. (Personal contact with the Dutch Diabetic Fund)

4.4.2. Obesity

Obesity, or overweight, refers to a person's overall body weight and where the extra weight comes from. Overweight is having extra body weight from muscle, bone, fat, and/or water. Obesity is having a high amount of extra body fat. The most useful measure of overweight and obesity is the body mass index (BMI). BMI is based on height and weight and is used for adults, children, and teens. The BMI is the person's weight in kg divided by the height^2 in meters. The current value settings are as follows: a BMI of 18,5 to 25 may indicate optimal weight, a BMI lower than 18,5 suggests the person is underweight while a number above 25 may indicate the person is overweight. A BMI below 17,5 may indicate the person has anorexia or a related disorder. A number above 30 suggests the person is obese and over 40 the person in morbidly obese.(http://en.wikipedia.org/wiki/Body_mass_index)

Millions of people worldwide are overweight or obese. Being overweight or obese puts you at risk for many diseases and conditions. The more body fat that you carry around and the more you weigh, the more likely you are to develop heart disease, high blood pressure, type 2 diabetes, gallstones, breathing problems, and certain cancers.

A person's weight is a result of many factors. These factors include environment, family history and genetics, metabolism (the way your body changes food and oxygen into energy), behaviour or habits, and other factors.

Certain things, like family history, can't be changed. However, other things like a person's lifestyle habits can be changed. You can help prevent or treat overweight and obesity if you;

- Follow a healthful diet, while keeping your calorie needs in mind
- Are physically active
- Limit the time you spend being physically inactive
- Weight loss medicines and surgery also are options for some people who need to lose weight if lifestyle changes don't work.

Also for people who are overweight teff may have a good influence. Because of the low GI people don't get hunger quick after the meal.
(http://www.nhlbi.nih.gov/health/dci/Diseases/obe/obe_whatare.html)

5 Conclusion and discussion.

There are no doubts than teff appears as an interesting crop to be investigated. If it represents 70% of the human nutrition in Ethiopia, and, if it is true that in this African country the incidence of diseases as Celiac, anemia, osteoporosis and obesity is low, then it is necessary to go deep in the relationship between this cereal and these diseases.

Teff is an interesting raw material for new food products development. Other than enjera, it is possible, as shown in chapter 2.2 in this work, to elaborate all kind of bakery products, fermented or non fermented beverages, porridges breakfast foods, and can be used to control sauces and gravies texture. Food technologist, we can modified the medium of the teff starch, and obtain processing behaviour according with our needs. It is enough to add some chemical compounds as salts, acids and alkalis, to change some physical properties as foaming capacity and protein solubility.

It is then, an interesting activity for scientist as nutritionists, chemists, food engineers and for trade people, to re-discover this attractive little grain that is really ancient but new for developed countries. It has a very small size but it contains a giant nutritional value.

Potential safety of teff for consumption by patients with celiac disease
The most important medical effect of teff is on celiac disease. If teff is totally gluten free (as shown in some investigations recently made, as in one by Spaenij-Dekking et al, 2005 of the Leiden University Medical Center), teff can be included on the diets of celiac patients, without any risk. However, the authors of this work, we think that it is necessary to be careful, because they are some research results that can give doubts about the safety to use teff as an important ingredient of free gluten diets. For example, we use the following discussion, found in a recently edited article: "**61% of the CD patients with a gluten free diet (GFD) which do not consume teff reporting symptoms of CD. This percentage was comparable with the percentage of symptoms reported by teff users before teff was introduced into their GFD. However, a significant reduction of symptoms from 58% to 17% was reported after adding teff to the GFD. The patients who get CD symptoms after eating teff reported that the symptoms are fewer and significantly shorter in duration than before**". The article was written by E. Hopman *et al*, 2007. The title of the research is "Teff in the diet of celiac patients in The Netherlands". We found in this discussion terms that are relative, and shouldn't be use in scientific reports, as "The symptoms are fewer and significantly shorter in duration than before". Additionally, in the article written by Spaenij-Dekking et al, 2005, they said: In conclusion, within the limits of the currently available methods, no gluten or gluten homologues could be detected in the teff varieties tested. This finding indicates that teff may be suitable for use in the diet of patients with celiac disease. Ultimately, the study of teff consumption by patients with celiac disease in remission will be needed in order to determine whether teff is safe for these patients".

Some of the authors involved in this kind of discussion, they say that a lot of investigations were subject to significant methodological limitations: small study populations, short periods of investigation, and/or no available tests to measure the direct effect of oats and other cereals on the intestinal mucosa. There is not a matter to have doubts that humans need to continue with the research in this important topic.

About the contribution of teff, because its high iron content, to the low incidence of anemia between the Ethiopian population, we have a lot of doubts too. We firstly accepted both of the statements cited below: High iron teff content, and low anemia incidence in Ethiopia. But analyzing all the references obtained our criteria changed, and the doubts take place in our mind. We expose immediately some extracts of articles, responsible of our doubts:

- "While Ethiopian people are eating a lot teff, which is rich of iron it is probable to think that Ethiopian people do not have iron deficient. Unfortunately this is not true".
- "Preschool children in Africa, which includes Ethiopia, have some of the highest rates of anemia in the world— nearly 56%(United nations). In Ethiopia, the magnitude and importance of iron deficiency anemia as a public health problem is still disputed. Some studies reported iron deficiency anemia rates of less than 18% while others have reported rates of 25% and above".

Additionally, there is a big dispute between writers (authors of the used articles), about the high iron content in teff. Authors as Bultosa, Turkensteen, Mamo, Parson and Yigsaw talk about the higher nutritious value of teff, because of the iron content and aminoacid balance. They say that the nutritious values of teff are higher than the nutritious values of other cereals. Some scientists think that the high results about the iron content in teff are due to ferruginous soil ground into the outside surface of the grains. As teff has a very small size, it is very difficult to clean it very well after harvest. It is necessary to think too, that teff grow with other little grains as amaranthus, and it begin impossible to separate them. Amaranthus develop as a weed. It is Known that amaranthus is a free gluten grain too, with a high protein content and a good minerals level. The presence of anemia in Ethiopia can be due to other factors, as a low consumption of vitamin C, which enhances the iron absorbance. The authors who said teff has a higher iron content that other grains, they say that zinc and iron content are higher too. ("Compared to wheat, barley and oats, Eragrain teff has a high content of minerals such as: iron, calcium, zinc and magnesium"). We can forget that a low Iron content is not the only cause of anemia. A shortage of folic acid or vitamin B12 can also be the cause of anemia. In the other hand, some authors they say: "iron actually embedded in the grain walls must be considered to be a dietary source of iron along with the actual true iron content of the grain itself".

The enjera processing procedure includes a large fermentation step (some authors talk about two), that gives enjera their sensorial characteristics, as flavour, aroma and colour. But the more important effect of teff fermentation is an increase in the nutritional content, because the decreasing of the relationships Iron:Phytates and Iron:Tannins. If it is true that content of Iron, Zinc and Calcium are higher in teff than in other cereals, it is true that it has a high phytate content. This characteristic is obtained because it is not possible to peel this grain. All its external layers have to be included in the milling processing, and they have a high phytate content. If a diet has a high phytate content, the disponiblity of some nutrients, as minerals will decrease, because the formation of chelates (Wise, 1983; Hallberg *et al*, 1987). Tannins, they react with minerals present in foods, decreasing their availability to be used by human enzymes. That's whysince fermentation can decrease the phytate content by a factor of 3–4, traditional household practices such as fermentation need to be encouraged to address the problem of zinc and iron deficiency, which is particularly prevalent in Ethiopia (Umeta et. al, 2007).

Finally, about the development of a teff production and teff processing activity in The Netherlands, we think that this can represents to the population another way to diversify its feeding. For the obesity and celiac diseases patients, this can be an opportunity to obtain important ingredients for their diets. For the companies involved in the activity, this can represent a good opportunity to increase their economical profits. We think it is very difficult than Ethiopian government or Ethiopian population obtain a benefice if Dutch companies increase their yield production. It is not possible to translate the yield results from The Netherlands to Ethiopia. Ethiopia soil is very poor, and we understood this from the articles that talk about the use of manure, in order to increase the nutrients level of Ethiopian soils and other fertility studies made in this African country.

6 Recommendations.

When the nutrition of a so important population is involved, as this of the Ethiopia country, the institutions as WHO, FAO and a lot of associations as The Celiac Sprue Association, the Finish Celiac Society, The Celiac Disease Foundation and others, they should put together all their resources to develop a big project. This has to be an interdisciplinary project, with the participation of institutes and universities of different countries. It is necessary to include the agronomical and yields aspects, varieties selection, social benefits, teff flour production new products development, and food safety system implementation.

It is necessary to begin doing an objective neutral chemical characterisation, to be sure about micro chemical teff composition, because they are a lot of doubts about this, an secondly, we recommend to investigate about the real anemia incidence in Ethiopia.

In order to improve the consumption of enjera, and the fermentation step in the manufacture processing, it is necessary to investigate details about this practice, the changes involved, the participating microorganisms and to standardize a procedure (protocol). It is necessary to be sure, and this is a topic related with microbiological, chemistry and food safety aspects, of the absence of mycotoxins. This dangerous substance has been found in teff grain and teff products by different authors.

It is not out of matter, to include in the project a research about teff starch modifications, in order to increase their properties to be used in the bakery industry. This topic involves Food Technology, Food Physics, Food Chemistry and Food Safety.

It is possible that the project design was made at Van Hall Larenstein part of Wageningen University, but it is necessary to begin to include and to be sure of the associations, universities and institutions as these mentioned below.

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TRANSFORMACIÓN GENÉTICA DE PLANTAS MEDIADA POR *Agrobacterium tumefaciens*

María Camila Balcázar[1,2], Elizabeth Hodson[2] y Wilson Terán[2]

[1] Departamento de Biología. Facultad de Ciencias y Tecnología. Universidad Mayor de San Simón (UMSS). Calle Sucre y parque la Torre Cochabamba.
[2]Unidad de Biotecnología Vegetal. Departamento de Biología. Universidad Javeriana. Laboratorio 206A -Edificio 54. Carrera 7 # 43-82 Bogotá.

Palabras clave: Transformación genética; *Agrobacterium tumefaciens*; Plásmido pBI121; gusA; Callogénesis; Organogénesis.

RESUMEN: La transformación genética en tubérculos y raíces de interés alimentario como la papa (*Solanum tuberosum* L.) y la yuca (*Manihot esculenta* Crantz) ha cobrado un gran interés en los últimos años debido a la gran importancia de estos cultivos para la alimentación mundial, particularmente en las regiones más pobladas y pobres de mundo. Se han venido desarrollando varios protocolos de transformación genética en estas dos especies con el fin de transfererir genes de resistencia a patógenos fúngicos y víricos, genes de tolerancia a sequía y salinidad, o genes metabólicos con fines de biofortificación. El presente trabajo pretende evaluar y estandarizar protocolos de transformación genética mediada por Agrobacterium tumefaciens para estas dos especies, que permitan obtener plantas transgénicas con una gran eficiencia y rapidez (dos a tres meses), de manera que puedan ser aplicados rutinariamente en futuros proyectos de transformación genética en estos importantes cultivos alimentarios.

ABTRACT: Genetic transformation of important tuber and root food crops such as potato (*Solanum tuberosum* L.) and cassava (*Manihot esculenta* Crantz) has gain interest in the past few years because of the importance of those crops for food security, particularly in the poorest and more populated regions of the world. Various transformation protocols have been developed for both species, in order to transfer either fungal or viral pathogen resistance genes, drought and salinity tolerance genes, or metabolic genes for biofortification purposes. The present work is aimed to evaluate and standardise Agrobacterium tumefaciens mediated transformation protocols for both species in order to obtain transgenic plants with high efficiency and shortened times (two to three months), that could be routinely used in future projects of genetic transformation for genetic improvement of those important food crops.

INTRODUCCIÓN

La ingeniería genética vegetal representa un importante hito en la ciencia agrícola moderna. El advenimiento de la tecnología de ADN recombinante a principios de los 70 y el posterior desarrollo de técnicas de transferencia de ADN proporciono grandes oportunidades para la inserción de genes extraños tanto de organismos procariotas como eucariotas en el genoma de plantas de importancia agrícola, permitiendo modificar y aumentar el *pool* de las variedades disponibles en poco tiempo [1,2], sin alterar el fondo genético de las mismas. Este hecho es de suma importancia ya que el objetivo de las diferentes técnicas de mejoramiento es la incorporación acumulativa de nuevas características benéficas, sin perder las mejoras logradas previamente [3].

Las plantas transgénicas que expresan rasgos nuevos ahora están siendo ampliamente cultivadas para la mejora de su rendimiento, calidad y otras característica de

valor añadido [1]. La producción de plantas transgénicas requiere la transferencia del material genético dentro de la célula vegetal, la integración estable del ADN introducido en el genoma de la planta y la regeneración de todas las plantas de manera que el gen introducido sea mantenido establemente en las subsecuentes generaciones, para esto es necesario que las células que han recibido el transgen en su genoma tengan la capacidad de regenerar en plantas fértiles [3,4,5]. Finalmente es también necesario evidencia física de la integración del T-DNA, la expresión de los genes introducidos y una transmisión estable a las generaciones futuras [5,6]. Las plantas transgénicas que transmiten el rasgo introducido a la progenie se obtienen utilizando diversos métodos de inserción de ADN, tales como la bio-balística, electroporación y la permeabilización de protoplastos por medio de la aplicación de polietilenglicol [7,8]. Sin embargo, el método más comúnmente utilizado para obtener plantas transgénicas es el de la transformación mediada por *Agrobacterium tumefaciens*. *Agrobacterium* puede transferir ADN a un amplio grupo de organismos (numerosas especies de angiospermas dicotiledóneas y monocotiledoneas, gimnospermas y hongos, incluyendo levaduras, ascomicetes y basidiomicetes). Recientemente, se reportó la transferencia de ADN a células humanas [7].

NATURALEZA DE LA INTERACCIÓN *AGROBACTERIUM*-PLANTA

A. tumefaciens, y *A. rhizogenes*, miembros del género *Agrobacterium* son bacterias Gram-negativas aeróbicas obligadas que viven en el suelo. Ellas son capaces de desarrollar un crecimiento saprofítico o parasítico al infectar una gran variedad de especies de Dicotiledoneas a través de heridas causadas en las plantas, por diferentes agentes externos. *A. tumefaciens* induce la formación de tumores en el tallo, enfermedad conocida como "agalla de corona", mientras que *A. rhizogenes* induce una proliferación excesiva de raíces, causando así la enfermedad conocida como "raíz en cabellera" [3,5,8,9].

La «agalla de corona» es una consecuencia de un proceso natural de transferencia de DNA de la bacteria a la célula vegetal, semejante a la conjugación bacteriana: un fragmento de DNA plasmídico, denominado T-DNA (*transferred DNA*), es transferido a la célula vegetal y es integrado en su genoma [8]. Este proceso de transformación es estimulado por compuestos exudados de células vegetales dañadas [9]. El T-DNA corresponde a un segmento definido de un plásmido de alto peso molecular (150-200 Kb) denominado plásmido Ti (*tumour-inducing*) en *A. tumefaciens* y Ri (*root inducing*) en *A. rhizogenes*. El T-DNA está delimitado por secuencias directamente repetidas de 25 pb conocidas como borde derecho y borde izquierdo [8,3].

El paso inicial en el proceso de infección es el ataque de *A. tumefaciens* a las células de la planta a través de una herida. En esta etapa la bacteria produce una red de fibras de celulosa que conectan fuertemente a la bacteria con la superficie de la célula vegetal, etapa en la que están involucrados genes cromosómicos (*chv*A, *chv* B, *chv* E, *cel*, *psc* A y *att*) [10,3]. Este proceso de infección se produce debido a que la bacteria responde a ciertos componentes fenólicos de la planta como la acetosiringona e hidroxiacetosiringona, los cuales son liberados en las heridas de plantas susceptibles. Estas pequeñas moléculas, actúan induciendo los genes de virulencia (*vir*) que son codificados en el plásmido Ti [10].

Los genes *vir* están localizados en una región de 35-kb en el plásmido Ti, situados fuera de la región del T-DNA [11]. Existen 20 genes *vir* organizados en 8 unidades transcripcionales denominadas *vir*A—*vir*H, que son co-reguladas formando un regulón. Solamente cuatro loci (*vir*A, *vir*B, *vir*D y *vir*G) son absolutamente esenciales para la

tumorogénesis, mientras que los otros afectan la eficiencia de la transferencia y el espectro de hospederos. El locus *vir*A codifica una proteína de membrana que percibe la presencia de los metabolitos liberados por las células de la planta en respuesta a las heridas. VirA fosforila la proteína codificada por el gen *vir*G que, entonces, activa a los demás genes *vir*. La proteína VirD2 (una endonucleasa), reconoce y corta los bordes derecho e izquierdo que delimitan el T-DNA y se une covalentemente al extremo 5´ de la molécula de cadena simple de T-DNA. La proteína VirE2 se une entonces a la superficie da la unidad T-DNA-VirD2, protegiéndola de nucleasas y formando el llamado complejo T. El paso del complejo T por la membrana bacteriana y por la pared celular de la célula vegetal es garantizada por proteínas codificadas por el *locus vir*B. El complejo-T es transferido al núcleo a través de un poro de la membrana nuclear. En el núcleo ocurre la integración del T-DNA al genoma de la célula vegetal [8].

Durante la inserción del T-DNA en el cromosoma de la planta, son también producidas pequeñas delecciones del DNA cromosómico en el sitio de unión entre el T-DNA y el DNA cromosómico de la planta. En tanto, mientras la inserción del T-DNA en el DNA de la planta ocurre en sitios al azar, los bordes del T-DNA muestran homología con el DNA de la planta en el sitio de inserción [10].

Una vez integrado en el genoma celular, ocurre la transcripción de genes presentes en el T-DNA y la consecuente traducción de enzimas que, a su vez, llevan a cabo la síntesis de dos productos principales: hormonas vegetales (auxinas y citoquininas) y opinas. Las auxinas y citoquininas regulan el crecimiento y desarrollo celular de la planta, por lo tanto, la síntesis de ambas desencadena numerosas divisiones celulares, y el crecimiento de tejido en forma de tumor como consecuencia del aumento de las cantidades de auxina y citoquinina endógenas, generado por la síntesis de sustancias de las células transformadas [8,10]. Las opinas son formas modificadas de aminoácidos o azúcares, formadas también como consecuencia de la activación de genes localizados en el T-DNA. Las opinas son sintetizadas junto con la agalla de corona y posteriormente secretadas. Pueden ser usadas como fuente de carbono y algunas veces también como fuente de nitrógeno. Los genes para el catabolismo de las opinas se encuentran en el plásmido Ti y no es parte de la región del T-DNA [10].

El tipo de opina formado depende de la cepa bacteriana infectante. Más de 20 diferentes opinas ya fueron identificadas. Los diversos tipos de plásmidos Ti son clasificados en base a la opina mayormente sintetizada en los tejidos vegetales y metabolizada por la bacteria inductora [8].

MANIPULACIÓN GENÉTICA CON *Agrobacterium tumefaciens*

La patogénesis de plantas desarrollada por las especies del género *Agrobacterium,* es un hecho extraordinario de la naturaleza, ya que permite la transferencia, integración y expresión de un fragmento de DNA (T-DNA), de origen procariota en células eucariotas. Este mecanismo puede ser aprovechado para la introducción de genes de interés en las plantas, ya que los genes que codifican la síntesis de hormonas vegetales y opinas, que a su vez no son necesarios para la transferencia del T-DNA, pueden ser reemplazados por los genes de interés, sin alterar el proceso de transferencia. Gracias a esto, y al hecho de que los genes *vir* no necesitan estar en el mismo plásmido que el T-DNA para ser funcionales, y que el plásmido Ti tiene un tamaño de 200 kilobases, es que se han desarrollado dos

estrategias diferentes para la transferencia de genes mediada por *Agrobacterium*. Una que utiliza vectores co-integrados y la otra que utiliza vectores binarios [3,11].

Los vectores co-integrados consisten en un plásmido de *E. coli,* donde se ha introducido el gen de interés; esta secuencia es posteriormente integrada al plásmido Ti desarmado entre los bordes derecho e izquierdo del T-DNA. Al transferir el plásmido de *E. coli* a *Agrobacterium* el gen insertado queda entre los bordes del T-DNA por recombinación homóloga. El resultado es un plásmido en donde el material genético nuevo se sitúa en *cis* con respecto a los genes *vir* en el mismo plásmido. En los vectores binarios la región *vir* y el T-DNA residen en plásmidos separados o en dos replicones diferentes dentro de Agrobacterium y actúan en *trans* uno con respecto al otro; la región *vir* se encuentra en el plásmido Ti desarmado de la cepa de *A. tumefaciens* y el T-DNA se encentra en el otro plásmido vector. El plásmido junto con el T-DNA constituye el vector binario mientras que el plásmido que contiene los genes *vir* se conoce como ayudador (*vir* helper). El plásmido ayudador con los genes *vir* generalmente posee una completa o parcial delección de la región del T-DNA confiriéndoles el carácter de cepas no virulentas a las cepas de *Agrobacterium* que los llevan. Actualmente este tipo de vectores es el más utilizado ya que su construcción es más sencilla [12,13].

Tanto los vectores co-integrados como los vectores binarios contienen los siguientes elementos básicos:

- Un origen de replicación que permite que el plásmido sea replicado en *E. coli*. En los vectores binarios, se añade también un origen de replicación que funciona en *A. tumefaciens*, mientras que en los vectores co-integrados no, con objeto de favorecer la recombinación homóloga [10].
- El borde derecho del T-DNA. Esta región es absolutamente necesaria para la integración del T-DNA en el DNA de la célula vegetal. Sin embargo muchos vectores contienen tanto el borde derecho como el izquierdo [10].
- Secuencias promotoras, que son regiones del DNA ubicadas corriente arriba de regiones codificantes, las cuales contienen secuencias específicas para el reconocimiento y unión de proteínas involucradas en el control del nivel y patrón de la expresión génica. Se han identificado promotores tejido-específicos, de expresión temporal y de expresión constitutiva; siendo los últimos los más utilizados actualmente ya que presentan altos niveles de transcripción y facilitan la expresión del gen en cualquier tipo de tejido. Tal es el caso del promotor CaMV 35S (del virus del Mosaico de la Coliflor) [11].
- Secuencias terminadoras, las cuales actúa como señalizadoras para la detención del proceso de transcripción. La secuencia de terminación más utilizada es la proveniente del gen de la nopalina sintetasa (NOS) de *A. tumefaciens* [13].
- Entre las secuencias promotoras y terminadores reside el transgen de interés, pero también otros genes como los genes de selección de la planta transgénica y en algunos casos genes reporteros.

GENES MARCADORES

Considerando que un porcentaje relativamente pequeño de células se torna establemente trasformada con el empleo de cualquier método de introducción de DNA exógeno en las células vegetales, es esencial poder detectar el DNA foráneo que ha sido

integrado en el DNA genómico de la planta, de modo que aquellas células que han sido transformadas puedan ser identificadas [10,8]. Con los genes marcadores es posible reconocer y seleccionar las células que han sido trasformadas, estos son co-transformados con los genes de interés que queremos expresar en la planta. Hay dos tipos de marcadores, de selección e informadores o reporteros [13,14].

El gen marcador de selección, otorga una importante ventaja a las células transgénicas que lo expresan, ya que les permite crecer en presencia de un agente selectivo. Esto se logra ya sea por medio de una selección negativa o una selección positiva. En la selección negativa, el gen marcador de selección codifica una proteína que transfiere a las células que lo expresan resistencia a agentes fitotóxicos como antibióticos o herbicidas, permitiéndoles crecer y desarrollarse en presencia de los mismos, mientras que las células que no lo expresan (no transformadas), no pueden hacerlo. En el caso de la selección positiva, la ventaja está dada por el uso diferencial de un sustrato. Un ejemplo de este caso es el del gen *man*A de *E. coli* el cual codifica una enzima que permite a las células que lo expresan utilizar la manosa como fuente de carbono. Sin embargo cabe destacar que algunas especies pueden poseer resistencia a los agentes selectivos mencionados, por tanto es importante evaluar este aspecto antes de elegir el gen de selección, el agente selectivo y las concentraciones del mismo, antes de ser utilizados [3].

Dentro de estos genes el más utilizado es el gen de la neomicina fosfotransferasa (*npt*II) que confiere resistencia a antibióticos aminoglicósidicos, como la kanamicina, mediante la fosforilación del mismo. Son pocas las excepciones en las que se ha utilizado el gen de resistencia a higromicina (*bpt*) o el gen de resistencia al herbicida "basta" [15,16]. Como la mayoría de los genes de selección son de origen procariótico, es necesario poner bajo el control de la planta señales de regulación transcripcional, incluyendo tanto las secuencias promotoras como las de terminación, para asegurar su efectiva expresión en las células vegetales [10].

Adicionalmente, los genes marcadores reporteros o informadores dan a la célula que ha incorporado el gen una característica que la hace distinguible de las demás. Estos genes codifican proteínas que producen un fenotipo característico de fácil y rápida observación. Los más utilizados son genes que codifican enzimas que hidrolizan sustratos cromogénicos, fluorogénicos, o emisores de luz, de tal manera que se logra identificar a las células transformadas al añadir el sustrato adecuado [3].

El gen reportero más utilizado es el gen *uid*A o *gus*A aislado de *E. coli* y que codifica la enzima ß-glucuronidasa (GUS). Esta enzima es una hidrolasa ácida que hidroliza una amplia variedad de ß-glucurónidos. La presencia de GUS puede ser detectada histoquímicamente adicionándose una sustrato cromogénico, como el ácido 5-bromo-4-cloro-3-indol-ß-D-glucorónido (X-Gluc), el cual en presencia de la enzima, forma un precipitado azul, resultado de la dimerización del producto de hidrólisis de X-Gluc. [8]. Con esta enzima también se puede usar el sustrato 4-metil-umbeliferil-ß-D-glucorónido (4-MUG) el cual al ser hidrolizado forma un compuesto fluorescente, 4-metilumbeliferona (MU), que se puede medir por técnicas fluorimétricas [8,17].

La desventaja en la utilización del gen *gus*A es la consecuente muerte de las células vegetales en el momento del análisis o tinción. En este sentido, como alternativa, se vienen utilizando otros genes reporteros, como los genes *lux* y *gfp*, cuyos productos pueden ser

identificados mediante un análisis no deletéreo. Los genes *lux* codifican la enzima luciferasa de *Photinus pyralis* (luciérnaga). La luciferasa cataliza la reacción de oxidación de luciferina, en presencia de ATP, produciendo una luz verde-amarilla. El gen *gfp*, a su vez, fue aislado de *Aequorea victoria* (medusa) y codifica la proteína de fluorescencia verde (green fluorescent protein, GFP). La GFP absorbe luz ultravioleta o luz azul, haciendo que las células que expresan este gen, emitan una luz verde fluorescente [8,18].

REGENERACIÓN DE TEJIDOS VEGETALES

Uno de los elementos básicos requeridos para el éxito en procedimientos de transformación genética de plantas es un sistema de cultivo de tejidos que permita regenerar plantas completas y fértiles, a partir de células transformadas con el gen de interés, en medios de cultivo selectivos. Esto se logra gracias a la propiedad de totipotencia de las células y tejidos vegetales, [3], es decir la capacidad que tienen de crecer, dividirse y finalmente diferenciarse para formar una planta completa [19].

Antes de que una célula vegetal sea capaz de expresar su propiedad de totipotencia, es necesario que primero pase por un proceso de desdiferienciación, es decir una reversión del estado diferenciado al meristemático, y posteriormente una nueva diferenciación que la capacite para expresar su potencial organogénico. Este proceso denominado "organogénesis somática", puede realizarse de dos maneras diferentes, indirecta cuando se realiza con una callogénesis intermedia, o directa cuando se realiza sin callogénesis [20].

El cultivo *in vitro* de protoplastos con concentraciones definidas de las fitohormonas axina y citoquinina, puede derivar en la formación de una masa de células indiferenciadas conocidas como callo. El callo con una transferencia regular a nuevo medio sólido y fresco, puede ser cultivado de manera indefinida, o puede ser cultivado bajo condiciones hormonales que induzcan la formación de brotes. Los brotes extraídos de los callos pueden ser cultivados en medio fresco e inducidos a iniciar la formación de raíces, y finalmente formar una planta completa. Aunque este procedimiento general es válido para la mayoría de las plantas, las condiciones hormonales varían entre especies y deben ser determinadas empíricamente, [19] esto se debe a que existe una importante influencia del genotipo, en la respuesta a diferentes condiciones del cultivo *in vitro* [3].

OBJETIVOS

OBJETIVO GENERAL

- Estandarizar un protocolo de transformación genética de papa (*Solanum tuberosum* L.) y yuca (*Manihot esculenta* Crantz.) mediada por *Agrobacterium tumefaciens*.

OBJETIVOS ESPECÍFICOS

- Transformar las cepas de *Agrobacterium tumefaciens* GV2260 y LBA4404 con el plásmido binario pBI121.
- Aplicar y evaluar dos protocolos de transformación de papa y yuca con dos cepas distintas de *A. tumefaciens* portadoras del plásmido binario pBI121.
- Obtener callos y plántulas de papa y yuca transformadas.
- Verificar la inserción y expresión del gen reportero *gus*A en los tejidos de las plantas transgénicas obtenidas.

METODOLOGÍA

MATERIAL VEGETAL

Se trabajó con plántulas de yuca (*Manihot esculenta* Crantz) y papa (*Solanum tuberosum* L, variedad Diacol Capiro.), las cuales provienen de plantas establecidas *in vitro* a partir de segmentos nodales, en medio Murashige & Skoog (MS), a 21 ± 2°C de temperatura, con un fotoperiodo de 16 horas luz/8 horas oscuridad y una humedad relativa del 70%.

Estas plántulas son parte del material establecido en el laboratorio de cultivo de tejidos de la Unidad de Biotecnología Vegetal de la Pontificia Universidad Javeriana**.**

CULTIVO Y TRANSFORMACIÓN DE CEPAS DE *Agrobacterium tumefaciens*

Para el proceso de infección y transformación se utilizaron las cepas de A. tumefaciens GV2260 (con resistencia a rifampicina y carbenicilina) y LBA4404 (con resistencia a estreptomicina). Estas cepas fueron transformadas con el plásmido binario pBI121 por el método de electroporación.

El plásmido pB121, tiene un tamaño de 12.8 kb, de acuerdo con su mapa de construcción (Jefferson, et al.1987) [17]. El T-DNA se inicia con la secuencia correspondiente al borde derecho, seguido del promotor NOS (nopaline synthase), luego el gen *nptII* (neomycin phosphotransferase II) que confiere resistencia a varios antibióticos aminoglicósidos incluyendo kanamicina, neomicina y G418, este es seguido por la secuencia terminadora NOS. A continuación esta la secuencia promotora CaMV 35S proveniente del virus del mosaico de la coliflor, la región codificante GUS (β-Glucoronidasa), que constituye el gen reportero, nuevamente la secuencia terminadora NOS y finalmente la secuencia correspondiente al borde izquierdo. Entre los diferentes constituyentes se localizan varios sitios de corte con enzimas de restricción, como *Eco*RI y *Hin*dIII.

El plásmido pBI121 fue aislado de una cepa de *Escherichia coli* DH5α previamente transformadas, cultivada en medio LB líquido con kanamicina (50 µg/ml), durante toda la noche a 37 °C. Posteriormente se realizó la extracción del plásmido haciendo uso del kit de purificación de plásmidos Quantium Prep. Plasmid Miniprep Kit. de BIO RAD. Una vez purificado el plásmido se procedió a su cuantificación por espectrofotometría haciendo uso del espectrofotómetro Nano Drop ^{TM} 1000.

Para verificar el tamaño del plásmido se realizaron dos análisis de restricción, el primero con la enzima *Eco*RI y el segundo con las enzimas *Eco*RI y *Hin*dIII. El producto de estas reacciones se visualizó en gel de agarosa al 0.8% (p/v), teñido con bromuro de etidio (0.5 µg/ml).

Finalmente se procedió a la transformación de las cepas de *A .tumefaciens* GV2260 y LBA4404 mediante electroporación. Se cultivaron estas bacterias en medio LB líquido con los antibióticos de selección respectivos durante toda la noche a 27 °C y en completa oscuridad. Posteriormente se realizaron tres lavados de las bacterias con agua destilada estéril a 4°C. Luego de los lavados, las bacterias se resuspendieron en 250 ml de agua destilada estéril. Para la electroporación, se mezclaron 40 µl de la suspensión bacteriana con 3.5 µl del plásmido (500 ng) purificado entre los dos electrodos de la celda de electroporación. Se dejo reposar por cinco minutos y finalmente se electroporó con un pulso de 1.8 kV. Las bacterias electroporadas se pusieron a crecer en 1 ml de medio LB durante 1 h a 27°C y se sembraron alícuotas de 100 µl por agotamiento en medio LB sólido

suplementado con Kanamicina (50 µg/ml) y los respectivos antibióticos de selección de cada cepa, a 27°C, por 3 días y en completa oscuridad.

TRANSFORMACIÓN Y REGENERACIÓN DE PLANTAS

CORTE DE EXPLANTES FOLIARES

Para obtener los explantes a infectar, se tomaron hojas jóvenes y saludables de yuca (*M. esculenta*) y papa (*S. Tuberosum* L*)* a las que se les retiró el pecíolo y 1 mm de la base. Luego, con el objetivo de producir heridas en las hojas, se realizaron varios cortes sobre la nervadura central dejando un espacio de aproximadamente 1-2 mm entre cada uno.

INFECCIÓN DE EXPLANTES FOLIARES

Para la el proceso de infección se probaron 2 protocolos diferentes para *M. esculenta* y *S. tuberosum* L.

La infección de explantes foliares de *M. esculenta* se realizó con las dos cepas de *A. tumefaciens* (GV2260 y LBA4404), transformadas previamente con el plásmido pBI121. Estas bacterias fueron cultivadas toda la noche en medio LB líquido con los antibióticos de selección respectivos (Rifampicina, 100 µg/ml; Kanamicina, 50 µg/ml; Carbenicilina, 100 µg/ml; para la cepa GV2260 (pBI121) y Streptomicina, 10 µg/ml; Kanamicina 50 µg/ml; para LBA4404 (pBI121)), a 27°C, 150 r.p.m. y en completa oscuridad hasta alcanzar una lectura de turbidez OD_{600} = 0.4 – 0.5. Posteriormente la bacteria fue colectada, lavada y resuspendida en medio LB líquido libre de antibióticos para prevenir la inhibición del crecimiento de las plantas. Con la ayuda de pinzas estériles se tomaron los explantes foliares previamente cortados y se sumergieron en cada una de las suspensiones bacterianas por 15 minutos. Este procedimiento se realizó con 5 explantes para cada cepa. Pasados los 15 minutos los explantes fueron retirados de la suspensión bacteriana, secados en papel filtro estéril y colocados con el lado adaxial hacia abajo en medio MS sólido libre de antibióticos. Simultáneamente a este proceso se tomaron 4 explantes foliares previamente cortados y fueron colocados en una placa de Petri que contenía medio MS sólido estéril y libre de antibióticos, sin pasar previamente por las suspensiones bacterianas, los cuales constituyeron los explantes control no infectados. Finalmente, las 3 placas de Petri (con hojas infectadas y con hojas control) fueron incubadas por dos días a 26 °C y en completa oscuridad, para el co-cultivo con *A. tumefaciens*

Para la infección de explantes foliares de *S. tuberosum*, sólo se trabajó con la cepa LBA4404 (pBI121). Estas bacterias fueron cultivadas en medio YEP líquido con los antibióticos respectivos (Streptomicina, 10 µg/ml; Kanamicina 50 µg/ml), a 27°C, 150 r.p.m. y en completa oscuridad hasta alcanzar una lectura de turbidez OD_{600} = 0.3 – 0.4. Posteriormente las bacterias fueron colectadas, lavadas y resuspendidas en 11 ml de medio de infección (*IM* Infection Medium) líquido y libre de antibióticos. Luego, 200 µl de esta suspensión fueron adicionados a placas de Petri que contenían 20 ml de medio *IM* líquido, estéril y libre de antibióticos. Con la ayuda de pinzas estériles se tomaron 20 explantes foliares previamente cortados y se sumergieron con el lado adaxial hacia abajo en las placas que contenían las suspensión bacteriana. Se repitió este proceso con 50 explantes en total. Adicionalmente se tomaron 10 explantes foliares previamente cortados y se sumergieron también con el lado adaxial hacia abajo, en medio *IM* estéril. Esta última placa sirvió como control no infectado. Las placas de Petri con los explantes foliares fueron agitadas a 35 rpm por 15 min., a temperatura ambiente y luego fueron incubadas por 48 horas en completa

oscuridad y a la misma temperatura, para el co-cultivo con la bacteria y su respectivo control.

INDUCCIÓN DE «CALLO»

A diferencia de otros métodos de transformación no se realizó ningún proceso de lavado o secado de los explantes antes de transferirlos a medio de regeneración (*MGC* Callus induction medium). En esta etapa también se realizaron procedimientos diferentes para *M. esculenta* y *S. tuberosum*.

Para *M. esculenta*, después de la etapa de co-cultivo con *A. tumefaciens* los explantes fueron transferidos al medio inductor de callo MGC^A, colocándolos con el lado adaxial hacia abajo. Posteriormente fueron incubados a 21 ± 2°C de temperatura, con un fotoperiodo de 16 horas luz/8 horas oscuridad y una humedad relativa del 70%. En esta etapa los explantes fueron transferidos a nuevo medio cada 5 a 7 días, para garantizar el crecimiento de "callo" y la selección contra *A. tumefaciens* y otros contaminantes. Esta etapa se mantuvo por 6 semanas hasta la aparición de callos friables resistentes a Kanamicina.

Para *S. tuberosum,* después del periodo de co-cultivo, los explantes fueron sembrados con el lado adaxial hacia abajo en medio inductor de "callo" MGC^B y posteriormente incubados en cámara de crecimiento a 21 ± 2°C de temperatura, con un fotoperiodo de 16 horas luz/8 horas oscuridad y una humedad relativa del 70%, por un periodo de 2 semanas. En esta etapa se transfirieron los explantes a nuevo medio de cultivo después de 8 días, para garantizar la selección contra *A. tumefaciens* y otros contaminantes.

INDUCCIÓN DE BROTES

Una vez formados los "callos", los explantes fueron transferidos a medio inductor de brote (*MGS* Shoot induction medium) e incubados como en el paso anterior, durante 3 a 4 semanas.

COMPOSICIÓN DE MEDIOS

Los medios usados en este estudio fueron solidificados con 2.3 g/l de fitagel y el pH fue ajustado a 5.8.

Medio de infección IM. Este consistió en un medio Murashige & Skoog (MS) libre de cualquier fitohormona y suplementado con 20 g/l de sacarosa.

Medio inductor de callo MGC^A. Consistió en un medio MS suplementado con las fitohormonas auxina: ácido α-naftalinacético (ANA) 0.1 µg/ml y citoquinina: 6-benzilaminopurina (BAP) 0.5 µg/ml; los antibióticos de selección Kanamicina 100 µg/ml y Cefotaxime 500 µg/ml; y 30 g/l de sacarosa.

Medio inductor de callo MGC^B. Medio MS suplementado con 16 g/l de glucosa, 5 mg/l de la auxina ANA, 0.1 mg/l de la citoquinina BAP, 250 mg/l de Cefotaxime y 50 mg/l de Kanamicina.

Medio inductor de brote MGS. MS suplementado con 16g/l de glucosa, 2.2 mg de la citoquinina zeatin-ribosa, 0.02 mg/l de la auxina ANA, 0.15 mg/l de la giberalina: ácido giberélico GA_3, 250 mg/l de cefotaxime y 50 mg/l de Kanamicina.

DETECCIÓN DE LA TRANSFERENCIA: EXPRESIÓN DEL GEN *gus*A

Para verificar la transferencia del T-DNA a las células vegetales, se llevó a cabo un procedimiento de tinción histoquímica de las plantas regeneradas a partir de los explantes

de hojas infectadas con *A. tumefaciens.* Se utilizó el protocolo descrito por Jefferson (1987) evaluándose de este modo la expresión del gen *gusA*, la cual se evidencia por una reacción colorimétrica detectable a simple vista de la enzima β-glucoronidasa codificada por dicho gen, al reaccionar con el sustrato X-gluc.

A las 5 semanas de incubación de los explantes de *M. esculenta* en medio MGC^A se llevo a cabo un procedimiento de tinción histoquímica, como primer ensayo de verificación de la transferencia del T-DNA a las células vegetales. Para esto, se extrajeron "callos" de un explante control, un explante infectado con la cepa LBA 4404 (pBI121) y un explante infectado con la cepa GV2260 (pBI121), y se sumergieron en 1 ml de solución de tinción con sustrato X-gluc, la cual tenía la siguiente composición: 1 mM de X-gluc (diluido en dimetil formamida), 50 mM NaH_2PO_4 (pH 7.0), 10 mM de EDTA y 0.1% de Triton X-100. La reacción de tinción se realizó toda la noche a temperatura ambiente y en completa oscuridad., tras lo cual se incubaron los callos teñidos en etanol al 70 % (vol/vol) para su conservación.

RESULTADOS

PURIFICACIÓN DEL PLASMIDO BINARIO pBI121

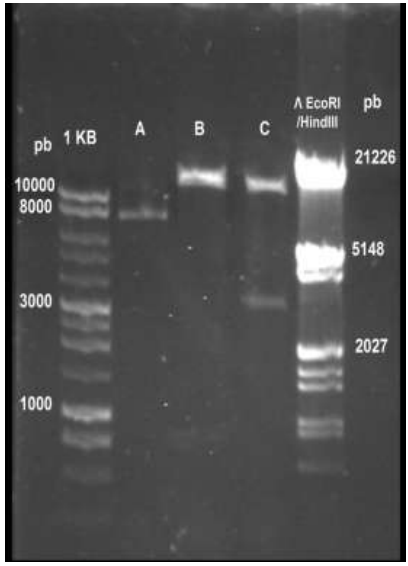

Figura 1.- Purificación y Digestión del plásmido binario pBI121 con las enzimas de restricción *Eco*RI y *Hin*dIII. A: Plásmido purificado (Sin cortar). B: Plásmido digerido con *Eco*RI. C: Plásmido digerido con *Eco*RI y *Hin*dIII

Para verificar la integridad del plásmido binario pBI121 extraído de células de *E. coli* almacenadas en glicerol a -80ºC, se realizó un análisis electroforético y de restricción en gel de agarosa (Figura 1). En este gel se logró verificar la calidad del plásmido extraído, libre de DNA contaminante y sin evidencias de degradación (carril A). Adicionalmente, el análisis de restricción empleando dos enzimas con sitios de restricción únicos (*Eco*RI y *Hin*dIII), permitió confirmar la integridad del plásmido al obtenerse fragmentos de restricción de tamaños esperados (carriles B y C). Así la digestión simple permitió evidenciar un tamaño de aproximadamente 15 kb, acorde con el tamaño teórico del plásmido, y la doble digestión arrojó dos fragmentos de tamaños esperados: uno de aproximadamente 12 Kb y el otro de 3 Kb.

TRANSFORMACIÓN Y CALLOGÉNESIS

Los explantes de *M. esculenta* infectados con *A. tumefaciens* y que fueron cultivados en medio inductor de callo (MGC^A), empezaron a desarrollarlo a partir de la tercera semana de su transferencia a medio MGC^A. Tres semanas después, fueron transferidos a medio inductor de brote (*MGS*), es decir, seis semanas después de su co-

cultivo con *A. tumefaciens*. En esta etapa pudo comprobarse que sólo los explantes infectados con cepas de *A. tumefaciens* desarrollaron abundante callo en presencia del agente de selección (kanamicina) (Figura 2. A y C), mientras que los explantes control no presentaron callogénesis (Figura 2.B) tal como se esperaba. Esto evidencia que los callos obtenidos en los explantes infectados, provienen de células transformadas y son muy probablemente transgénicos.

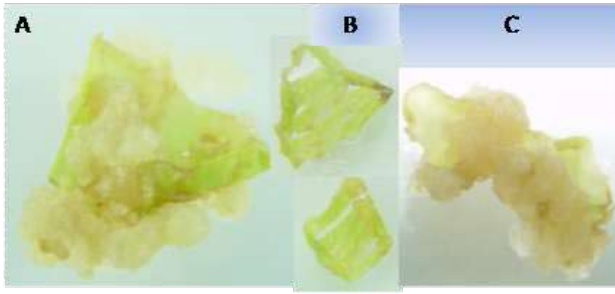

Figura 2.- Callos formados en los explantes de *M. esculenta* a las 6 semanas de cultivo en medio inductor de callo (*MGC*[A]). A: Explante infectado con la cepa GV2260 (pBI121). B: Explantes control. C: Explante infectado con la cepa LBA4404 (pBI121).

Es importante mencionar que los explantes de *M. esculenta* infectados con la cepa GV2260 (pBI121) desarrollaron callo con una eficiencia del 100 %, mientras que los explantes infectados con la cepa LBA4404 (pBI121) lo hicieron con una eficiencia del 80%, lo cual podría indicar una mayor eficiencia en la transferencia del T-DNA a células de tejidos foliares de yuca por parte de la cepa GV2260, comparado con la cepa LBA4404 (Tabla 1).

Tabla 1. Frecuencia de transformación de explantes de hojas de *M. esculenta* y eficiencia en la producción de callo

Explante	N° inicial de explantes	N° de explantes que desarrollaron callo	% de eficiencia	N° de explantes transferidos a *MGS*
Infectado con LBA4404 (pBI121)	5	4	80	4
Infectado con GV2260 (pBI121)	5	5	100	4

Para la transformación de explantes de papa (*S. tuberosum* L.), no se observó a la fecha formación de callos, tras dos semanas en medio inductor de callo *MGC*[B], no obstante se esperan resultados similares a los obtenidos en yuca.

CONFIRMACIÓN DE LA TRANSFERENCIA Y EXPRESIÓN DEL GEN REPORTERO

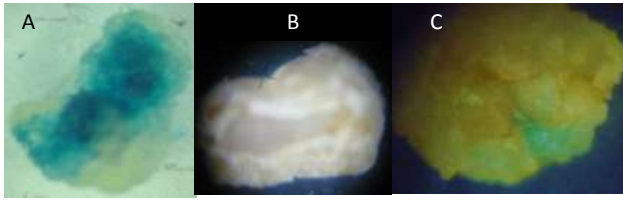

Figura 3.- Tinción histoquímica con buffer X-Gluc de callos formados en explantes de *M. esculenta* a las 6 semanas de cultivo en medio inductor de callo (MGC^A). A: Callo formado en un explante infectado con la cepa GV2260 (pBI121). B: Callo formado en un explante control. C: Callo formado en un explante infectado con la cepa LBA4404 (pBI121).

Al haberse obtenido callos de *M. esculenta* resistentes a kanamicina, se decidió verificar la transferencia del gen reportero *gusA* y su expresión en estas células de callo, mediante tinción histoquímica en presencia del sustrato X-Gluc para la enzima β– glucuronidasa (GUS). La tinción logró confirmar la transferencia y expresión del transgen *gusA* en células de callo proveniente de explantes infectados con ambas cepas de *A. tumefaciens*, gracias a la coloración azul que presentaron las células, indicadora de la presencia de la enzima reportera GUS (Figura 3). Como control negativo, se realizó la tinción de un callo de 6 semanas proveniente de un explante de *M. esculenta* no transformado, e inducido en medio MGC^A sin agente de selección. Cómo esperado, este callo no presentó coloración azul en ninguna de sus células. Adicionalmente, cabe destacar que el callo obtenido de un explante infectado con la cepa GV2260 (pBI121) muestra una mayor cantidad de células transformadas que el callo obtenido de un explante infectado con la cepa LBA4404 (pBI121), lo cual estaría corroborando que la cepa GV2260 parece ser más eficiente en la transferencia de T-DNA a células de tejido foliar de *M. esculenta* que la cepa LBA4404.

RESULTADOS ESPERADOS

Transformación de yuca (*M. esculenta* Crantz): Al haberse obtenido callos transgénicos que expresan el gen reportero *gusA*, se puede esperar obtener tras unas dos a tres semanas en medio inductor de brotes (MGS) al menos dos plántulas de yuca transformadas, teniendo en cuenta que la eficiencia de transformación esperada es de aproximadamente un 40% (W. Terán, comunicación personal).

Transformación de papa (*S. tuberosum* L.): Aunque es aún muy prematuro el poder observar callogénesis en los explantes infectados, el protocolo evaluado para la transformación de tejidos foliares de papa está reportado como un protocolo altamente eficiente, con rendimientos de transformación superior al 60%. Se espera así obtener callos embriogénicos tras una semana adicional en medio inductor de callo, y las primeros brotes transgénicos tras 3 semanas adicionales en medio inductor de brotes. Unos ocho días de cultivo de estos últimos en medio de enraizamiento, permitiría finalmente la obtención de plántulas transformadas de papa.

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