ADDIS ABABA UNIVERSITY, COLLEGE OF SCIENCE

SCHOOL OF GRADUATE STUDIES

FOOD SCIENCE AND NUTRITION PROGRAM

Enrichment of Tef [*Eragrostis tef* (ZUCC.) *Trotter*] Flour With Soy Protein Concentrates For Injera Making

BY

SANDOCAN DEBEBE

A thesis submitted to the School of Graduate Studies of Addis Ababa University in partial fulfillment of the requirement for the Degree of Master of Science in Food Science and Nutrition.

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Abstract

In the continuous search for solution to the problem of malnutrition in its various forms, mainly among the people of the developing countries, views have been expressed of the need to improve the nutritive quality of staple local foods through better processing and enrichment. Consequently, this study was initiated with the aim of enriching tef (Eragrostis teff (ZUCC.) Trotter) flour (used to make injera: widely used staple food in Ethiopia) with soy protein concentrate (SPC) and thereby enhance its protein content and to analyse the effect on other nutritional component and organoleptic properties of injera. Very white tef (Quncho variety) and defatted Soy flour (Glycine max) were collected from Debre Zeit Agricultural Research Center and Ethiopian Spice Factory, respectively. Then, SPC was prepared using ethanol/water (3:1 ratio) as a solvent. The prepared soy protein concentrate (with known proximate composition) was blended with tef flour in 5%, 10%, 15% and 20% ratios. The result divulges that the protein content of the injera increased from 11.31% for the control and 14.30%, 17.30%, 20.76 % and 25.06% for 5%, 10%, 15% and 20% SPC blended injera, respectively. Increase in protein content at 5% blend of SPC was not statistically significant from that of control injera. Other proximate compositions (moisture, ash and crude fiber) of the injera were also increased. However, fat, carbohydrate and gross energy content of injera were decreased. The mineral contents of enriched flour injera were found to show increment for all Fe, Zn and Ca content. Moreover, although increments in phytate and tannin content are observed in blended injera, the ANOVA performed on phytate content implies that the changes are not statistically significant ($p < 0.05$) with that of the control injera (100% tef flour) until 20% blend. The sensory analysis conducted was resulted in acceptance of injera by the panelist until 15% SPC blend. Mixture Design Expert software used to optimize the exact combination of targeted protein value (20.76%) and ranged overall acceptability (5.7-7.75) has demonstrated the optimum region blend for tef and SPC with the linear models was found to be 87.35% and 12.65%, respectively. Thus, this study has revealed that it is possible to minimize the protein deficiency in developing countries through enriching various staple cereals with SPC.

1 INTRODUCTION

1.1 Background

In developing countries, one of the greatest problem affecting millions of people, particularly, children is lack of adequate protein intake. Especially, in tropical and sub-tropical areas, feeding the fast-growing population is becoming a serious problem. These areas are also characterized by a shortage of animal protein and incidences of protein energy malnutrition (Hernandez *et al.*, 1996). In the case of Ethiopia, 52% of children suffer from stunting and 11% wasting, which result from the long-term effects of protein energy malnutrition (UNICEF, 2004).

Multiple policy measures have been taken to combat nutritional problems such as enrichment mechanism. "Enrichment" is defined as "the addition of one or more essential nutrients to a food whether or not it is normally contained in the food, for the purpose of preventing or correcting a demonstrated deficiency of one or more nutrients in the population or specific population groups" (FAO/WHO/UN, 2002). In general cereals have low protein content; enrichment of cereals with locally available legume which are having high protein can increase protein content of blended cereal and legume (Bolaji *et al.*, 2010). Of the legumes soybean is the most promising source of protein enrichment (Boonyaratpalin *et al.*, 1998).

High protein foods of animal origin such as meat, fish, milk and eggs are very expensive to low income households. Enrichment of staple foods with non-animal protein is one alternative mechanism although (Uche et al., 2008) efforts to increase the local production of these animal protein sources at affordable prices are still ongoing (Akerele, 1967).

Tef *(Eragrostis teff (ZUCC.) Trotter)* is one of the popular and indigenous cereal crops believed to have been originated in Ethiopia (Bultosa, 2007). Annually 1.32 million metric tons of *tef* is produced in Ethiopia (CSA, 1998). The principal use of tef grain for human food is the Ethiopian injera (a fermented, spongy, sour and circular flat bread), that is responsible for about 70 % of the Ethiopian population (Davison *et al.*, 2004). However, tef and its products are low in protein and deficient in essential amino acids (Ketema, 1997). It is also believed to have poor qualitative

and quantitative protein content. Thus, continuous dependence on tef without supplementing with meat, fish and/or other protein-rich sources would result in protein deficiency. On the other hand, due to high cost of animal proteins, most people cannot afford to use supplements. Equally ignorance about the importance of protein also contributes to the low protein intake.

There is, therefore, a need to look for cheaper but good quality protein sources that are readily available to supplement *tef*. Soybean, is one of protein rich legume with a good essential amino acid profile, is potentially useful for this purpose. Comparing soy beans with most other legumes, soy beans are much higher in protein (35% of energy) (Messina, 1995). It's oil is 61% polyunsaturated and 23.4% monounsaturated (Gunstone *et al.*, 1986). In addition, soy beans are cheap, have high quality protein source and are readily available in many countries where cereals are consumed in large quantities (Edema *et al.*, 2001).

Thus, the purpose of this study is to present the process of enriching tef grain flour with soy protein concentrates to develop protein enriched tef product and evaluate the effect on organoleptic and nutritional quality of the enriched injera.

1.2 Objective of the study

1.2.1 General objective

- ✓ To enrich tef flour used to make injera (widely used staple food in Ethiopia) with soy protein concentrate and thereby enhance its protein content.

1.2.2 Specific objectives

- ✓ To analyze the nutritional composition of newly developed injera
- ✓ To analyze the values of the minerals in the injera: Ca, Zn and Fe
- ✓ To analyze anti nutritional factors in the injera: Phytate and Tannin in the injera
- ✓ To carryout sensory evaluation of the new injera

2 LITERATURE REVIEW

2.1 Malnutrition in Ethiopia

According to Todd (2005), malnutrition is a physical condition or process that results from the interaction of inadequate diet and infection and is most commonly reflected in poor infant growth; reduced cognitive development, anemia, and blindness in those suffering severe micronutrient deficiency; and excess morbidity and mortality in adults and children alike. He also restated that undernutrition and overnutrition are two forms of malnutrition. Undernutrition is malnutrition due to inadequate food consumption or poor absorption or biological use of nutrients consumed due to illness, disease, or nutrient imbalances.

The food-bias on nutrition in Ethiopia starts from the interpretation of the definition of food security. This indeed is the core of the matter in linking the nutrition security strategy to food security strategy. Nutrition affects the availability of food and food in turn affects nutrition status of a household in rural farming families. The food side of the nutrition problem starts at the individual level but extends to household up to national level.

Malnutrition in the Ethiopian context has been described as a long-term year round phenomenon due to chronic inadequacies in food intake combined with high levels of illness. Malnutrition is the most common health problem affecting both children and adults in Ethiopia (Gillespie & Haddad, 2003). Gillespie and Haddad indicated that malnutrition occurs at all stages of the life cycle.

National Nutrition Surveys were carried out by Central Statistical Authority (CSA) in 1983, 1992, and 1998, which focused on rural Ethiopia. Based on data collected, the three indicators of protein-energy malnutrition (PEM) include weight-for-age (underweight), height-for-age (stunting) and weight-for-height (wasting) could be computed. As shown in Table 1, data collected in 1983 and 1992 indicated that the underweight prevalence rate for rural areas increased from 37.3 percent to 46.9 percent respectively for children 6 to 59 months of age. The

prevalence declined to 42 percent in 1998 in children of 3 to 59 months of age. The decline during this period needs to be qualified due to the inclusion of the lower age group.

Table 1: Trend in prevalence rates of underweight, stunting, and wasting, percent of children under 5 years of age

Status of Children	CSA Surveys			Ethiopia DHS
	1983	1992	1998	1999/00
Underweight (weight-for-age)	37.3	46.9	42.0	47.2
Stunting (height-for-age)	60.7	64.0	52.0	51.5
Wasting (weight-for-height)	8.0	9.3	9.0	10.5

Source : CSA, 1999; CSA & ORC Macro 2001

The prevalence of underweight children 6 to 59 months of age is estimated to be 47 percent in the Ethiopia Demographic and Health Survey (DHS) 2000 study (CSA & ORC Macro 2001). This represents a further increase of 5 percent, which is probably due to the drought of 1990/00. The height-for-age (stunting) prevalence for rural Ethiopia increased from 60.7 percent in 1982/83 to about 64 percent in 1992. However, there was a noticeable decline in stunting reported in 1998 with 52 percent of the children 3 to 59 months of age being classified as stunted (CSA, 1999). The Ethiopia DHS of 2000 estimated that 51.5 percent of children under 5 years of age were stunted (CSA & ORC Macro, 2001). The weight-for-height (wasting) prevalence picked up slightly from 8 percent of children 3 to 59 months of age in 1992 to 9.3 percent in 1992 for rural Ethiopia and then showed a marginal decline to 9 percent in 1998 (CSA, 1999). The proportion of wasted children increased to 10.5 percent in the DHS 2000. The higher level in 2000 is probably due to prevailing drought in that year, since wasting is a measure of acute malnutrition. The trend for all measures of malnutrition are found to be among the highest in the world, with figures not showing any significant improvement over the last twenty years, except for the prevalence rate of stunting which shows a decline of about 0.5 percentage points per year. Studies on malnutrition of adults (PEM) are scarce in Ethiopia except in some pocket studies

conducted by NGOs (Getachew *et al.*, 2001) and the Ethiopia DHS of 2000. The body mass index (BMI) is used as an indicator to measure the nutritional status of women and a high prevalence of chronic energy deficiency. The DHS found that 30.1 percent of women 15 to 49 years of age have a low BMI (< 18.5 kg/m2).

Survivors of child malnutrition can suffer from impaired physical development and limited intellectual abilities, which in turn may diminish their working capacity during adulthood with negative effects on economic growth. Child malnutrition may also lead to higher levels of chronic illness and disability in adult life and these may also have intergenerational effects as malnourished females are more likely to give birth to low-weight babies (Todd, 2005).

2.1.1 Causes of malnutrition in Ethiopia

While the problem of malnutrition in Ethiopia is relatively well-documented (Alemu *et. al.*, 2005), its specific determinants are not well understood. To reduce malnutrition one must understand its causes. Not only are extant studies based predominantly on small-scale surveys that focus on particular regions of the country, but there is a lack of agreement about the relative importance of factors affecting nutritional status. For example, some empirical studies stress the importance of parental education and/or nutritional knowledge, while others recommend the need to focus on improving the poverty/wealth status of households. This question is not only of academic interest but of considerable policy relevance, both among national and international policy-makers (Alemu *et. al.*, 2005)

The conservation, proper utilization, and management of land and water resources, literacy, and skill development for rural youth and food and nutrition-focused education to women are key determinants at the national level. Developing household livelihood systems that maximize the creation and building of their assets and change in the conventional cultural practices that are detrimental to the nutritional status of children and women should be considered as major determining sets of factors to positively impact nutritional status at the household level (Todd, 2005).

2.1.2 Protein energy malnutrition

The term Protein Energy Malnutrition (PEM) includes a wide spectrum of malnutrition primarily affecting children in developing countries (infants, pre-school). It's severe clinical forms are: Marasmus, Kwashiorkor and Mixed feature called marasmic-kwashiorkor. The milder forms of it like stunting (chronic form) and wasting (acute) forms of malnutrition are highly widespread in developing countries (Tefera *et. al.*, 2001). Low birth weight (< 2500 g) is now recognized as a major cause of stunting in childhood which will be present in an estimated 17±4 million infants (16±4% of newborns) born in developing countries in 2000 (Table 2). Low birth eight is particularly common in South Central Asia, where 28±4%of newborns are affected and is a major problem also in middle and western Africa (21±3% and 17±2%, respectively) (Stephenson *et al.*, 2000).

If these cases of PEM can be recognized early enough by routine weight and height measurements (growth monitoring in under five clinics) and relevant action taken, then severe malnutrition can often be prevented easily. It is not sufficient to treat only severe cases of malnutrition coming to the health institution, as those coming to the health institution are the tips of an iceberg (Tefera *et. al.*, 2001). Therefore, further approaches at the grass root community level are required

Table 2. Estimated incidence of low birth weight (LBW) and intrauterine growth retardation-low birth weight (IUGR-LBW) and expected number of affected newborns in year 2000

	LBW		IUGR-LBW	
United Nations regions and sub_regions	**Incidence % (<2500g)[a]**	**Total No. (thousands)[b]**	**Incidence % (<2500g; ≥ 37 weeks)[a]**	**Total No. (thousands)[b]**
Africa	–	–	–	–
Eastern	–	–	–	–
Middle	21.3	853	14.9	597
Northern	–	–	–	–
Western	17.2	1451	11.4	962
Asia[e]	18.0	13774	12.3	9344
Eastern[e]	5.8	1250	1.9	409
South Central	28.3	10917	5.6	8062
South-East	10.3	1190	5.6	647
Western	8.3	417	4.5	226
Latin America and Caribbean	11.5	1329	6.5	755
Caribbean	11.7	91.0	6.7	52
Central America	12.3	422.0	7.2	247
South America	11.1	816.0	6.2	456
Oceania[d]	15.0	29.2	9.8	19
Melanesia	15.4	29.0	9.9	19
Micronesia	–	–	–	–
Polynesia	4.0	0.2	0.2	0.03
All Developing countries[e]	16.4	17436	11.0	11677

[a] **Sources**: WHO, 1996*a*; [b] Total live births for 2000 are based on the UN World Population Prospects (United Nations 1998). [c] Excludes Japan. [d] Excludes Australia and New Zealand; -, not applicable because coverage of live births !80%. [e] Weighted average of incidences in each country.

2.1.3 Ways to combat the problem

Aid

The food security situation in Ethiopia has been extremely precarious for some eight million people due to the combination of environmental, socio-political and developmental instabilities (Todd, 2005). Drought is a recurrent feature of the climate of Ethiopia, and its effects are severe since the agriculture is predominantly rain-fed. In general, the culinary and eating habits in each of the regions are homogeneous and non-diversified, mainly based on cereals and pulses. This sort of diet may result in kwashiorkor, stunted growth and Motor neuron disorders (MNDs). The food aid rations have not met the needs of the vulnerable populations for macro- and

micronutrients (Todd, 2005). Food aid to Ethiopia has been based on wheat, substituting the customary consumption of tef. This can contribute even further to MNDs, such as iron (Dorit et al., 2001).

Complementary Food

In developing countries, where malnutrition remains a major health problem in infants and pre-school children, considerable efforts to improve the health and nutritional status of growing children have focused on the production of nutritious low-cost complementary foods. As cereals are generally low in protein and are limiting in some essential amino acids, notably lysine and trytophan, supplementation of cereals with locally available legumes that are high in protein and lysine, although often limiting in sulphur amino acids, increases protein content of cereal-legume blends and their protein quality through mutual complementation of their individual amino acids (Osundahunsi & Aworh, 2003).

Enrichment

Stable food in Africa in 1970s and 1980s, when PEM was prevalent, was cereals like maize and wheat. And the natural cultigens of maize are known to be deficient in one of the essential amino acids (lysine) have has relatively low protein content (Egal, 2011). In the continuous search for solution to the problem of malnutrition in its various forms, mainly among the people of the developing countries, views have been expressed of the need to improve the nutritive quality of our local food through better processing and enrichment. Low-cost fortified blended foods were originally developed in the United States of America during the 1960s. Initially milk powder was used as the complementary source of protein. For economic and various other reasons, soybeans are now being used. Corn-soya blend and wheat-soya blend are the best known examples of fortified blended foods (WFP, 2002). To solve the problem of nutrient deficiency, the enrichment of staple food is routine and has proven to be very efficient for certain macro and micronutrients (Darnton & Nalubola, 2002). Maize, wheat and now tef are some of basic foods worthy of attention. These are some of the foods widely eaten through out Ethiopia (Uche et al, 2008).

Here soya is rich with lysine and have high protein (Egal, 2011) hence was used to produce the CSB (Corn Soya Blend -80 percent maize and 20 percent soy beans) product for this purpose.

2.1.4 Benefits of enriching foods

Since the early 20th century, food enrichment\fortification has reduced and eliminated nutrient deficiencies in many countries (Micronutrient Initiative, 1997 The efficacy of food enrichment in reducing the prevalence of nutritional deficiencies has been proven mainly in countries with relatively sophisticated food processing, distribution and marketing systems. In many developing countries where macro and micronutrient malnutrition remains a pubic health problem, a variety of products are voluntarily enriched (Micronutrient Initiative, 1997). The approach to food enrichment in order to end malnutrition focuses not only on whether the technology works, but rather on how it can be cost-effectively adapted in situations where production and distribution systems are relatively unsophisticated and where consumers have relatively low purchasing power.). If consumption of a particular food is consistent in some groups but only periodic in others, enriching several vehicles provides for complementary coverage.

Along with a growing understanding of the extent and impact of macro and micronutrient malnutrition, a number of interventions have demonstrated both the feasibility and the benefits of correction and prevention (Todd, 2005). Distributing inexpensive capsules, diversifying to include more micronutrient rich foods, or fortifying commonly consumed foods can make an enormous difference.

Thus in doing so, one has to focus on crops that have been cultivated for a long period of time in a country, and are able to provide reliable yield under unreliable agro-climatic conditions and make ranking first against area coverage, demand and market value. In Ethiopia case also the need to improve the nutritive quality of our local food through better processing and enrichment should obtain considerable focus. There for, *Injera* is one of such basic foods worthy of attention.

2.2 Tef

Tef, Eragrostis tef (Zucc.) Trotter, belongs to the family Poaceae, subfamily Eragrostoideae, tribe Eragrosteae and genus Eragrostis (Ketema, 1997). The grain tef is very small, oval in shape and uniform in size (1.0 – 1.2 mm in length) (Umeta *et al.*, 1996). There are approximately 350 species in the genus Eragrostis consisting of both annuals and perennials which are found over a wide geographic range. Eragrostis tef is one of those species (Patricia, 2008). It comes in a range of colours from milky white to almost dark brown. The most common colours are very white, white, light brown and dark brown (Bultosa *et al.*, 2002).

Fig 1: Tef farm

Ethiopia is the center of origin and diversity for tef (Costanza *et al.*, 1980; Demissie, 2001; Vavilov, 1951). According to CSA (2008) report, tef [Eragrostis tef (Zucc) Trotter] has the largest share of area (23.42 %, 2.6 million hectares) under cereal cultivation and third (i.e. after maize and wheat) in terms of grain production (18.57 %, 29.9 million quintals) in Ethiopia. The crop is highly adapted to diverse agro ecological zones including conditions marginal to the production of most other crops. It can grow from sea level up to 3000 meters above sea level and performs well between 1700 and 2400 m. Tef is cultivated in high rainfall areas with long growing periods. It can also grow in low rainfall and drought prone areas characterized by

protracted growing seasons and frequent terminal moisture stress (Kelemework *et al.*, 2000). It is pest resistant, drought tolerant, fast maturing, and does well in a tremendous range of soil conditions, including vertisolic substrates that are prone to water logging (Tadesse, 1993; Jones, 1988; Tefera and Ketema, 2001). One of the identified problems with tef is low productivity, and thus, yield improvement is a main focus of modern breeding programs (Ketema, 1983). The cultivation of tef in Ethiopia has partly been motivated by its relative merits over other cereals in the use of both the grain and straw (Kelemework *et al.*, 2000).

2.2.1 Characteristics of tef

The micro- and macronutrients level of grain tef is apparently higher than that of barley, wheat and sorghum .The nutrient composition of grain tef indicates that it has good potential to be used in foods and beverages worldwide (Bultosa *el al.*, 2002).

2.2.2 Chemical characteristics

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. 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 (Patricia, 2008).

Lipids: The crude fat content of tef grain ranges from 2.0-3.0% with mean of 2.3% according to Bultosa (2007). He also reported that, even though, germ in tef is known to occupy large proportion as in other small grain its crude fat is known to be not as such high. Fat content of tef starches has slightly lower hydrolyzed lipids (mean 8.9 mg/g) than maize starch (9.9 mg/g). The crude fat (ether extract) content of the tef starches (mean 0.29%) is 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 tef which is around 2% (DM) (National Research Council, 1996). Crude fat (petroleum ether extract) consist mostly of non-starch lipids *i.e.*, it is not endogenous to the starch (Bhojwani *et al.*, 1986). The low crude fat content in tef starch is most probably related to the low crude fat

content of the grain. Bultosa *et al,* (2002) found that the tef 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 tef, starch is the largest proportion (Umeta *et al.*, 1988). The tef flour starch amylase % has reported to range from 20.0 - 25.8% with mean 23.0% (Bultosa, 2007). Amylose in maize and tef starches analyzed along with the tef flour varieties has showed that tef has lower amylase content than maize, which are 28.4% and 27.7%, respectively. The presence of other trace flour components in the tef flour sample could somehow interferes and suppresses the starch sample dissolution and the iodine binding with amylase of the blue color formation similar as reported in other cereals and contribute for lower amylase % in the tef flour (Bultosa, 2007)

Belta *et al,(*2002) found an amylose content ranges from 24.9-31.7 %, depending on the method used for the determination and on the tef variety analyzed. Amylose determination was made with two different methods. Both methods showed that the amylose content of the tef varieties studied is typical of normal native cereal starches like maize, sorghum and wheat with no waxy- or amylo-type starches (Whistler & James, 1997).

During the tef products processing, and depending on the degree of enzyme (Whistler & James, 1997) or acid treatment, starch can be depolymerised to different types of oligo and monosaccharides (maltodextrins and glucose).

Protein: Bultosa (2007) has reported the protein content of grain tef as it ranges from 8.7- 11.1% with mean 10.4%. Compared to other cereals, tef has higher protein content than, like maize (8.3%), sorghum (7.1%), barley (9.0%), millet (7.2%) and almost equivalent to wheat (10.3%) (Asrat & Frew, 2001). The amino acid composition of grain tef is reported to be comparable to that of egg protein, except for its lower lysine content (Patricia, 2008).

Tef starch has protein contents in the range of 0.16 – 0.23% (Watson, 1998). He also found a mean protein content of the tef starches of 0.19%. This is higher than that of maize starch

(0.07%). The protein content among the tef varieties probably varied depending upon the degree of contamination of the starch by the proteins of the endosperm.

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 tef has a very big nutritive value (Yigzaw *et al.*, 2001). Besides providing protein and calories, tef 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 tef has, is the lack of gluten in the grain. This made it useful for patients with the celiac disease. The tef starche has 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.

2.2.3 Physical characteristics

Physical characteristics of a food are the result of the macro components concentration, their relationship and their behavior under different environment conditions. Endosperm texture has been identified as a factor that consistently affects the processing and food making properties of cereals like tef and sorghum (Rooney & pflugfelder, 1986). The endosperm matrix protein and protein associated with starch which affects endosperm texture also influences the milling, pasting and fermentation properties of the grain (Yetneberk, 2004). The tef starch granule is a compound type from which many simpler (2–6 μm in diameter) polygonal shaped granules are

Table 3: Proximate composition and flour starch amylose % of the 13 tef grain varieties

Variety	Moisture(%)	Grain Protein(%)[&]	Ash (%)[&]	Crude fat(%)[&]	Crude fiber(%)[&]	Amylose(%) from flour[&]
DZ-01-354	11.07±0.02hi*	10.60.7c-f	2.18±0.0b	2.1±0.2a	3.3±0.2c-e	25.8±0.7d
DZ-01-99	10.83±0.13fg	10.8±0.36-f	3.16±0.0f	2.1±0.0a	3.8±0.3e	22.9±1.2bc
DZ-01-196	9.69±0.03b	10.4±0.6b-e	2.14±0.0b	2.4±0.7ab	3.2±0.2bc	22.1±11.0bc
DZ-01-787	10.85±0.12fg	10.4±0.5c-f	2.06±0.0a	2.5±0.5ab	3.5±0.1c-e	23.8±2.1cd
DZ-Cr-44	9.30±0.04a	10.7±0.0c-f	2.82±0.1d	2.6±0.6ab	2.7±0.1ab	25.6±0.3d
DZ-Cr-82	10.79±0.14f	10.6±0.3c-f	2.15±0.0b	3.0±0.4b	3.5±0.2c-e	22.3±0.6bc
DZ-Cr-37	11.15±0.08i	11.0±0.2ef	2.54±0.0c	2.0±0.3a	3.3±0.4cd	22.7±0.6bc
DZ-Cr-255	10.24±0.08d	11.1±0.1ef	3.10±0.1f	2.5±0.6ab	2.6±0.2a	20.0±0.4a
DZ-01-974	10.33±0.10d	10.0±0.5bc	2.16±0.0b	2.1±0.2a	3.5±0.3c-e	22.7±0.6bc
DZ-Cr-358	10.49±0.06e	10.1±0.2b-d	1.99±0.0a	2.2±0.0ab	3.5±0.4c-e	22.4±0.5bc
DZ-01-1281	11.22±0.11i	11.1±0.2f	2.52±0.0c	2.5±0.7ab	3.4±0.5c-e	22.7±1.4bc
DZ-01-1285	9.96±0.06c	8.7±0.1a	2.02±0.0a	2.5±0.6ab	3.1±0.4bc	24.2±2.1cd
DZ-01-1681	10.96±0.04gh	9.7±0.3b	2.99±0.0e	2.0±0.0a	3.7±0.1de	21.2±0.5ab
Mean	**10.53±0.58**	**10.4±0.7**	**2.45±0.42**	**2.3±0.5**	**3.3±0.4**	**23.0±1.8**
Range	**11.22-9.30**	**11.1-8.7**	**3.16-1.99**	**3.0-2.0**	**3.8-2.6**	**25.8-20.0**
Maize Starch (n=7)						28.4±2.0
Tef (DZ-Cr-37) starch (n=7)						27.7±1.9

*Values within the same column with different letters are significantly different ($p < 0.05$) and are means of at least three determinations.

[&] Values are on dry matter basis

Source : Bultosa (2007)

released on milling (Umeta and Parker, 1996). The compound granule surface is smooth, with no evidence of pores. The small granule size of tef 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 (a measure of how much the flour component is soluble in water, is useful as it is a reflection of the strength of the network within the starch granules (Qian *et al.*, 1998) than maize starch (Bultosa *et al.*, 2002). An anatomical study of tef grain has revealed that it contains compound starch granules (Umeta and Parker, 1996), similar to those of rice and amaranthus.

Seed protein; in general, are composed of three groups, namely storage proteins, structural proteins and biologically active protein (enzymes) (Fukushima, 1991). The storage protein have been described as a sink for surplus nitrogenous compound required for physiological processes (Tsai *et al.*, 1978).

In relation of pasting properties o starches, lipids are reported to cause a higher pasting temperature and a lower starch pas viscosity (Fortuna et al 200). Free faty acids, however, increase starch paste viscosity (Nelles et al., 2000)

2.2.4 Nutritional value of tef

Nutritionally tef is comparable to other major cereals (i.e., wheat, rice, oats, maize, and sorghum) and it provides about two-thirds of the daily dietary protein intake for most Ethiopians (Ketema, 1997). And the reason that tef grain is recognized for its better nutritional value than common cereal grain is because it is consumed as a whole grains mostly as injera (a fermented, spongy, sour circular flat bread) (Ketema, 1997) and free from the type of gluten found in wheat (Spaenij-Dekking *et al.*, 2005). Various studies (Yetneberk et. al., 2004; Zegeye, 1997) indicate that tef grain injera is superior among other cereal grains.

Tef consist proximate composition of moisture, ash, protein (%N×6.25), crude fat and crud fiber had ranged 9.30 - 11.22 (mean 10.53), 1.99 - 3.16 (2.45), 8.7 - 11.1 (10.4), 2.0-3.0 (2.3), 2.6 - 3.8 (3.3), respectively (Bultosa, 2007). Starch is the largest proportion in the carbohydrate fraction of grain tef (Umeta and Parker, 1996). Matrix change of starch was reported to be a major

contributor to the texture of *injera (*Parker *et al.,* 1989). It is also considered to have an excellent amino acid composition, with lysine levels higher than wheat or barley, as well as very high calcium, phosphorous, iron, copper, aluminum, barium, and thiamine (Mengesha, 1965). While the reported high iron content (0.05%) of tef seed has been refuted the lack of anemia in Ethiopia is considered to be due to the available iron from injera (Ketema, 1997).

2.2.5 Uses of tef

In Ethiopia, tef is a major cereal crop and has been grown in other African countries as a hay crop. Tef flour is primarily used to make a fermented, sourdough type, flat bread called Injera (Davison *et al.*, 2004). Tef 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) In addition, tef can be substituted for seeds, nuts or other small grains when baking, used as a thickener for soups, stews, gravies and puddings, in making grain burgers, and in stir-fries and casseroles. The seeds can also be sprouted and the sprouts used in salads and on sandwiches (Ratchanee, 2009.). Tef straw is the preferred binding material for walls, bricks and household containers made of clay in Ethiopia. In addition, the tef plant is used as a livestock forage or pasture crop. It is primarily grown in Africa, India, Australia and South America. In the United States, tef is grown on limited areas in the Pacific Northwest and Midwest (Michael, 2008). Recently alternate tef dishes, such as tef breakfast cereal, tef waffles, and tef banana bread are becoming popular in the United States of America (EARO, 2000).

2.2.6 Tef and legumes blend

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 (Patricia, 2008). It could also decrease certain anti nutritional factors like phytates, protease inhibitors and flatulence factors. Tef has been used by Yigzaw et al. (2001) to be mixed 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 crop with tef is sunflower (Patricia, 2008).

Although mixing tef 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. (2001) did not want to go higher than 8:2 (tef: grass pea) ratio as a compromise between nutritional adequacy and sensory value (Patricia , 2008).

Another trial on blending tef with full fat soybean, which was done by Jemal (2010), and targeted on the effect of tef-soy flour blend ratio on physical properties and hardness of extrudates and resulted with optimum blend ratio (5% soy, 10% moisture and 135°C temperature) considering sensory and nutritional value of the product.

2.2.7 Processing of Injera

The principal use of tef grain for human food is the Ethiopian bread (injera, flat bread that is responsible for about 70 % of the Ethiopian population) (Davison *et al.*, 2004). Injera is sourdough type flat bread. It is described as a soft, porous, thin pancake, which has a sour 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), enjera has a tasty sweet flavour. It can be made also from other cereals like wheat, barley, sorghum, or maize or a combination of some of these cereals.

Tef is ground into flour, fermented for three days then made into injera. 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 (Patricia, 2008)

Yeast, Candida guilliermondii (Cast.), is the micro-organism primarily responsible for the fermentation process (Stewart & Getachew, 1962). Recently alternate tef dishes, such as tef breakfast cereal, tef waffles, and tef banana bread are becoming popular in the United States of America (EARO, 2000).

2.3 Soy protein

The soybean plant (Glycine max) belongs to the legume family. Since the 1960s, soy protein products have been used as nutritional and functional food ingredients in many food categories available to the consumer (Joseph, 2001). It is one of the very few plants that provide a complete protein source and it has high quality protein with minimum saturated fat. Protein from the oilseed soybean is an economical and nutritious source with which to supplement the world's protein supply (Smith & Circle, 1972). Furthermore, the cost of soy protein is dramatically less than that for expensive and inefficiently produced animal protein. In addition, the nutritional quality of soy protein compares favorably with that from the animal (Meyer, 1971). In order to maximize the potential of soybean as a world food source, it is necessary to provide the food in a form that is acceptable to the culture for which it is intended (Meyer, 1971). Protein concentrates and isolates of the soybean offer the greatest opportunity for incorporation into a wide variety of familiar food forms.

2.3.1 Nutritional effects of soy protein blend

Many applications for soy protein products involve combinations with cereal grains and/or alternate protein sources. Their addition raises the quality (as with alternate proteins) and the quantity (as with cereal sources). Soy protein amino acid profiles (rich in lysine, limiting in sulfur amino acids; Joseph, 2001) fit nicely with grain proteins (limiting in lysine, rich in sulfur amino acids). The resulting protein quality, if properly blended, is superior to the individual components. Substantial percentages of soy flour have been incorporated successfully into bread (Joseph, 2001). By adding 12% soy flour in bread, the lysine content of the bread is more than doubled, and the protein content is increased by up to 50% (Dubois & Hoover, 1981). Blending nonfat dry milk (NFDM) and SPC at any level yields a Protein Digestibility-Corrected Amino acid Score (PDCAAS) value of 1.0. Blending soy protein concentrate with rice flour at a 10% level raises the PDCAAS of the mixture from 0.65 for 100% rice flour to 0.98 for the 90/10 blend. Similar results have been obtained when blending soy protein concentrate with wheat flour or barley flour (Joseph, 2001).

Table 4: Nutritional content of soyfoods

NUTRIENT CONTENT OF SOYFOODS													
SOYFOOD	**Kcal**	**Protien**	**Fat**	**CHO**	**Crude Fiber**	**Calcium**	**Iron**	**Zinc**	**Thiamin**	**Riboflavin**	**Niacin**	**Vit B6**	**Folate**
100 grams		(g)	(g)	(g)	(g)	(mg)	(mg)	(mg)	(mg)	(mg)	(mg)	(mg)	(mcg)
MISO	206	11.8	1-Jun	28	2.5	66	2.74	3.32	0.1	0.25	0.86	0.22	33
NATTO	212	17.7	11	14.4	1.6	217	8.6	3.03	0.16	0.19	0	N/A	N/A
SOY FLOUR defatted	329	47	1.2	38.4	4.3	241	9.24	2.46	0.7	0.25	2.61	0.57	305.4
SOY FLOUR full-fat raw	436	34.5	20.6	35.2	4.7	206	6.37	3.92	0.58	1.16	4.32	0.46	345
SOY PROTEIN concentrate	332	58.1	0.5	31.2	3.8	363	10.78	4.4	0.32	0.14	0.72	0.13	340
SOY PROTEIN isolate	338	80.7	3.4	7.4	0.3	178	14.5	4.03	0.18	0.10	1.44	N/A	176
SOY SAUCE tamari	60	10.5	0.1	5.6	0	20	2.83	0.43	0.06	0.15	3.95	0.2	18.2
SOYBEANS boiled	173	16.6	9	9.9	2	102	5.14	1.15	0.16	0.29	0.4	0.23	53.8
SOYBEANS, roasted	474	35.2	25.4	33.6	4.6	138	3.9	3.14	0.1	0.15	1.41	0.21	211
SOY MILK	33	2.8	1.9	1.8	1.1	4	0.58	0.23	0.16	0.07	0.15	0.04	1.5
TEMPEH	199	19	7.7	17	3	93	2.26	1.81	0.13	0.11	4.63	0.3	52
TOF, raw, firm	145	15.8	8.7	4.3	0.2	205	10.47	1.57	0.16	0.1	0.38	0.09	29.3
TOFU, raw, regular	76	8.1	4.8	1.9	0.1	105	5.36	0.8	0.08	0.05	0.2	0.05	15

Source: USDA, (1986)

2.3.2 Physico-chemical effects of soy protein blend

Incorporation of soy protein can significantly impact the mechanical, physico-chemical and micro-structural properties of foods. Several studies have investigated the addition of soy protein to extruded starch products with conflicting results (Chang *et al.*, 2001; Faubion and Hosney, 1982; Ghorpade *et al.*, 1997; Li et al., 2007; Zasypkin and Lee, 1998). Chang *et al.* (2001) found that radial expansion increased on addition of up to 25% soy protein concentrates (SPC) to a cassava starch-based matrix. Further addition of SPC decreased the radial expansion. Additionally, with increasing soy protein concentrate, hardness decreased, water absorption index (WAI) increased, and water solubility index (WSI) decreased. Faubion and Hosney (1982) reported that the expansion of wheat starch with 1–8% soy protein isolate was higher than that of pure starch. However, at 10% soy protein isolate, expansion decreased. Ghorpade *et al.* (1997), on the other hand, showed that increasing the percentage of soy protein isolate from 10% to 30% in corn starch extrudates did not significantly affect bulk density and the percentage of open pores. Zasypkin and Lee (1998) showed that increasing the proportion of soybean flour to 10% in a wheat flour-soybean flour blend resulted decrease in expansion ratio (ER), at 16% in-barrel moisture content (MC), and an increase in ER at 17–18% MC. Further addition of soy flour up to 40% led to continuous decrease in expansion ratio. Beyond 40%, the trends for ER were again dependent on MC and also a possible phase inversion occurring beyond that level of soy flour. (Li et al., 2005) reported that the addition of soybean flour to corn meal in the range of 0 to 40% increased ER.

2.3.3 Anti-nutritional factors in soybean seeds

According to their temperature resistance the anti-nutritional factors can be classified in heat labile and heat stable Anti-nutritional factors (Table 5)

Table 5. Anti-nutritional factors in soybeans

Heat labile	**Heat stable**
Protease inhibitors	Saponins
Lectins	Estrogens
Goitrogens	Cyanogens
	Phytate
	Oligosaccharides
	Antigens
	Tannin

Source: Peisker (2001)

Protease inhibitors: Among the anti-nutritional factors present in soybean seed, the main ones are protease inhibitors. Protease inhibitors represent 6% of the protein present in soybean seed (Mikic *et al.*, 2009). Protease inhibitors are actively inhibiting the digestive enzymes trypsin and chymotrypsin and lower protein digestibility.

Lectins: Lectins are proteins that are widely distributed in plant kingdom and have unique property of binding carbohydrate-containing molecules with a high specificity, causing agglutination of red blood cells (Mikic *et al.*, 2009). Soybean agglutinin (SBA) causes the waste away of the microvilli, reduces the viability of the epithelial cells and increases the weight of small intestine because of hyperplasia of crypt cells (Grant *et al.*, 1987). The inactivation of soybean lectin by a moist heat treatment is parallel with the destruction of trypsin inhibitors. Soybean lectin is quite resistant to inactivation by dry heat treatment.

Goitrogens lead to an enlargement of the thyroid gland. All three are easily to be destroyed or inactivated with appropriate heat processing, e.g. toasting, extrusion, and treatment with hot air or microwaves (infrared) after wetting (Peisker, 2001).

Saponins are glycosides and characterised by a bitter taste. They may hemolyze red blood cells. Estrogens (Isoflavonids) can cause an enlargement of the reproductive tract and cyanogens are split in the digestive tract to the poisonous hydrogen cyanide.

Phytate: Phytic acid is another anti-nutritional factors present in soybean seed and products to the extent of 1-1.5% of DM (Mikic *et al.*, 2009). It is able to chelate mineral elements, such as zinc, magnesium, iron, calcium and potassium and makes these elements longer absorbed from intestines. About two thirds of the total phosphorus from soybean seed is bound to phytic acid (Nelson , 1968). Several soybean genotypes have been developed with a low phytic acid content (Spear and Fehr, 2007).

Oligosaccharides form the majority of the carbohydrate fraction in soybeans. Only about 2% of the carbohydrate fractions are starch and 6% cellulosic compounds (Peisker, 2001).The non-starch oligosaccharides impair digestion (intestinal cramps, diarrhoea, and flatulence) due to lack of the appropriate digestive enzymes of the host specie.

Antigenic factors (glycinin and β-conglycinin) cause the formation of antibodies in the serum of preruminant calves and small piglets. They prevent the proliferation of certain beneficial bacteria in the gastro-intestinal tract. (Refstie, 1998).

However, the presence of heat stable factors in soybeans is with the exception of the oligosaccharides and the antigenic factors, rather small and not very likely to cause problems under practical conditions. But removal of the oligosaccharides and glycinin/β-conglycinin from defatted flakes contributes to a significant increase in the nutritional value. Further, the protein concentration in the SPC is much higher than in soybean meal, which makes it an interesting ingredient for high protein, high energy diets (Peisker, 2001).

2.3.4 Processed soybean

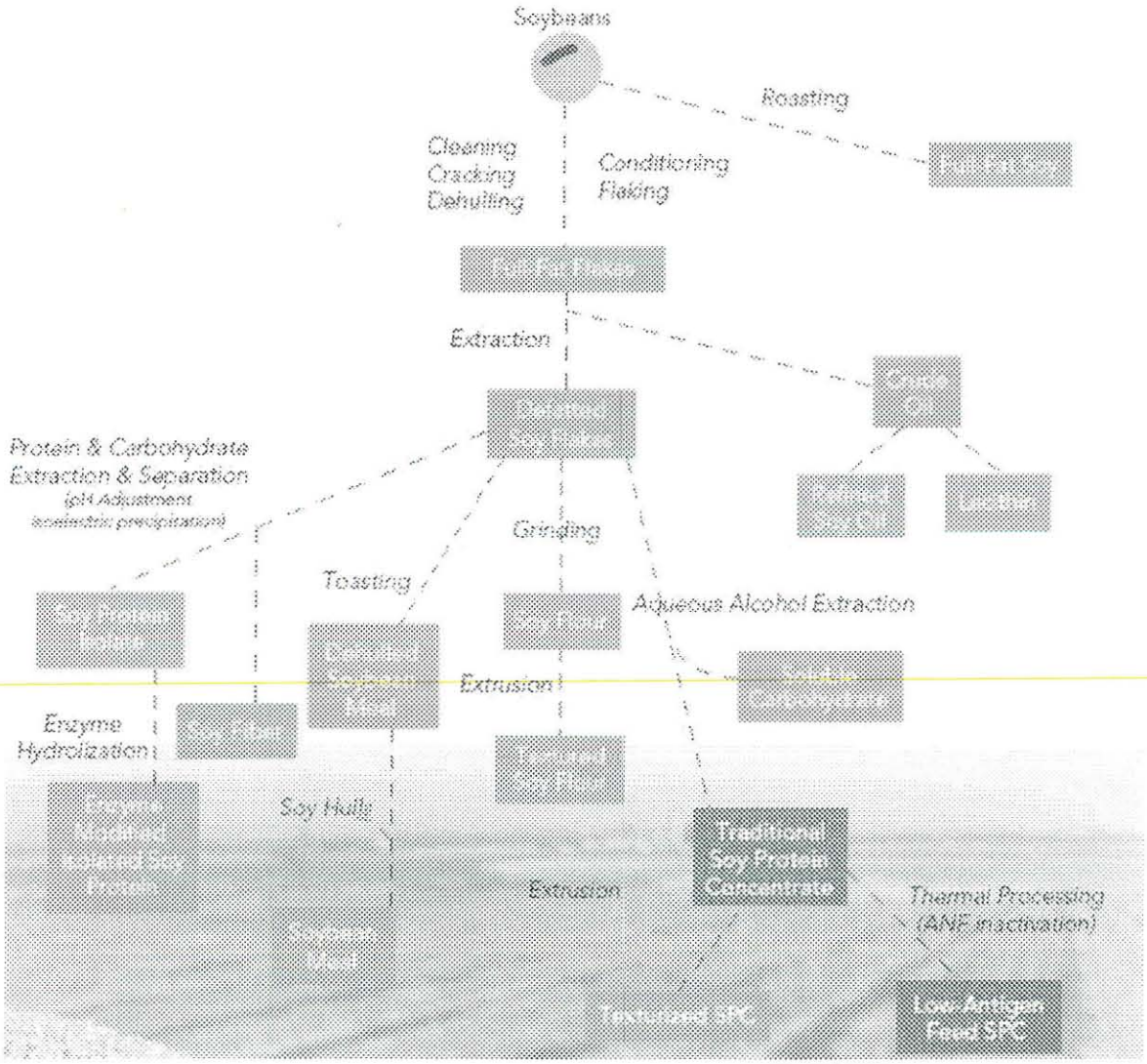
Soy flour processing falls into three major groups. These groups are based on protein content, and range from 40% to over 90%. All three basic soy protein product groups (except full-fat and partially defatted extruded-expelled flours) are derived from defatted flakes. They are:

Soybean grits. This is a coarsely ground product, ~ 10-30 mesh, produced by grinding dehulled defatted soybean flakes. On a moisture-free basis, grits contain >50% protein and 32% carbohydrate. Grits can be used in baked products, meat substitutes and extenders. (Barbara, 1995)

Soybean flour. Ground soybean flakes, which may be defatted by hexane extraction, are the base material for soybean flour. A variety of soy flours can be produced, defined by their lipid content (Fulmer, 1989). Like soybean grits, Soybean flours usually contain ~50% protein and 30% carbohydrate (moisture-free basis), but the percentage of fat can be varied. (Barbara, 1995)

Soybean concentrates. To achieve a concentrate with >65% protein and 27% carbohydrate on a dry weight basis, manufacturers must start with a defatted milled product such as coarse grits, soy flour or feed stock (Beery, 1989). The concentrate is made by immobilizing the protein by isoelectric precipitation with aqueous acid, 60-80% aqueous alcohol, moist heat or mixed organic solvents. Soluble proteins are removed by these procedures, along with some non protein components, including a portion of the carbohydrates and salts. Soy protein concentrates (SPC) can be dried, rehydrated, flavored and made into flakes, fibers and granules. Typical composition of SPC is 71.7% protein on a dry weight basis (8.5% moisture), 0.5% fat, 6.2% ash, 3.5% crude fiber and 15.8% carbohydrate. (Barbara, 1995)

Fig 2: Soybean processing flow chart showing the techniques used to produce various soy products for human and animal use.



Source : USSEC (2008)

Isolated soy proteins. The most purified form of commercial soy protein ingredients is isolated soy protein (ISP) (Hoogenkamp 1993, Waggle *et al.,* 1989). These are high-quality food ingredients that are generally recognized as safe by the U.S. Food and Drug Administration. The major protein fractions are removed to obtain 90% protein (N X 6.25) on a moisture-free basis. The traditional process for isolating soy proteins was developed in the 1950s. Although there are

modifications and patents, there are some essential steps. The dehulled flaked beans are extracted with hexane to remove oil, followed by water or alkali extraction of soluble proteins and carbohydrates. The major proteins are precipitated at pH 4.5, centrifuged and washed to remove carbohydrates and whey proteins that are soluble at that pH. (Lusas and Riaz, 1995, Waggle *et al.*, 1989).

Soybeans are also high in iron, calcium and zinc (Slavin, 1991). It is known that phytates bind minerals, particularly zinc and iron, and can therefore decrease absorption. It is possible that mineral absorption is better from fermented soyfoods because of their reduced phytate content (Klein, 1996). Calcium absorption from soy foods has been shown to be similar to that of milk (Slavin, 1991). In the case of iron, research has shown that iron absorption is increased when a vitamin C source is eaten with soy foods Soyfoods are good sources of the B vitamins, especially thiamin, B6 and folate.

Whole soybeans contain large amounts of the fat soluble vitamin E. A recent review of the literature (Slavin, 1991) concluded that after degumming, refining, bleaching and deodorizing, soy oil retains an average of 68.3% of its vitamin E. One tablespoon of soy oil provides about a third of our daily requirement for vitamin E.

2.3.5 Soybean products

Using soy proteins successfully in traditional foods depends on formulating products in such a manner that the traditional characteristics of that product are maintained. When plant proteins replace animal proteins, it is critical that traditional food characteristics and quality not be changed. In new foods, soy products must also contribute to the overall appeal of the product.

Proteins affect the sensory properties of foods, i.e. the appearance, color, flavor, taste, and texture, which are key attributes determining consumer acceptance. The flavor of soy proteins, and their interaction with both desirable and undesirable flavors, is extremely critical. This determines the application of soy proteins and suggests choices between products and usage level. Table 6 outlines the major food uses of soy protein products.

Bakery Products

In bakery products, soy protein ingredients are being used for a variety of functional and nutritional reasons. As a general rule, when adding soy flour to various baked goods formulations, up to 3% of the wheat flour may be replaced with soy flour without any further formula adjustments other than water. Usually, for every pound of soy flour substituted in the formula, an additional 1 to 1.5 pounds of water must be added. Higher levels of water can be added when SSL (Sodium Stearoyl Lactylate) is used as an emulsifier (Joseph, 2001).

Pasta Products

High protein pasta products, such as spaghetti, can be prepared from durum semolina (Seyam *et al.*, 1979) or hard wheat farina fortified with soy protein products. All soy protein products increase the water absorption of spaghetti dough and affect its processing conditions. Of the soy products tested, soy protein isolates perform best (Joseph, 2001).

Breakfast Cereals

Expanded emphasis on nutrition in breakfast cereals has led to an increased use of soy protein to boost protein value and quantity. This is especially true now that the FDA has allowed a health claim to be made for the addition of soy protein to foods to fight CHD (Laignelet & Feillet, 1974). Soy proteins are used extensively as ingredients in hot cereal mixes and as components of compound breakfast bars.

Dairy-Type Products

To lower costs, improve nutrition, reduce allergy response, and improve functionality, a number of dairy analog products have been developed with soy protein products (Laignelet & Feillet, 1974). These include soy milk, soy cheese, nondairy frozen desserts, coffee whiteners, yogurt, and others. Isolates and functional concentrates are the most acceptable products in dairy applications because of their fine particle size, dispersibility, high protein content, and low flavor profile (Joseph, 2001).

Table 6: Important Food Uses for Soy Protein Products

Product	soy Protein isolate	soy Protein concentrate	soy flour (grits)	Textured soy Protein
Bakery Products				
Milk Products	X	X	X	
Bread, rolls			X	
Breads (specialty)	X	X	X	
Cakes, Cake mixtures	X	X	X	
Cookies, biscuits, crackers, pancakes, sweet pastry, snacks, etc.	X	X	X	
Doughnuts		X	X	
Pasta products	X	X	X	
Breakfast cereals				
Dairy-type products	X	X	X	
Beverage powders		X		
Cheeses		X		
Coffee whiteners		X		
Frozen desserts		X		
Whipped toppings		X		
Infant formulas	X	X	X	
Milk replacers for young animals	X	X	X	
Meat food Products				
Emulsified meat products				
Bologna, frankfurters	X	X		
Miscellaneous sausage	X	X		
Luncheon loaves	X	X		
Luncheon loaves(canned)	X	X		
Seafood	X	X		
Coarsely ground meat products				
Chili con came, sloppy Joes	X	X	X	X
Meat balls	X	X	X	X
Patties	X	X	X	X
Pizza toppings	X	X	X	X
School lunch/military	X	X		X
Seafood				X
Whole muscle meat				
Analogs	X	X		X
Ham		X		
Meat bits(dried)				X
Poultry breast		X		

Seafood(Surimi)	X	X		X
Stews	X	X		X
Miscellaneous applicantions				
Candies, Confection, desserts	X	X		X
Dietary items	X	X		X
Asian foods		X		
Pet foods			X	X
Soup mixes, gravies	X	X		X

Source: Rakosky, 1974

2.3.6 Acceptability of soy-containing products

Factors influencing consumer attitudes and acceptance of a new product may include product quality, attributes, price, as well as consumer socio-demographics, and possible interactions between these factors. According to Information Theory (Nelson, 1970; Darby and Karni, 1973), consumers assess the quality and attributes of a good based on three types of information they obtain about that good, including search, experience, and credence characteristics. Search characteristics are those that can be assessed prior to purchase (e.g., appearance of a product); experience characteristics are those that can be obtained as the product is consumed (e.g., taste of a product); and credence characteristics are those that cannot be discerned by normal use (e.g., healthiness of a product). The functional health benefits of nutrition-enhanced foods can thus be defined as being credence characteristics. Even after consumption, consumers may not be able to evaluate the health effects of the product. In order to make a choice, consumers will have to rely on either information received from other sources (e.g., advice from health practitioners, health claims on food labels, and health information from media sources) or their previous experience with other products that are similar to the base-product of a functional food (Yanning et al., 2006)

Expanded use of soy in major food items such as breakfast cereals has been limited, despite the increase in public awareness of its health benefits, due to its objectionable sensory characteristics. Soy foods are associated with beany aroma and bitter taste. Furthermore, addition of soy into extruded products can have negative impacts on texture, such as decrease in

expansion and increase in breaking strength (Faubion and Hoseney, 1982). In baked goods, the creamy color and beany or nutty flavor can contribute to the sensory quality (Barbara, 1995). The protein quantity and quality of soybean-containing foods have been studied. Studies showed that bread made with 12% defatted soy four is acceptable by the consumer and contains more protein of higher nutritional quality than common wheat bread (L-Rahman & Youssef, 1978)

3 MATERIALS AND METHODS

3.1 Study setting

All the experiments were carried out in Addis Ababa, which is the capital city of Ethiopia with the altitude of 2444 meter above sea level with a mean annual rainfall of 1196 mm and located 9001 N and 038045 E. The minimum and maximum temperatures were 9.9 ^{0}c and 24.6 ^{0}c, respectively (Fissha and Olsson, 2004). Experiments on SPC were conducted in Ethiopian Spice Extraction Factory laboratory. Analysis of raw materials and products proximate composition, anti nutritional factors and minerals were done in the Ethiopian Health and Nutrition Research Institute and in Food Science and Nutrition research laboratory of AAU.

3.2 Study design

The studies were laboratory based experiments. Initially, proximate composition of selected tef variety was done using standard AOAC and AACC methods and packed by using packaging (polyethylene bag) materials. Secondly, soyprotein concentrate was prepared using ethanol/water (3:1 ratio) as a solvent. Then, the obtained soy protein concentrate (with known proximate composition) was directly blended with prepared tef flour in different ratio (Table 7), taking preliminary experiment and reviewed literatures as a ground. And finally, the sensory analysis, proximate composition, minerals and anti nutritional factors were performed on *injera*, which was prepared from those blended tef flour. And each analysis was done in duplicate.

Table 7: Blend proportion

Sample code	**Blend proportion**	
	Proportion of tef flour%	**Proportion of SPC flour%**
RRR	**100%**	**0%**
YYY	**95%**	**5%**
SSS	**90%**	**10%**
PPP	**85%**	**15%**
TTT	**80%**	**20%**

3.3 Materials

Very white tef called *Quncho* grain [Eragrostis tef (Zucc.) Trotter] grown in 2010/11 cropping year, was obtained from Debre Zeit Agricultural Research Center (DZARC) and defatted Soy flour (*Glycine max*) was found from Ethiopian Spice Factory. One cultivar of each was used for the study. *Quncho* tef variety is selected due to its especial characteristics of high yield, high quality injera and highly recommended variety by research centers for all tef producing areas at this time. And also, today, in Ethiopia, Quncho tef is becoming more popular due to its relatively better in its composition.

3.4 Raw material preparation

The tef grains collected for this study were cleaned to remove stones, dust and light materials, glumes and stalks. Cleaning was done by winnowing and hand sieving. Properly cleaned tef grain was milled into flour using commercial miller followed by sieving to pass through 710 μm sieve size. And finally, the flour was sealed in plastic bags, and stored at room temperature till next stage.

3.5 Soyprotein concentrates preparation

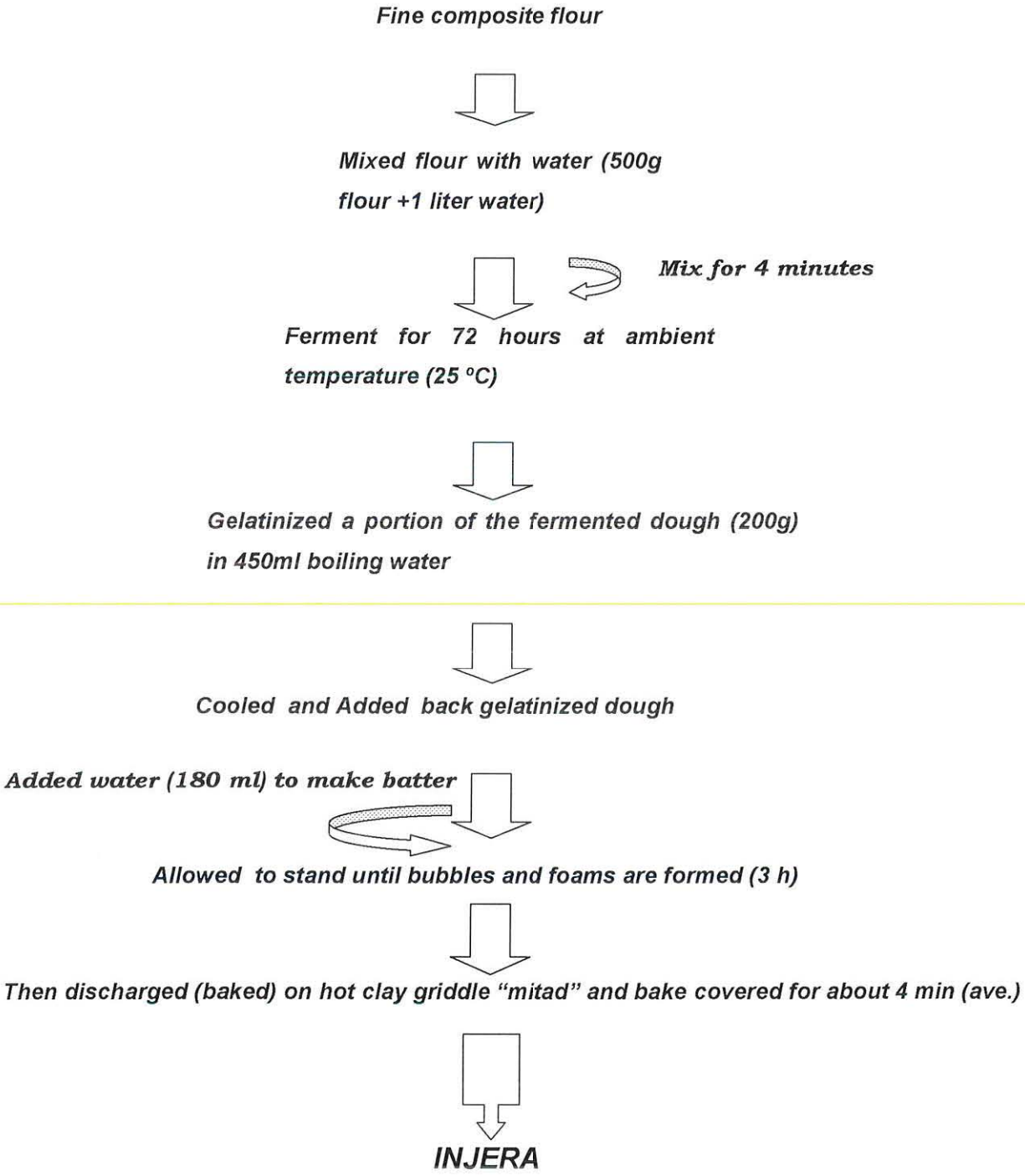
Production of protein concentrates from soy flakes was based on the concept of immobilizing the major protein fractions while permitting the soluble carbohydrates, salts, and other soluble low molecular weight components to diffuse from the cellular matrix of the flakes (Smith & Circle, 1972). Thus, the purchased defatted soya flour from Ethiopian spice extraction factory was prepared in the following way. Soybean seeds were cleaned, dehulled and flaked. The flakes were defatted with n-hexane. The defatted flour with the solvent was then lifted to steam tank and treated with live steam at atmospheric pressure for 1 h with 140 °c so as to remove the solvent and to minimize anti nutritional factors. Finally, dried flour was further powdered in a blender to make it more uniform. As a result, this defatted soy flour was used for the preparation of protein concentrate. Then defatted flour was treated or washed with aqueous alcohol (ethanol\water with 3:1 ratio) to leach out selectively the soluble carbohydrates, salts, and other

soluble low molecular weight components until clear oil color solvent was obtained which in turn leads to soyprotein concentrate (Peisker, 2001).

3.6 Injera preparation

The traditional method of injera preparation varies from household to household and from region to region. The flow diagram of the standardized injera making procedure is presented in Fig. 3. Properly prepared and blended flour with desired ratios has directly used for preparation of a dough, and fermentation after adding starter culture (a batter from a previous batch) and fermenting at room temperature for ≈72 h. After fermentation, gelatinize a portion of (25% of) the fermented dough (200g) in 450ml boiling water. The objective of gelatinization (cooking) part of dough was primarily to increase the amount of gluey material between the dough particles to form more cohesive starch matrix in the injera (Yetneberk *et al.*, 2004). And also it increases water absorption capacity and rolling quality of dough injera. The gelatinized batter was cooled at room temperature and added back to the fermenting dough. After thorough mixing, 100 ml of water was added and the batter was fermented at room temperature for 2–3 h. At this stage of fermentation, considerable amount of gas evolution takes place (Yetneberk *et al.*, 2004). About 300 g of the partially gelatinized and fermented batter was poured in a circular manner on a 47cm diameter hot clay griddle, covered, and baked for ≈4 min. This generates a steam essential for cooking injera; completely gelatinize most of the starch within batter (Parker *et al.*, 1989).

Fig 3: Flow chart for injera preparation



3.7 Chemical analysis

Approved methods of American Association of Cereal Chemists (AACC, 2000) and Association of Analytical Chemist (AOAC, 2000) were used for proximate chemical analysis of the samples.

Determination of moisture content

The crucibles were dried in an oven at 105°C for 1 hour and placed in desiccators to cool. The weights of the crucibles (W_1) were determined. 5g of the prepared Samples were weighed in the dried crucible (W_2) and inserted into drying oven with air circulation at 105^{0}C for 3h, cooled in a desiccator to room temperature and it was again weighted (W_3). Then the amount of moisture was calculated by using Eq. (1).

$$\text{Moisture content in percent (\%)} = \left(\frac{W_2 - W_3}{W_2 - W_1}\right) \times 100 \qquad (1)$$

Determination of crude protein

Protein content was determined according to AOAC (2000) using the official method 979.09. In a cleaned Tecator flask about 0.5g of sample was weighed, 6mL of concentrated sulphuric acid was added and let to stand for 12h. After 12 h 3.5ml of H_2O_2 (30%) was added step by step. When the violent reaction stopped it was shacked and left in the rack. 3g of accelerated reagent (a mixture of copper sulphate pentahydrate and anhydrous potassium sulphate) was added and left for 15 minutes. The mixture was digested in a digest stove (HYP-1008 eight holes) at 370^{0}C for 4hH.After digestion it was cooled in the hood on the rack, 25ml of distilled water was added to dissolve the precipitate, 25 ml of 40% NaOH was added to the digested sample and placed in the distiller (KDN-102F, nitrogen analyzer distillation device). 25ml of H_3BO_3 (saturated solution), 25mL of distilled water and 3 drops of methyl red were added in the 250ml conical flask and placed in the distiller (KDN-102F, nitrogen analyzer distillation device). In the distillation when about 150-200 ml distillate was collected it was titrated with 0.1N HCl and the amount of HCl was recorded. The amount of protein was calculated by using the following formula.

$$\text{Nitrogen (\%)} = \frac{V_{HCL}inL \times N_{HCL}(ca.0.1) \times 14.00 \times 100}{W_o} \quad (2)$$

Where:

V is volume of HCl in L consumed to the end point of titration, N is the normality of HCl (used often is 0.1N), W_o is sample weight on dry matter basis and 14.00 is the molecular weight of nitrogen. The % of nitrogen is converted to % of protein by using appropriate conversion factor (% protein = 6.25 x % N for both the tef and soy flours).

Determination of crude fat content

The crude fat was extracted according to AOAC (2000) official method. The cleaned flask (cylinder) and boiling chips was dried in the drying oven at 100^0C for 1h, cooled in the desiccators for 30min and weighed. Two grams of sample was weighed in thimble containing fat free cotton. The thimbles were placed in the thimble holders, 50 mL of petroleum ether (boiling range of 60-90 ^{0}C) was poured in the flask, the thimble was immersed in the petroleum ether (in the flask) and heated at 80 ^{0}C in the fat determinator (SZC-C fat determinator) for 1hr, hanged the thimble and heated at the same temperature for 2hrs and then the solvent was recovered for 15 min. The heater was switched off, the flask was dried in the drying oven at 90 ^{0}C for 30 min, cooled in the desiccators for 15 min and then weighed the flask with the extract. The amount of extractable fat was calculated by using the following formula

$$\text{Crude fat, percent by weight} = \left(\frac{W_2 - W_1}{W}\right) \times 100 \quad (3)$$

Where: W_1 = weight of the extraction flask (g)

W_2 = weight of the extraction flask plus the dried crude fat (g)

W = weight of sample flour. (g)

Determination of ash content

Porcelain dishes were placed in a muffle furnace (Carbolite, Aston Lane, Hope, Sheffield, England, UK) for 30 min at 550 ^{0}C. The dishes were cooled in a desiccator (with granular silica gel) for about 30 minutes and weighed m_1. About 2.5000 g of fresh sample (in triplicate) was placed in dish (m_2). Dishes were placed on a hot plate under a fume-hood and the temperature was slowly increased until smoking ceases and the samples become thoroughly charred (turn black). The dishes with sample were placed inside the muffle furnace at 550 ^{0}C until ashing complete and cooled in a desiccator. The ash was clean and white in appearance. The residue were then weighed (m_3). The total ash was expressed as percentages on dry basis as follows:

$$\text{Total Ash (\%)} = \left(\frac{M_3 - M_1}{M_2 - M_1}\right) \times 100 \quad (4)$$

Where: (M_2-M_1) is sample mass in g on dry base and (M_3-M_1) mass of ash in g

Determination of crude fiber

Crude fiber analysis was conducted using the method of AOAC (2000) official method 962.09. About 1.5 g sample was transferred into a 600 ml beaker and about 200 ml 1.25% sulfuric acid was added and boiled for 30 minutes. Recording took place by placing a watch glass over the mouth of the beaker. After 30 minutes heating by gently keeping the level constant with distilled water, 20 ml of 28% KOH was added and again boiled gently for further 30 minutes, and then the solution was filtered through sintered glass crucibles. Subsequently, washing was conducted with hot distilled water, 1% sulfuric acid, 1% NaOH solution and finally with acetone. Then, filtered and dried it in the electric oven (memmert 854 Schwabach, West Germany) at 130 °C for 2 h. Furthermore, it was cooled at room temperature for 30 minutes in a desiccator and weighed, then transferred it to crucible to muffle furnace (GALLENKAMP, Model FSL 340-0100, U.K.) for 30 minutes ashing at 550^{0}C. Finally, it was cooled again in a desiccator and re-weighed. The crude fiber content was determined by using the formula

$$\text{Total Crude fiber} = \left(\frac{M_1 - M_2}{M_3}\right) \times 100 \quad (5)$$

Where M3 is the weight of sample

Determination of *Carbohydrate*

Carbohydrate content will be determined by difference.

$$\%C=100-[\%M+\%P+\%F+\%A] \quad (6)$$

Where:

C-Carbohydrate content, M-Moisture content, P-Protein content, F-Fat content, Fb-Fiber content and A-Ash content

Determination of mineral analysis

Calcium, zinc and iron were determined using atomic absorption spectrophotometer method of Osborne and Voogt (1978). The ash obtained after dry ashing at 550^0C was treated with 5 ml of 6N HCl to wet it completely and carefully dried on a low temperature hot plate. 7 ml of 3N HCl was added and the dish was heated on the hot plate until the solution just boils. Then, it has been cooled and filtered through a filter paper in to a 50mL volumetric flask. Again 7 ml of 3N HCl was added to the dish and heated until the solution just boils. Finally, cooled and filtered into the volumetric flask. For the determination of calcium, lanthanum chloride (1% w/v) was added to both standards and samples to suppress interference from phosphorus. Using atomic absorption spectrophotometer (Varian, spectra-10/20, Australia) a calibration curve was prepared by plotting the absorption or emission values against the metal concentration in mg/100g. Reading was taken from the graph, which depicted the metal concentrations that correspond to the absorption or emission values of the samples and the blank. The metal contents were calculated by using the formula

$$\textbf{Metal content (mg/100g)} = [(a\text{-}b \times v)]/10w$$

Where, W = weight of samples (g)

V = volume of extract (ml)

A = concentration of sample solution (µg/ml)

B = concentration of blank solution (µg/ml)

Determination of anti-nutritional factors

Determination of phytic acid

Phytic acid was determined by using Vaintraub and Lapteva (1988). About 1g of sample was extracted with 10 ml 2.4% HCl for 1hr at an ambient temperature and centrifuged (3000 rpm/30min) (Nüve, bench-top centrifuge, NF 800R, 2001, Ankara, Turk). The supernatant was used for phytate estimation. About 2 ml of Wade reagent (0.03% solution of $FeCl_3.6H_2O$ containing 0.3% sulfosalicylic acid in water) was added to 3 ml of the sample solution and centrifuged. The absorbance at 500 nm was read using spectrophotometer (BECKMAN, DU-64, Japan). The phytate concentration was calculated from the difference between the absorbance of the control (3ml of water + 1ml of Wade reagent) and that of assayed sample. The concentration of phytate was calculated using phytic acid standard curve (by preparing a series of standard solution containing 3.9, 7.8, 15.5, 23.4, 31.2 and 39µg/ml phytic acid in 0.2N HCl) using water to zero the spectrophotometer, and results were expressed as of phytic acids in mg/100gm dry weight by the following formula.

Phyticacid (Mg/100g) = [(absorbance –intercept) / (slope × density × weight of sample)] ×10/3

Where, Density= density of the solvent

Determination of tannins

The amount of condensed tannin was determined by Maxson & Rooney (1972). About 1g of sample was weighed, extracted with 10mL 1% HCl in methanol, at room temperature in mechanical shaker for 24 h. The mixture was centrifuge at 1000G for 10 minutes. 1ml supernatant was mixed with 5 ml of vanillin- HCl reagent in another test tube. When the reaction was completed (after 20 minute), the absorbance was read at 500 nm using spectrophotometer (BECKMAN, DU-64, Japan). Dcatechen was used as standard value of tannin in mg D-catechen per gram of sample. 40 mg D-catechen was dissolved in 100 mL of 1%HCl in methanol and from this 0, 0.2, 0.4, 0.6, 0.8 and 1ml was taken in a test tube and the volume was adjusted to 1mL with 1% HCl in methanol. 5ml of vanillin-HCl reagent in each test tube was added. After

20 minutes the absorbance was read at 500nm.The absorbance of the blank was subtracted from the absorbance of the corresponding vanillin-containing sample. Finally, the tannin contents were calculated. Values of tannins were expressed in milligram of D-catechin equivalent per gram of sample by the following formula.

Tannin (mg/g) = [(absorbance –intercept) / (slope ×density × weight of sample)] ×10

(/100) dilution factor

3.8 Sensory analysis

10 judges (7males and 3 females) selected from the staff and graduating class students of Food Science and Nutrition program of Addis Ababa University. Panelists were instructed to evaluate each sample's sensory attributes; visual color, taste, fluffiness, flavor and overall acceptability using a nine point hedonic scale rated from 1 (extremely dislike), 5(neither like nor dislike) to 9 (extremely like) (Lawless & Heymann, 1998). The five samples were presented turn by turn in identical containers (white plates), coded with three digit random numbers. Water was provided to rinse the mouth between evaluations and covered expectoration cups were also provided when panelists didn't wish to swallow the samples (Olaoye *et al.*, 2006).

3.9 Statistical Analysis

Nutritional composition, mineral composition and anti-nutritional factors data were statistically analyzed using one way analysis of variance (ANOVA) and Fisher's least significant difference (LSD) and Duncan test ware used to compare among different treatments. The statistical package used was SPSS version 16. Significant differences were determined at the $P< 0.05$ level. Results were expressed as mean + standard error. The hedonic scores for sensory evaluation were also analyzed by ANOVA.

3.10 Numerical Optimization

Design Expert is a piece of software intended to help with the design and interpretation of a wide range of designs, including factorials, fractional factorials and composite designs.

Moreover, it also enables to determine the exact value of combination according to supplied response.

Mixture Design Expert software enables setting criteria for all variables, including components by assigning "Optimization Parameters" or "Goal" to construct desirability indices. Desirabilities range from 0 to 1 for any given response. The program combines individual desirabilities into a single number and then searches for the greatest overall desirability. A value of 1 represents the ideal case. A value of 0 indicates that one or more responses fall outside desirable limits.

Criteria have been set for all components and responses in the Mixture design software, optimization part. Consequently "Goal" has been allocated on the basis obtained experimental values to obtain exact value of blend proportion in the ranges of experimental result ranges; having a target protein value (20.76) and in range overall acceptability (5.7 - 7.75).

4 RESULTS AND DISCUSSION

The effects of blending (5%, 10%, 15% and 20%) soyprotein concentrate (SPC) with tef (obtained from Debre Zeit Agricultural Research Center (DZARC)) flour on the organoleptic, nutrient compositions and levels of certain anti-nutritional factors (phytic acid and tannins) of the injera (prepared form blend of SPC and tef flour) were studied. And finally, sensory analysis and numerical optimization of the enriched injera has also been done. All results are in dry weight basis except the injera results reported in its edible portion (fresh injera).

4.1 Proximate composition of raw materials

Table 8: Proximate composition of raw materials used for enrichment*

Raw materials	Moisture%	Ash%	Protein%	Fat%	Fiber%	CHO%	Calories(kcal)
Tef flour	11.14±.14^{a}	1.68±.05^{b}	9.08±.10^{c}	3.20±.19^{a}	3.00±.20^{b}	74.90±.39^{a}	364.72±.56^{a}
Defatted Soy flour	8.70±1.10^{ab}	7.80±.60^{a}	57.40±1.37^{b}	2.40±.20^{b}	4.30 ±.30^{a}	23.70±2.87^{b}	346.00±7.81^{a}
SPC flour	7.16±.14^{b}	6.99±.03^{a}	68.25±.79^{a}	1.60±.10^{c}	3.60±.10^{ab}	15.99 ±.79^{b}	351.38±.93^{a}

Means in the same column followed by the same letters are not statistically significant using one-way ANOVA test ($P<0.05$)

* mean value ± standard error and n=2

4.1.1moisture Content

The moisture analysis conducted indicates that the moisture content of row tef and soyprotein concentrate (SPC) is 11.14% and 7.16%, respectively (Refer Table 8). The result of tef is in agreement with the finding of Bultosa (2007) that the moisture content of tef is between 9.30 to 11.22 %. However, the SPC result is somewhat lower than Barbara (1999) finding that SPC

moisture content is 8.5%. This different perhaps can be attributed to the disparity in variety, storage conditions and temperature during harvesting and processing.

4.1.2 Total Ash Content

The total ash content of row tef, defatted flour and SPC were found to be 1.68%, 7.80% and 6.99%, respectively (Refer Table 8). The ash content of row tef obtained in this study is slightly lower than Bultosa (2007) and National Research Council (1996) findings that the ash content of raw tef is between 1.99 to 3.16 and (2.0%), respectively. While the ash result of SPC is a little bit lower than Barbara(1995) result that the ash content of SPC is 6.2%. The disparity of ash content in SPC may be attributed to the extraction method, climatic factors, the soil type, and the variation in cultivar. The high ash contents of the SPC in the samples would illustrates that there is an appreciable quantity of minerals in SPC flour.

4.1.3 Protein

Our finding depicts that the protein content of tef flour is 9.08%, which is in agreement with the result Bultosa (2007) finding. Accordingly, he has reported that the protein content of tef ranges from 8.70 % to 11.10%. However, our result is slightly lower than the result obtained by Patricia (2008) that is 9.6% The protein content of defatted soy flour is found to be 57%, whereas for the soy protein concentrate it is 68.25% crude protein content that is slightly lower than result reported by Barbara (1995) which is 71.7% protein. Variations in protein content may be attributed to difference in the variety, soil, agronomic practices and processing methods.

4.1.4 Crude Fat

The crude fat content of row tef, defatted flour and SPC were found to be 3.20%, 2.40% and 1.60%, respectively. In this study the content of fat in the tef flour is slightly higher than both Bultosa (2007) and WHO (1991) findings that is in the range of 2.0% to 3.0% and 2.1%, respectively. However, our finding is in agreement with National Research Council (1996) which range from 2.0% to 3.19 %. These variations could be due to variety differences. The content of fat in the defatted and SPC in this study are higher than the results reported by USDA (1986) which are 1.2% and 0.5%, respectively. The result reported by Barbara (1995) is 0.5% and

Peisker (2001) is 1% which are also lower than the result obtained in this study. Partly, the variations could be explained by the difference in the methodology used to prepare defatted soy flour and soyprotein concentrate. Besides, the modification of the aqueous alcohol mixture, temperature and time of processing can further reduce the composition of the SPC (Peisker, 2001).

4.1.5 Crude Fiber

Regarding the content of fiber in tef, defatted flour and SPC, is found to be 3.00%, 4.30% and 3.60%, respectively. According to the result obtained by Bultosa (2007), the fiber content of tef flour found to be in between 2.6% to 3.8%, which incorporate the fiber content obtained in this study (Refer Table 8). As to defatted and SPC fiber content our finding is consistent with USDA (1986) study that the content of fiber in defatted as well as SPC is 4.30% and 3.6%, except the faint difference in the magnitude of SPC. Barbara (1995) also depicted that the content of crude fiber in SPC is 3.5%. This variation could be explained partly by the difference in variety and level of removal of the seeds hull during row material processing (Majed et al, 2006).

4.1.6 Utilizable Carbohydrate

Utilized carbohydrate (CHO) for row tef flour was found to be 74.90%, which was higher than the result of CHO reported by National Research Council (1996), Bultosa (2007) and (Kebede *et al.*, 2010) which is 72.00%, 74.32% and 71.72%, respectively. However, result obtained in this study was lower than Griffith and Castell (1998) report which is 80.10%. The difference perhaps due to the method used for the determination, and on the tef variety analyzed (Belta *et al.*, 2002).Crude CHO for defatted and SPC found to be with results of 23.70% and 15.99%, respectively. There is significant difference ($p < 0.05$) between row tef flour and processed soy flours. There is also a difference in CHO between defatted flour and SPC (Refer Figure 8) due to the removal of soluble carbohydrate using aqueous alcohol. CHO content of SPC was in agreement with the result reported by Barbara (1995). However, CHO content of defatted and SPC were very much lower than USDA (1986). These differences could be due to the more leaching out of soluble carbohydrate during removal of solvent in the process of defatting and protein concentration (Esenwah & Ikenebomeh, 2008).

4.1.7 Gross Energy

In this study the amount of gross energy contribution of the row tef flour was found to be 364 kcal/100g (Refer Table 8), which is slightly higher than the findings of Bultosa (2007) and Laike et al., (2010) which is 359.62 kcal/100 g and 352.32 kcal/100 g, respectively. The gross energy content of defatted soy flour and SPC were found to be 346.00 kcal/100g and 351.38 kcal/100g, respectively. Our finding is lower than the results reported by USDA (1986), which are 329 kcal/100 g and 332 kcal/100 g, respectively. However, Barabara's (1995) finding is higher (354.3 kcal/100 g) than the result obtained in this study. The difference may be due to the difference in cultivar and level of processing.

4.2 proximate composition of enriched injera (Tef flour and SPC)

The proximate compositions of enriched injera are given in Table 9 and Table 10 in dry basis and edible portion, respectively.

4.2.1 Moisture Content

The moisture contents of blended injera with 0%, 5%, 10%, 15 and 20% were showed increment fashion from 6.86 % for control (0%) to 6.47%, 7.06%, 7.14% and 7.36%, respectively (Refer Table 9). The moisture content of injera as edible portion also ranges from 65% to approximately 67% as shown in Table 10. These results were in agreement with EHNRI (1997) study that composition of different varieties of injera ranges from 55.80% to 68.80% moisture content. This difference perhaps can be attributed to higher fiber content of SPC (Refer Table 8). Moreover, the variation could also possibly be due to temperature of baking; in most cases this is the major drawback of traditional method of preparing injera for the fact that during baking the electrically heated *mitad* used for baking is not thermostatically controlled, batter is manually poured and spread on the *mitad*. Thus, the thickness of the injera depends on consistency of the operator. The elapsed time between applications of batter on the hot clay griddle need to be kept as constant as possible to limit variations in batter cooking temperature which is responsible for the difference for injera moisture content and its texture (Yetneberk et al., 2004).

Table 9: Proximate composition of enriched enjera using tef flour and SPC (dry weight base)*

SPC%	Moisture%	Ash%	Protein%	Fat%	Fiber%	CHO%	Calories%
0	6.86±00^{ab}	2.15±.04^{b}	11.31±1.04^{e}	2.15±.14^{a}	1.54±.04^{b}	77.52±.86^{a}	375.68±.50^{a}
5	6.47±.44^{b}	2.21±11^{b}	14.30±.49^{d}	1.95±.07^{ab}	3.03±03^{a}	75.06±.09^{b}	374.00±.92^{a}
10	7.06±.04^{ab}	2.73±.03^{ab}	17.30±.52^{c}	1.89±.07^{ab}	3.06±.04^{a}	71.02±.58^{c}	370.30±39^{b}
15	7.14±.02^{ab}	3.05±.39^{a}	20.76±1.07^{b}	1.68±.03^{b}	3.09±.03^{a}	67.53±.56^{d}	368.29±1.76^{bc}
20	7.36±.12^{a}	3.17±.15^{a}	25.06±1.62^{a}	1.33±.09^{c}	3.12±.20^{a}	63.07±1.73^{e}	364.50±1.36^{c}

Means in the same column followed by the same letters are not statistically significant using one-way ANOVA test ($P<0.05$)

* mean value ± standard error and n=2

4.2.2 Total Ash Content

The injera obtained from enriched flour has shown an increment in ash content with results of 2.15%, 2.21%, 2.73%, 3.05% and 3.17% for 0%, 5%, 10%, 15% and 20%, respectively (Refer Table 9). Analysis of variance conducted on ash content of the sample portrayed that the ash content of control (0%) is significantly different ($p < 0.05$) from enriched injera sample. This result contradicted with Rita *et al, (2010)* finding that there was no difference observed in ash content when soy blended with wheat flour. The increment in ash content indirectly shows that there is high mineral content in blended injera, which can be helpful to reduce the huge mineral deficiency in the society.

The content of ash in an edible form of injera is also depicted that it is 0.74%, 0.73%, .90%, 1.02% and 1.09% for 0%, 5%, 10%, 15% and 20%, respectively. It was found that there is a significant difference ($p < 0.05$) between the control and blended injera's: at 15 % and 20% (Refers to Table 10). Our finding is lightly higher than EHNRI (1997) finding. These disparities perhaps due to in an improvement in the SPC blend with tef flour.

4.2.3 Protein

In this study one of the significant changes observed in the proximate composition of injera is in crude protein content. The results portray that the protein content increased from 11.31% for the control and 14.30%, 17.30%, 20.76 % and 25.06% for 5%, 10%, 15% and 20% SPC blended injera, respectively. Analysis of variance, (ANOVA) conducted on the protein contents of all blended injera indicated that there is a significant deference ($P<0.05$) in protein content proportionally with increased amount of SPC. Apart from the high content of protein in SPC (Refer Table 8), the observed increase in protein content of injera in the sample is attributed to the synthesis of amino acids during the fermentation process (Oboh & Elusiyan, 2007).

Protein content in 15% and 20% SPC blend can effectively meet the daily protein requirement of children in age range from 4 to 8 years old only using 100g consumption of this flour (Ellie & Sharon, 2008)

Table 10: Proximate composition of enriched enjera using tef flour and SPC (as edible portion or fresh injera)*

SPC%	Moisture%	Ash%	Protein%	Fat%	Fiber%	CHO%	Calories(kcal)
0	65.45±.02^{b}	0.74±.01^{b}	3.90±.36^{d}	0.74±.04^{a}	.53±.01^{b}	29.16±.28^{a}	138.94±.09^{a}
5	66.93±.32^{a}	0.73±.03^{b}	4.72±.11^{cd}	0.64±.03^{ab}	1.00±.00^{a}	26.96±.43^{b}	132.57±1.55^{b}
10	66.77±.16^{a}	0.90±.01^{ab}	5.75±.20^{c}	0.62±.02^{b}	1.01±.01^{a}	25.94±.07^{c}	132.41±.74^{b}
15	66.46±.10^{a}	1.02±.12^{a}	6.96±.38b	0.56±.01^{b}	1.03±.01^{a}	24.98±.14^{d}	132.84±.89^{b}
20	65.61±.26^{b}	1.09±.05^{a}	8.62±26^{a}	0.46±.02^{c}	1.07±.04^{a}	24.22±.02^{d}	135.50±1.34^{ab}

Means in the same column followed by the same letters are not statistically significant using one-way ANOVA test ($P<0.05$)

* mean value ± standard error and n=2

The protein content in the edible portion of injera also revealed that there is an increment from 7.40% (w/w) for tef flour alone to 9.56%, 11.89%, 12.87% and 16.19% on substitution of tef flour with 5%, 10%, 15% and 20% SPC flour, respectively. This protein contents obtained at 15% and 20% of blend can also be used to meet the daily protein requirement for children with 150g and 125g consumption this flour, respectively (Ellie & Sharon, 2008)

In general, blending SPC have resulted more than a double increment in protein content from 100% tef injera. Thus, this increment in protein content directs that it is possible to supplement protein deficiency which is widely visible in developing country due to high cost of animal protein source.

4.2.4 Crude Fat

Analysis performed on crude fat content of enriched injera were obtained to have a crude fat content of 2.15% for the control (0% SPC or 100% tef flour) and 1.95%, 1.89%, 1.68% and 1.33% for 5%, 10%, 15% and 20% SPC, respectively. As the amounts of SPC flour in the composition increased, the content of fat in the injera decreased. Our variation analysis also depicted that there is a significant difference ($P < 0.05$) in content of crude fat in the blended injera samples compared with pure tef injera This low content of fat in the composite product might be due to the low content of fat in the SPC compared to the content of fat in tef flour (refer to Table 8). Heat treating of the blended sample during baking of injera could also led to the protein-lipid or carbohydrate-lipid complexs which might in turn reduce the crude fat obtained in this study and that would be facilitated here by higher amount of protein (Anastase *et al.*, 2007).

Moreover, crude fat content in the edible form of injera (see Table 10) is slightly lower than dry weight base (see Table 9). This is actually due to the high content of moisture in the fresh injera. Fresh injera thus, found to have a crude fat content of 0.74%, 0.64%, 0.62%, 0.56% and 0.46% for 0%, 5%, 10%, 15% and 20%, respectively. The results have showed a slight decrement than a result reported on by EHNRI (1997) (0.60%), food composition table, composition of various varieties of injera, which could be a result of lower value of fat content consisted by SPC.

4.2.5 Crude Fiber

Analysis of variance performed on crude fiber content revealed that injera made from 100% tef flour has a significant difference in fiber content from the rest prepared from blend of SPC (5%, 10%, 15% and 20%). However, the blended flours have showed insignificant difference in crude fiber with each other. This was with result of 1.54% for control one and 3.03%, 3.06%, 3.09% and 3.12% were for 5%, 10%, 15% and 20%, respectively as showed in Table 9. Further fermentation during the process is capable to minimize crude fiber content of injera which could enable enzymatic degradation of the crude fiber by the enzymes excreted by the microorganisms involved in the fermentation process (Tilahun, 2009). There is a significant difference ($p < 0.05$) in the fiber content of the different blended.

The fiber content of 0%, 5%, 10%, 15% and 20% edible injera is 0.53%, 1.00%, 1.01%, 1.03% and 1.07% respectively. Fiber content of the controlled injera sample is found to be slightly lower than the result reported by EHNRI (1997) food composition table result. However, the result obtained for SPC enriched injera were not obtained out of the range of EHNRI (1997) table, for the composition of various varieties of injera.

4.2.6 Utilizable Carbohydrate

The carbohydrate content of injera significantly decreases ($p < 0.05$) with increase in SPC concentration. Accordingly, the carbohydrate content of control injera is 77.52% and for the remaining blended injera is 75.06%, 71.02%, 67.53% and 63.07% respectively for 5%, 10%, 15% and 20%. This partly explained by the high protein and ash content found in SPC. Moreover, the decrease in total carbohydrates could be due to degradation and a subsequent decrease in starch and soluble sugars which are principal substances fermentation by microorganisms (Ejigui et al., 2005). Similarly, heat treating of the blended sample during baking of injera could also lead to carbohydrate-lipid complexes, which might contribute for the decrease in CHO as the protein concentration increase in the sample flour (Anastase et al, 2007).

Injera in edible form which were enriched with 5%, 10%, 15% and 20% SPC have shown to have CHO content of 26.96%, 25.94%, 24.98% and 24.22%, respectively and. Injera prepared from control flour was found to be 29.16% of CHO (Table 10). CHO value reported previously

by EHNRI (1997) for 100% tef injera is 31.90% in edible form, which is a little bit higher than the value obtained in this study. ANOVA carried out on injera in its edible form reviled that there is a significant difference ($p < 0.05$) in CHO within the means.

4.2.7 Gross Energy

In addition, the content of gross energy of injera decreased with increase in SPC in the blended tef flour. Accordingly, the amount of energy obtained from 0%, 5%, 10%, 15% and 20% SPC blended tef flour is 375.68 kcal/100 g, 374.00 kcal/100 g, 370.30 kcal/100 g, 368.29 kcal/100 g and 364.50 kcal/100 g, respectively. The analysis of variance indicates that the total calorie of injera is significantly difference ($p < 0.05$) between the control and blended injera. There is also a significant difference ($p < 0.05$) among the enriched injera too (Refer Table 8).

Injera in it edible form was found to have 138.94 kcal/100 g, 132.57 kcal/100 g, 132.41 kcal/100 g, 132.84 kcal/100 g and 135.50 kcal/100 g for 0%, 5%, 10%, 15% and 20%, respectively. The result obtained in this study is lower than the one reported by EHNRI (1997). Moreover, other results obtained in this study are consistent with the finding reported by EHNRI (1997).

4.3 Mineral content of enriched injera (Tef and SPC)

The results of iron zinc and calcium composition of blended product (injera) are presented in Table 11

4.3.1 Iron (Fe)

Our finding depicted the content of Iron increase when we increase the concentration of SPC. Accordingly, the content of Iron for 0% of SPC tef injera, 5% , 10%, 15% and 20% of SPC content of injera is found to be 11.96 mg/100g, 18.91mg/100g, 20.06mg/100g, 22.88mg/100g and 24.01mg/100g, respectively (see in Table 11). This corroborates with the findings Slavin (1991) that SPC content increase the content of iron in the blended product of injera. The variance analysis divulges that there a significant difference ($p<0.05$)in the Iron content between the 0% SPC injera and the remaining blended SPC injera.

However, the content of Iron in this study is in the 0% SPC injera much lower than the one reported by Patricia (2008), which is 30mg/100g. These enriched injera can achieve recommended daily allowance of iron intake (Ellie & Sharon, 2008). The difference might be attributed to the difference in climatic and soil where the row tef obtained from. Moreover, the moisture content of the sample during chemical analysis might also contribute for the observed difference.

4.3.2 Zinc (Zn)

The result obtained in this study has also indicated that the content of Zinc increase with increase in the concentration of SPC in the enriched injera. Consequently, the content of Zinc in 0% SPC blend product of injera, 5%, 10%, 15% and 20% is found to be 3.19mg/100g, 3.39 mg/100g, 4.66 mg/100g, 5.71 mg/100g and 7.01 mg/100g, respectively (refer Table 11). There is a significant difference ($p < 0.05$) between control and blend in the content of Zn. Veronica (2002), has also obtained a significant increase in the concentration of Zinc when she increase the concentration of SPC in millet.. The content of Zn in 0% SPC blended injera is found to be a bit higher the one reported by Patricia (2008) which is 1.41 mg/100g. Similarly the difference might be ascribed to the difference in environmental and soil effect.

Table 11: Mineral content of enriched injera*

SPC blend	Fe	Zn	Ca
(%)	(mg/100g)	(mg/100g)	(mg/100g)
0	11.96±.05^{d}	3.19±.19^{d}	172.91±.86^{e}
5	18.91±.90^{c}	3.39±.34^{d}	182.50±.50^{d}
10	20.06±.37^{bc}	4.66±.06^{c}	192.10±.98^{c}
15	22.88±1.85^{ab}	5.71±.21^{b}	201.37±.46^{b}
20	24.01±.24^{a}	7.01±.11^{a}	211.12±.89^{a}

Means in the same column followed by the same letters are not significantly different ($P<0.05$)

* mean value ± standard error and n=2

4.3.3 Calcium (Ca)

The result also indicated that the content of calcium increase with the increase in the concentration of SPC. Accordingly, the content of calcium in 0% SPC blended injera, 5%, 10%, 15% and 20% is found to be 172.91 mg/100g, 182.50 mg/100g, 192.10 mg/100g, 201.37 mg/100g and 211.12 mg/100g, respectively. This result suggests that there is a significant difference ($p < 0.05$) in the concentration of calcium between the controlled injera and that of the blended ones. This finding corroborates with the finding of Abiodun et al. (1998). Furthermore, Veronica (2001) has also reported the same fact that the concentration of calcium content in blended millets with soybean flour significantly increase.

4.4 Anti-nutritional factors

The anti-nutritional factors (phytate and tannin) contents of the processed injera sample are shown in Table 12

Table 12: Anti-nutritional factors of enriched injera (Tef and SPC)*

SPC Blend	Phytate	Tannin
%	mg/100g	mg/100g
0	119.08 ± 0.07 ^{b}	2.10 ± 1.78 ^{e}
5	123.14 ± 3.03 ^{b}	3.59 ± 0.18 ^{d}
10	129.27 ± 8.47 ^{b}	8.56 ± 0.10 ^{c}
15	137.87 ± 0.87 ^{b}	10.26 ± 0.28 ^{b}
20	174.19 ± 1.78 ^{a}	18.42 ± 0.12 ^{a}

Means in the same column followed by the same letters are not statistically significant using one-way ANOVA test ($P<0.05$)

* mean value ± standard error and n=2

4.4.1 Phytate Content

This finding portrays that when SPC is mixed with tef flour the concentration of phytate value increased.

Since soy bean is a legume, it is expected to have more anti-nutritional factors than cereals. As a result the phytate content for 0% SPC blend, 5%, 10%, 15 and 20% is found to be 119.08 mg/100g, 123.14mg/100g, 129.27mg/100g, 137.87mg/100g and 174.19 mg/100g, respectively(See in Table 12). The result of phytate content reported by Patricia (2008) for control injera tef was 126 mg/100g, which is slightly higher than the result obtained in this study. Even though soybean is well known for its high phytate content, moist heating, solvent washing unites, and fermentation (resulted by activity of native phytase and/or the fermentative microflora) for preparing defatted soy flour, SPC and finally injera might have significantly contributed to the removal of phytate content of both soy flour and tef (Abdelhaleem et al., 2008). The variation analysis indicated that the phytate content has insignificant difference between 0% SPC content and 5%, 10% and 15% , while we found a significant difference between 20% blend of SPC (see in Table 12).

4.4.2 Tannin Content

Tannins are known for deafening the concentration of bioavailability of proteins, particularly due to the minerals iron. In this study we found that increasing SPC concentration indicated increase in the content of tannin in tef flour. Accordingly, the content of tannin found to be 2.10 mg/100g, 3.59mg/100g, 8.56mg/100g, 10.26 mg/100g and 18.42 mg/100g for 0%, 5%, 10%, 15% and 20% SPC, respectively. The analysis of variance suggests that there is a significant difference ($p < 0.05$) in the concentration of tannin between the controlled injera and that of the blended ones (See Table 12). Like that of phytate, different unit operations in the processing have contributed to the reduction of tannin content. In addition, Urga et al (1997) reported that household processing through natural fermentation of tef flour dough able to reduce the tannin content by about 55% presumably through the action of polyphenol oxidase generated during fermentation.

4.5 Bioavailability of minerals

The results of Fe: phytate, Zn: phytate, phytate: Ca and [calcium x phytate]: [zinc] of enriched injera are given in Table 13.

For absorption and transportation of enterocytes to take place the minerals must be available in ionic form (either as cations or anions), which is suitable for uptake and transport (Egal, 2011). The highly-soluble monovalent minerals, such as sodium and potassium can be transported easily. However, the solubility of various minerals is often low at neutral pH. The solubility of these minerals is dependent on the presence of other compounds, which can make it relatively easy to precipitate or form non-absorbable complexes. Well-known complexes food components are phytic acid (phytate) and oxalic acid (Harland, 1989). The formation of such chelates depends on ratio of the content of zinc, iron or calcium compare to phytate's in the food (Umeta et al., 2005)

In this study phytic acid has created its inhibitory effect on the absorption of iron by forming insoluble complexes in the gut, in which phytate (iron molar ratio was obtained with greater than 0.15) is regarded as indicative of poor iron bioavailability (Umeta et al., 2005). In this study although the SPC concentration has shown reduction in phytate:iron ratio the results are still beyond the acceptable dosage to be taken as a good iron bioavailability.

Table 13: Bioavailability of minerals in the enriched injera*

SPC Blend (%)	**Phytate content** (mg/100g)	**Phytate: Fe** (molar ratio)	**Phytate: Zn** (molar ratio)	**Ca: Phytate** (molar ratio)	**[Calcium x phytate] /[zinc] (mol/kg)**
0	$119.08^{b} \pm 0.07^{b}$	$0.84 \pm .01^{a}$	$3.71 \pm .22^{a}$	$23.96 \pm .11^{a}$	$0.1604 \pm .00^{a}$
5	$123.14^{b} \pm 3.03^{b}$	$0.55 \pm .04^{b}$	$3.64 \pm .45^{a}$	$24.47 \pm .67^{a}$	$0.1662 \pm .02^{a}$
10	$129.27^{b} \pm 8.47^{b}$	$0.54 \pm .04^{b}$	$2.74 \pm .14^{ab}$	24.63 ± 1.73^{a}	$0.1318 \pm .00^{a}$
15	$137.87^{b} \pm 0.87^{b}$	$0.51 \pm .87^{b}$	$2.39 \pm .04^{b}$	$24.10 \pm .10^{a}$	$0.1206 \pm .00^{a}$
20	$174.19^{a} \pm 1.78^{a}$	$0.61 \pm .05^{b}$	$2.47 \pm .29^{b}$	20.20 ± 1.98^{a}	$0.1302 \pm .01^{a}$

Means in the same column followed by the same letters are not statistically significant using one-way ANOVA test ($P<0.05$)

* mean value ± standard error and n=2

However, the phytate result for Zn was obtained in the range of good bioavailability (the results are below 15, which is the threshold value for poor Zn absorption (Melaku *et al.*, 2005). Bioavailability of Zn at 0%, 5%, 10%, 15% and 20% of SPC were obtained to have 3.71, 3.64, 2.74, 2.47 and 2.39 molar ratios, respectively. These all results reveal that Zn is not poor in its bioavailability. The observed reduction in molar ratio might be contributed due to the increasing value in Zn content with increased proportion of SPC. ANOVA performed on here revealed that there is a significant reduction ($p < 0.05$) in molar ratio at 15% and 20% blend (Table 13).

Results obtained on calcium have similar effect with that of iron in the above discussion. Calcium is obtained with higher amount of molar ratios than the critical number 6:1, which is taken as poor absorption. ANOVA carried out on calcium has resulted with no significant change ($p> 0.05$) observed due to SPC inclusion.

High calcium level in foods is well known to have effect on bio availability of zinc when the [calcium x phytate]: [zinc] millimolar ratio exceeds 0.5 (Melaku *et al.*, 2005). However, in this study all values were found to show lees than 0.5, which indicate that the prepared injera are still poor in calcium content, and have a good Zn bioavailability. Thus, the possible contribution of calcium in such type blend foods would not exacerbate the low bioavailability of zinc.

4.6 Sensory evaluation of newly developed injera

The mean sensory scores for enriched injera products are summarized in Table 14. The sensory scores for test flavour, texture, Odor, flexibility and backside appearance indicate that all products have a mean value greater than 5, indicating that the products are well liked by the judges. Eyes, which are the main attributes for injera, were obtained to have less than 5 sensory score for 15% and 20% SPC blend, and also the overall acceptability of 20% injera was found having less than 5 sensory score, indicating that it is not liked by the panelist. Scores for all attributes of the product revealed that significant ($p<0.05$) differences exist between the products.

Table 14: Sensory values for the enriched injera*

Sensory	0%	5%	10%	15%	20%
Color	8.00±.36^{a}	6.40±.37^{bc}	7.00±.39^{ab}	5.80±.41^{c}	5.70±.44^{c}
Test	7.00±.51^{a}	5.90±.56^{b}	5.70±.71^{ab}	5.40±.52^{b}	5.00±.51^{b}
Flavour	7.00±.53^{a}	5.40±56^{b}	6.30±.39^{ab}	5.70±.26^{ab}	5.40±.37^{b}
Texture	6.50±.63^{a}	6.40±.30^{a}	5.10±.68^{a}	5.30±.40^{a}	5.40±.49^{a}
Oder	7.40±.37^{a}	7.10±.27^{a}	11.80±5.02^{a}	6.20±.32^{a}	5.80±.57^{a}
Flexibility	7.30±.51^{a}	6.40±.58^{ab}	6.00±.59^{ab}	5.20±.35^{b}	5.60±.42^{b}
Eyes	6.80±.29^{a}	6.90±.49^{a}	6.30±.48^{a}	4.70±.47^{b}	4.80±.53^{b}
Backside Appearance	6.30±.51^{a}	6.00±.42^{ab}	5.40±.47^{ab}	4.60±.47^{b}	5.00±.39^{ab}
Overall acceptability	7.50±.37^{a}	6.50±.34^{ab}	6.50±.37^{ab}	5.70±.49^{b}	4.30±.33^{c}

Means in the same column followed by same letters are not significantly different ($P< 0.05$)

* mean value ± standard error

4.7 Numerical Optimization

Mixture Design Expert Software has made a summary of the relation/correlation between protein proportion and overall acceptability (Refer Appendix 1, 2 and 3). Thus, protein value was set target (20.76) and overall acceptability was set in range of accepted value (5.7-7.75) by the panelist during the experiment performed. The program set the optimization value for the formulations with relatively better desirability that would resulted in responses with approximate targeted values. Consecutively, to produce the optimum protein and overall acceptability value, Mixture Design Expert Software preset tef and SPC combination to be 87.35% and 12.65, respectively with desirability of 0.68 (Refer appendix 4).

5 CONCLUSION AND RECOMMENDATION

5.1 Conclusion

This study has highlighted an alternative way for the people who are denied from protein intake just because of financial constraint and alternative even for those who deserve animal protein.

Attempt was made to enrich tef flour with soyprotein concentrate (SPC) to supplement protein in relatively a very cheap price. The result divulges that after mixing tef flour with soyprotein concentrate the the protein content of the injera increased from 11.31% for the control and 14.30%, 17.30%, 20.76 % and 25.06% for 5%, 10%, 15% and 20% SPC blended injera, respectively. Increase in protein content at 5% blend of SPC was not statistically significant from that of control injera. Other proximate compositions (moisture, ash and crude fiber) of the injera were also increased. However, fat, carbohydrate and gross energy content of injera were decreased. The mineral contents of enriched flour injera were found to show increment for all Fe, Zn and Ca content. Moreover, although increments in phytate and tannin content are observed in blended injera, the ANOVA performed on phytate content implies that the changes are not statistically significant ($p < 0.05$) with that of the control injera (100% tef flour) until 20% blend. The sensory analysis conducted was resulted in acceptance of injera by the panelist until 15% SPC blend. Mixture Design Expert software used to optimize the exact combination of targeted protein value (20.76%) and ranged overall acceptability (5.7-7.75) has demonstrated the optimum region blend for tef and SPC with the linear models was found to be 87.35% and 12.65%, respectively.

5.2 Recommendation

This study has tried to see the nutritional and organoleptic effect of enriching SPC with tef flour on its end product, *injera*. Accordingly we found a very promising result. Thus, blending 12.65% SPC and 87.35 tef flour enables to improve the protein composition of injera and other nutrients above 75% with relatively minimal cost. Therefore, this study has revealed that it is possible to

minimize the protein deficiency in developing country through enriching SPC with various staple cereals. However, much work is needed

- On bioavailability and stability of the nutrients
- To improve the processing of these foods
- To strengthen soy flour producing industry in Ethiopia
- On awareness to be given to bring them into the society attention.
- On functional properties of enriched flour injera.

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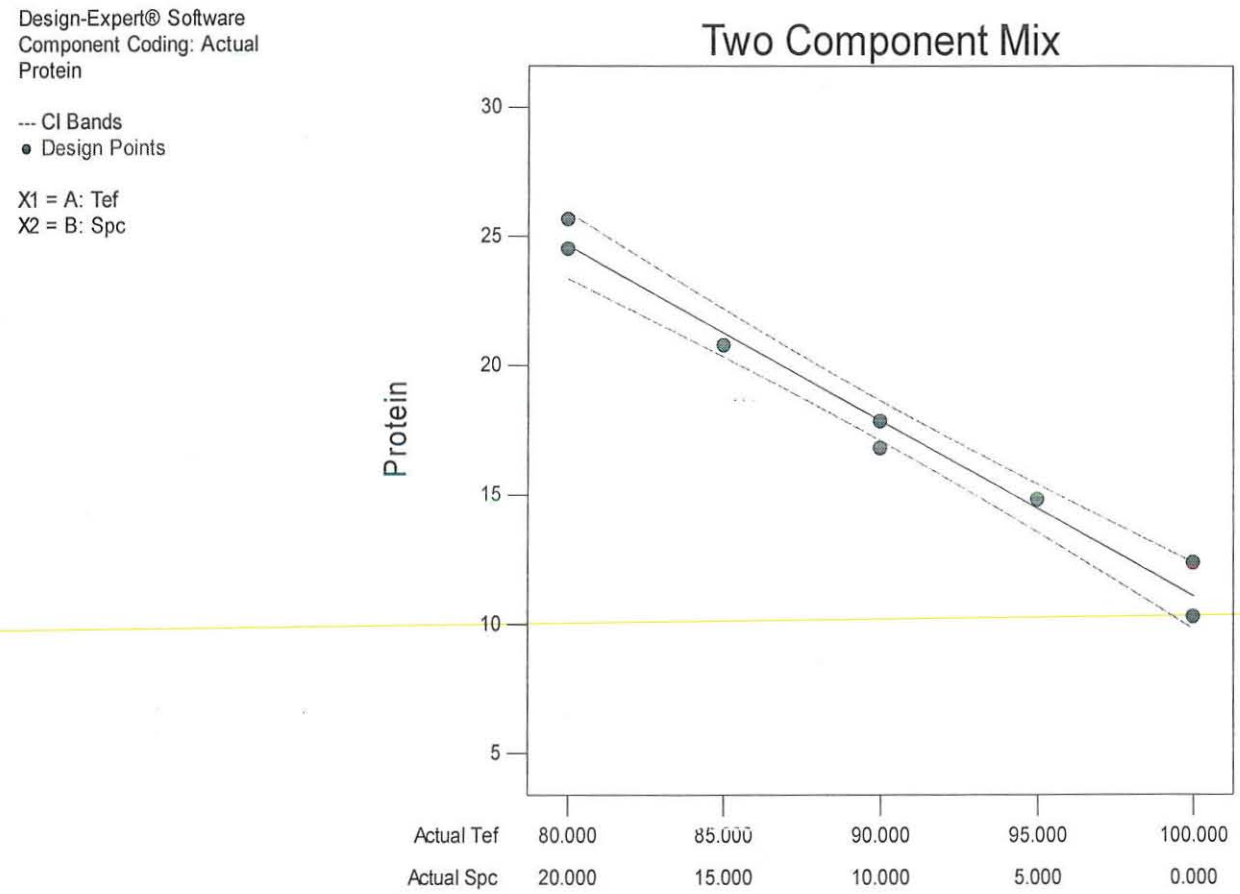
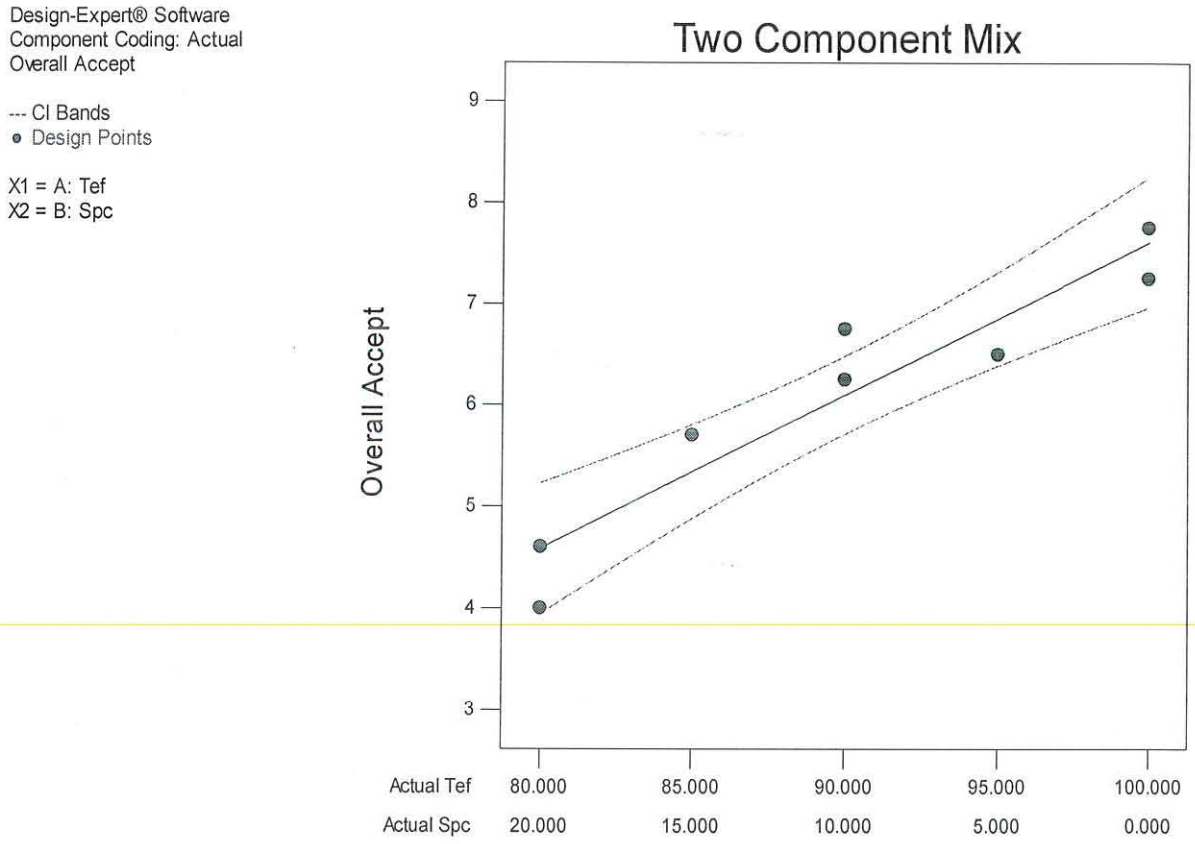
Appendix

Appendix 1: Effect of component mixture on protein value



Appendix 2: Effect of component mixture on the Overall acceptability.

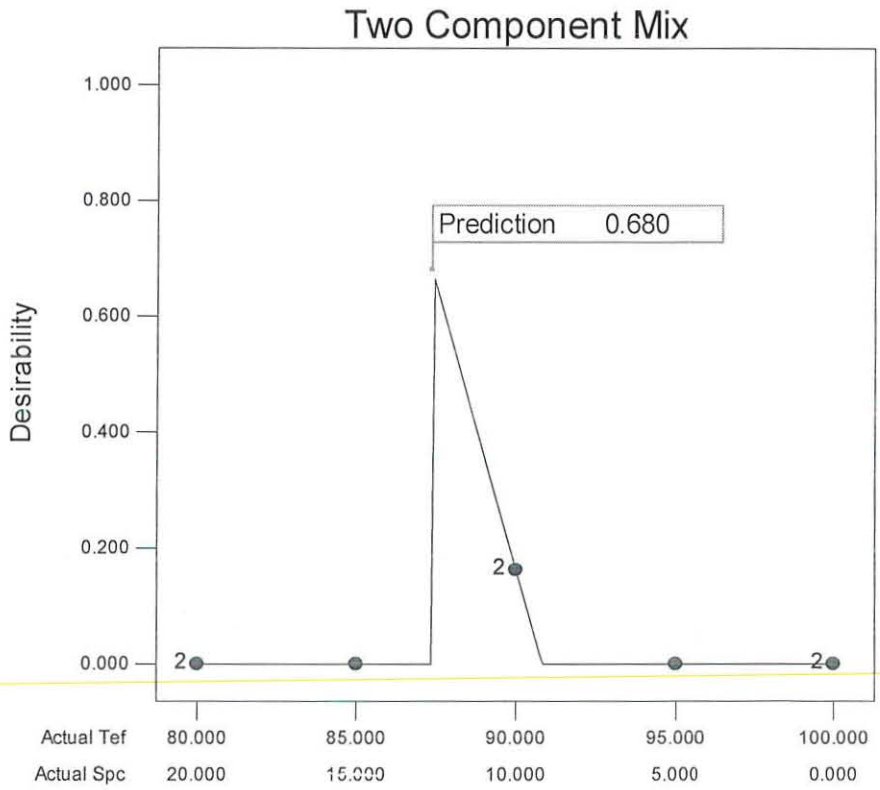


Appendix 3: Effect of component mixture on desirability

Design-Expert® Software
Component Coding: Actual
Desirability

• Design Points

X1 = A: Tef
X2 = B: Spc



Appendix 4: Mixture Design Expert software prediction values

Name	Goal	Lower Limit	Upper Limit	Lower Weight	Upper Weight
A:Tef	is in range	80	100	1	1
B:Spc	is in range	0	20	1	1
Protein	is target =20.76	17.3	25.63	1	1
Overall Accept	is in range	5.7	7.75	1	1

Solutions Number	Tef	Spc	Protein	Overall Accept	Desirability
1	87.35	12.65	19.65	5.7	0.68

Declaration

I, the undersigned, declare that this thesis is my original work and that all sources of materials used for the thesis have been correctly acknowledged.

Name: Sandocan Debebe

Signature:.....

Date:.....

The thesis has been submitted with my approval as a supervisor.

Name: Dr. Gulelat Desse Signature:.....

Date:.....

Professor Negussie Retta Signature:.....

Date:.....

Mr. Feleke Sibhatu Signature:.....

Date:.....