Addis Ababa University

Addis Ababa Institute of Technology

School of Chemical and Bio Engineering

"INVESTIGATION ON MINERAL CHARACTERIZATION OF POWDER INJERA"

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A thesis submitted to the School of Chemical and Bio Engineering, Addis Ababa Institute of Technology, in partial fulfillment of the requirements for the Degree of Master of Science in Chemical Engineering (Biochemical Engineering)

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This is to certify that the thesis prepared by Mulatu Betsegaw, entitled, Development and nutritional characterization of powder injera" and submitted in partial fulfillment of the requirements for the degree of Master of Science in Chemical Engineering (Biochemical Engineering) complies with the regulations of the University and meets the accepted standards with respect to originality and quality.

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DECLARATION

I, the undersigned, declare that this thesis is my original work, has not been presented for a degree in this or any other university, and all sources of materials used for the thesis have been fully acknowledged.

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ABSTRACT

Injera is a fermented, sour bread consumed as a staple food in Ethiopia and Eritrea. The bread can be prepared from various cereals but tef [Eragrostis tef (Zucc.) Trotter] is the most preferred ingredient. A deep study on its inherent characteristics related to processing of injera is needed for its use in various food applications. This research attempt to use spray dryer technology to cook injera, as an alternative method of injera processing. And the product is forwarded for the use of as a food supplement. Also the researcher study the impact of type of tef white and red on nutritional composition of powder injera and impact of fermentation time at 24h, 48h, and 72h on moisture content, ash content, fiber content, iron content, zinc content, calcium content, potassium and sodium content of nutritional composition of powder injera was tested using statistic and regression analysis. Microsoft excel and two factorial three level design expert 11 is used to analyze the experimental result.

It is observed that red tef has higher calcium, zinc, and iron value than the white tef but the white tef powder injera has sodium and potassium content than the red tef powder injera. And it is observed that at fermentation time of 48h the powder injera has higher mineral content than at fermentation time of 24h and 72h.

Key words: red tef, white tef, fermentation time, powder injera, nutritional composition, food-supplement.

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ACRONYMS

EFSA	The European Food Safety Authority
EPI	Ethiopia Public Health Institute
$LaCl_3$	lanthanum chloride
HNO_3	Nitric acid
N	Normality
FAAS	Flame Atomic Absorption Spectroscopy
Zn	zinc
HCl	hydrochloric acid
ANOVA	Analysis of Variance
g	gram
mg	mille gram
L	liter
µl	micro liter
ml	mille liter
ppm	parts per milion
%	Percent
Cu	Copper
Ca	Calcium
Fe	Iron
W1	Weight of fresh sample and box
W2	Weight of dried sample and box
SA	Weight of sample

CHAPTER ONE

1. INTRODUCTION

1.1. Background

Injera (ingerra, enjera, enjerra), a spongy, sourdough, crepe-like flatbread is the staple of the Ethiopian meal accounting for approximately 70% of dietary calories (Gamboa, 2008). In the northern Ethiopian highlands and around Addis Ababa injera is traditionally and preferentially made from the flour of tef (t'ɐf) (*Eragrostis tef* (Zucc.) Trotter), but regionally, wheat, barley, sorghum, millet, maize and rice are all incorporated (Ashenafi, 2006). Tef injera is getting popular in the developed world as well because of its being a whole grain product and gluten free nature, the cause for celiac disease (Abiyu, 2013); (Gamboa, 2008).

Injera is also considered as good sources of energy, fiber, iron, calcium and vitamins although the fermentation process during preparation results in significant reduction of most of the nutrients found in the cereals flour (Mezemir, 2015). Unfortunately, even white tef injera color darkens after baking and during storage. Moreover, the shelf life of white injera is only 3–4 days essentially due to mould spoilage (Abate Z, & Ashagrie D, 2012).

The traditional method of Injera preparation varies from household to household and from region to region. However, in general Injera preparation involves two fermentation stages. The first takes 24-48hr (depending on the sourness desired) from mixing the flour with water and adding the back-slopped culture. Then a portion of the fermented dough was cooked and added back to the fermented dough to initiate the second fermentation. A lot of Efforts have been made by many researchers to promote the use of composite flour for the making of injera by partially substituting tef with maize, barley, wheat, sorghum, with decorticated sorghum, and with sorghum and maize to improve the nutrient density and sensory acceptability injera. However almost no study has reported on productive diversification of injera. This study is planned to improve process technology of injera and diversify mode of use of injera.

Unfortunately, injera has a short shelf life of 3-4 days (Abate Z, & Ashagrie D, 2012). Among the numerous methods used for food conservation, drying is unquestionably the most ancient but still very much used nowadays. It is a process by which water is removed from the food, by vaporization or sublimation, thus reducing the water available for degradation reactions of chemical, enzymatic or microbial nature. Thus the dryer technology used in this research is advantageous for the increment of shelf life of injera.

Under the food supplements Directive (Directive 2002/46/EC) food supplements' are defined as foodstuffs that are meant to supplement the normal diet and which are concentrated sources of nutrients or other substances with a nutritional or physiological effect, alone or in combination, marketed in dose form, namely forms such as capsules, pastilles, tablets, pills and other similar forms, sachets of powder, ampoules of liquids, drop dispensing bottles, and other similar forms of liquids and powders designed to be taken in measured small unit quantities, where nutrients could be vitamins, minerals, herbal extracts and other ingredients.

The idea behind food supplements, also called dietary or nutritional supplements, is to deliver nutrients that may not be consumed in sufficient quantities. Food supplements can be vitamins, minerals, amino acids, fatty acids, and other substances delivered in the form of pills, tablets, capsules, liquid, etc. Supplements are available in a range of doses, and in different combinations. This research forward the use of powder injera as food supplement because of its nutritional composition. And the researcher attempt to study alternative technology to process this Ethiopian national food injera and forwarded the use of powder injera as a source of minerals and food supplement.

1.2. Statement of the Problem

Malnutrition is a major problem in many developing countries which affects infants during transitional phase of weaning (Sanda, 2010). Nutritional requirement in developing countries is remained questionable for long-time. In recent years, nutritionists and the general public have come to regard cereals as more than sources of energy and essential nutrients.

Certain minor components of foods are now recognized for their health-promoting properties, in particular for their roles in preventing or alleviating the effects of some of the chronic diseases such as cardiovascular disease and certain cancers (Bemihiretu Boka, Ashagrie Z Woldegiorgis, & Gulelat D , 2013). Thus Injera has many nutritional and health benefits but alteration and processes modification injera processing and product diversification are very crucial elements. A deep study on its inherent characteristics related to processing is still needed for its use in various food applications across. As tef has positive image in Ethiopian community, injera derivatives should be developed and its nutritional value should be improved to improve health of the society.

Traditional method of Injera processing takes up to three and more days. People with busy life style needs to use foods which are easily available and suitable to cook within short time. Traditional injera processing is not easy for everyone to easily cook and use. Relatively it has long step to convert tef to injera and takes longer time.

And has the same mode of use through the centuries. A lot of Efforts have been made by many researchers to promote the use of composite flour for the making of injera by partially substituting tef with maize, barley, wheat, sorghum, with decorticated sorghum, and with sorghum and maize to improve the nutrient density and sensory acceptability injera. However almost no study has reported on productive diversification of injera.

This study tries to contribute minimize the above problems by producing instant injera using spray dryer technology which can be called powder injera. This study is attempt to introduce powder injera as a food supplement and diversify the mode of use of injera derivatives.

1.3. Objective

1.3.1. General Objective

To investigate on mineral characterize nutritional of powder injera produced by spray dryer technology.

1.4. Specific objectives

- Develop powder injera by spray dryer technology.
- Preliminary characterize mineral content of powder injera composition
- To evaluate the impact of tef type on powder injera ash, fiber and mineral composition.
- To evaluate the impact of fermentation time on powder injera composition.

1.5. Significance of the Study

- This research introduce new mode of injera processing.
- This research introduce new mode of injera utilization.
- Production of potential indigenous food product.
- This research will bases for other researches and projects going to be done on injera derivatives
- Increase consumer interest in health prompted globally applicable powder Injera.

Academic importance, this research will be basis for other researches on injera and its derivatives.

1.6. Scope of the Study

The study focuses on the analysis of development and mineral characterizing of powder injera. The study also focus on the production of powder injera by following traditional way of fermentation and spray dryer technology method. And also focus on two type of tef, white and red tef and three fermentation steps 24h, 48h, and 72h to study its impact on fermentation time on nutrient content of powder injera.

CHAPTER TWO

2. LITERATURE REVIEW

2.1. General description about Injera

Injera is a thin, fermented Ethiopian traditional bread made from flour, water and starter (ersho) which is a fluid saved from previously fermented dough. Tef (Eragrostis tef (Zucc) Trotter) is the most popular grain for making injera; although other grains such as sorghum, maize, barley, wheat and finger millet are sometimes used. The major quality attribute of a good injera is its slightly sour flavour (Zegeye, 1997). Fellow (1997) reported that normal and typical injera is round, soft, spongy and resilient, about 6 mm thick, 60 cm in diameter with uniformly spaced honeycomb-like 'eyes' on the top. Injera has a very high nutritional value, as it is rich in calcium and iron. 'Wot' in the Ethiopian national language (Amharic) means a stew which is made from plant and animal products is served with injera (Abate Z, & Ashagrie D, 2012). As a result of this, injera is not only a kind of bread –it is also an eating utensil (Science of cooking, n.d). Injera is the undisputed national food of Ethiopians (Blandino et al., 2003).According to Ball et al., (1996) about two-third of Ethiopian diet consists of injera and it accounts for about 2/3 of the daily protein intake of Ethiopian population (Arogundade, 2006). Unfortunately, injera storage period does not usually exceed 3 days at ambient temperature under the traditional storage conditions essentially due to mould spoilage.

2.2. Tef general description

Africa is the centre of origin and still today the major producing area for several cereal crops, notably sorghum, pearl millet, finger millet, tef and African rice. These traditional African cereals are sometimes called "Orphan Crops", or even "Lost Crops'' (Council, 1995). This is despite the fact that they are staple foods for millions of people in the semi-arid regions of the world, and particularly those who live by subsistence farming.

Tef (*Eragrostis tef* (Zucc.)) Trotter is a self-pollinated, annual, warm season grass that is used throughout the world as grain for human consumption and as forage for livestock. The amount of

tef produced in the world is increasing rapidly due to the plant's popularity as an especial nutritious grain.

Tef [Eragrostis tef (Zucc.) Trotter] crop is an annual grass species (Poaceae) indigenous to Ethiopia (Vavilov, 1951.)where the major world center for the genetic diversity of tef is found (Ketema,1993)). It is a tropical C4 selfpollinated but chasmogamous tetraploid cereal plant having a chromosom number of 2n = 4X = 40 (Ketema, 1997).

Tef has been cultivated and utilized for human consumption mainly in Ethiopia since time in memorial. The crop was introduced into several other countries like Australia, India and South Africa (Costanza et al., 1979), Argentina (Nicora, 1939), Ukraine (Krasnokutskii & Konstanc, 1939), Malawi, Zaire, Sri Lanka, New Zealand, Mozambique, Uganda, Tanzania, Palestine, Kenya, Canada (Ketema, 1997), USA and parts of Asia (Castellani, 1948) and was grown mainly, but not exclusively, as a forage crop. However, currently with the understanding of the nutritional and health benefits of tef grain, the crop is gaining popularity in the Western world menus and serious efforts are being undertaken to expand its cultivation notably in the Netherlands and United States of America (Evert et al., 2009; van Delden, 2011) and Spain.

Tef can grow under wide and diverse agro-ecologies. It grows best between altitudes of 1800 and 2100 meters with an annual rainfall of 750-850 mm and a temperature range of 10-27 degrees centigrade, though in practice in much more varied areas with rainfall up to 1200mm. The length of growing period ranges from 60 to 180 days (depending on the variety and altitude) with an optimum of 90 to 130 days (Ketema, 1997).

Tef grain does not contain gluten and is an increasingly important dietary component for individuals who suffer from gluten intolerance. When grown as a grain it is normally ground into flour, which is used to make *injera*, flat bread eaten with every meal. It is also used as porridge, similar to cream of wheat or fermented and used to make an alcoholic beverage (Ketema,1997).

Tef is one of the most nutritious alternative grains in the world. It offers calcium, fiber, protein, and antioxidants. It's a great source of energy, protects bone health, and provides long-lasting satiation that can even help to support weight loss efforts. Compared with wheat, tef is higher in nutrients and easier on digestion, mainly because it is absent of gluten. It contains 11% protein, 80% complex carbohydrate and 3% fat. It is an excellent source of essential amino acids, especially lysine, the amino acid that is most often deficient in grain foods. (Doris Piccinin, 14,

2010) Tef provides over two-thirds of the human nutrition in Ethiopia, with grain protein content (10-12%) similar to other cereals (Narasimha, 1997). Tef proteins have non-gluten nature and owing to prevailing portion of prolamins belong to easily digestible ones (Anderson, 2010), which make it a suitable alternative to wheat in the case of celiac disease and gluten-free diet. Besides providing protein and calories, it has high nutritional content, including better amino acid composition, especially lysine, more mineral content (mainly iron, calcium, phosphorus and copper) than other cereal grains, contain B1vitamin and is rich in fibre (Narasimha, 1997).

2.3. Physical properties of tef

There are different tef varieties with different colors varying from white to dark brown. For most varieties, the plant height is about 50-120 cm. One tef plant is capable of producing about 9000-90 000 grains, depending on the variety and production conditions. The seeds are small and ovalshaped (length: 0.9-1.7 mm; diameter: 0.7-1.0 mm) (Wrigley, C., Corke, H., Seetharaman, K., & Faubion, J, 2016) Three thousand tef grains have a mass of approximately one gram . Compared to wheat, the mass of the tef grain is only 0.6-0.8% (Wrigley, C., Corke, H., Seetharaman, K., & Faubion, J, 2016) The seeds of the tef plants are probably the smallest among cereals, with hundred kernels weighing from 0.18 until 0.38 mg (Wrigley, C., Corke, H., Seetharaman, K., & Faubion, J, 2016)

2.4. Composition of tef grain

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

Like other cereals starch in tef constitutes 73% of the grain. Amylose was reported to range 25-32% from extracted starch granules (Bultosa, 2017) and 20-26% with a mean value of 23% in flour starches (Bultosa, 2017). So far no *waxy*- or *amylo*- type starch traits were reported in tef.

The X-ray diffraction study on tef starch granules indicated that they are A -type with similar crystallinity to rice (Bultosa, G., Hall, A.N., Taylor, J.R.N., 2002). The A-type starches were noted for their good digestibility. Study on 13 Ethiopian tef varieties by Bultosa (2007) revealed the presence of variation in the amylose content of different tef varieties where the highest amylose contents were observed for DZ-01-354 (25.8 %), DZ-Cr-44 (25.6 %), DZ-01-1285 (24.2 %) and DZ01-787 (23.8 %), and the lowest were for DZ-Cr-255 (20 %) and DZ-01-1681(21.2 %). The tef starch granules conglomerate and form starch compound granules of the endosperm (Bultosa, et al 2002; Wolter et al., 2013).

The crude protein contents of 13 tef varieties ranged from 8.7–11.1 % with mean 10.4 % (Bultosa, 2007). Hence, in terms of protein content tef is comparable to that of barley, wheat and maize, and higher than that of rice and sorghum (Table 2.1). Hager et al. (2012) reported that protein content of a tef flour obtained from a supplier in the Netherland to be 12.8%. However, such comparisons must be treated with caution as cereal grain protein content is strongly affected by cultivar and cultivation conditions. Protein is synthesized during the fruiting period, whereas starch synthesis starts later. If growing conditions in the late fruiting period are good, starch yield will be high but protein content will be relatively low (Lasztity, 1996). Study by Adebowale et al. (2011) and Hager et al. (2012) indicate that prolamins are the major protein storages in tef and, according to Adebowale et al. (2011); prolamins account approximately 40% of the total tef protein. Absence of wheat type gluten proteins is another important attribute which is being appreciated in tef (Hopman et al., 2008). Work by Spaenji -Dekking et al. (2005) confirmed the absence of wheat type gluten in pepsin and trypsin digests of 14 tef varieties. This indicates the grain to be a valuable ingredient for functional foods destined for gluten intolerant celiac patients.

In general tef grain can be regarded as a well balanced source of essential amino acids when compared to FAO reference pattern (FAO/WHO, 1973). It contains high amount of lysine that is most often deficient in grain foods. Compared to other cereals, tef grain proteins are known to have higher contents of isoleucine, leucine, valine, tyrosine, threonine, methionine, phenylalanine, arginine, alanine, and histidine (Table 2.1).

Despite the large proportion of germ in tef grain the fat content is known to be not as such high. The crude fat content of tef is higher than that of wheat and rice, but lower than maize and sorghum (Table 2.1). The crude fat content in 13 Ethiopian tefvarieties ranged

3.0-2.0% with mean of 2.3% (Bultosa, 2007) and among these cultivars the highest crude fat was for DZ-Cr-82 and the lowest was among DZ-01-354, DZ-01-99, DZ-Cr-37, DZ-01-974 and DZ-01-1681 ($p < 0.05$). El-Alfy et al. (2012) reported that tef grains are rich in unsaturated fatty acids, predominantly oleic acid (32.4 percent) and lino-leic acids (23.8 percent). Compared with tef grain the common cereal grains like rice, wheat and maize contain negligible amount of linoleic acid (LA) and only traces of α-linolenic acid (ALA) (El-Alfy et al., 2012). Furthermore, as tef is consumed as whole grain it can maintain the amount of crude fat and n-6 and n-3 poly-unsaturated fatty acids and this makes it a good source of fatty acids than refined ones. El-Alfy et al. (2012) reported that tef grains are rich in unsaturated fatty acids, predominantly oleic acid (32.4 percent) and lino-leic acids (23.8 percent).

The crude fiber, total and soluble dietary fiber, content of tef is much higher than those of wheat, sorghum, rice and maize (Table 2.1). The reasons are tef is a minute sized grain with higher proportion of bran versus endosperm and germ (Bultosa, 2007). In contrast to most common cereals, the amount of uronic acid in tef grain is high (Umeta, 1986). Among the Ethiopian tef cultivars the crude fiber contents of the brown tef varieties DZ-01-99 (3.8 %) and DZ-01-1681 (3.7 %) were the highest while those of DZ-Cr-44 (2.7 %) and DZ-Cr-255 (2.6 %) were the lowest.

Minerals are important for various physiological functions in the human body and cereal grains are important sources of minerals. The daily requirement of the major minerals (Na, Mg, K, Ca, Ph, and Cl) is estimated to be more than 100 mg while that of the trace elements (Fe, Cu and Zn) is less than 100 mg (Insel et al., 2004). Even though, there are high variability in the mineral content reported in different studies, tef is more rich in such minerals like Ca, Zn, Mg, Fe, Ph and Cu as compared to the contents found in other cereal grains (Table 2.1) compared to the other cereals. Hence, tef can be one of the best options in meeting the daily mineral requirement.

Study by McDonough et al. (2000) indicates that ferulic acid (285.9 μg/g) is the major constituent of phenolic acids in tef grains and Vanillic (54.8 μg/g), cinnamic (46 μg/g), coumaric (36.9 μg/g), gentistic (15 μg/g), syringe (14.9 μg/g) acids are also important constituents of tef phenolic acids. These major constituents of phenolic acids in tef do not

have galloyl and catechol functional groups and thus are less likely to hamper iron absorption (Baye et al., 2013). Hence, there is a possibility that the anti-oxidative properties of the polyphenols in tef can be exploited while not compromising on iron bioavailability.

In general the nutrient profile of tef grain indicates its potential ingredient to in formulation of nutritious and healthy for food formulatios. However, as most of these studies are not variety-based or undertaken on one or two tef types usually described as white and brown tef, the variation available between the different cultivars of tef including the recently released ones have not been explored yet (Baye, 2013).

Table 2.1. Proximate compositions and amino acid and microelement contents of tef grain compared with sorghum, brown rice and wheat.

Component	Tef	Sorghum	Brown Rice	Wheat
Starch (%)	73	62.9	64.3	71
Crude protein (%)	11.0	8.3	7.3	11.7
Amino acid (g/16g N)				
Lysine	3.7	0.3	3.7	2.1
Isoleucine	4.1	0.7	4.5	3.7
Leucine	8.5	2.1	8.2	7.0
Valine	5.5	0.8	6.0	4.1
Phenylalanine	5.7	0.9	5.5	4.9
Tyrosine	3.8	0.7	5.2	2.3
Tryptophan	1.3	0.2	1.2	1.1
Threonine	4.3	0.5	3.7	2.7
Histidine	3.2	0.4	2.3	2.1
Arginine	5.2	0.6	8.5	3.5
Methionine	4.1	0.3	2.7	1.5
Cystine	2.5	0.3	1.8	2.4
Asparagine + Aspartic acid	6.4	5.1		9.0
Serine	4.1	0.8	5.0	5.0
Glutamine + Glutamic Acid	21.8	29.5		17.0
Proline	8.2	1.3	5.0	10.2
Glycine	3.1	0.5	4.5	4.0
Alanine 1.6 5.5 3.6 10.1				
Crude fat (%) 3.9 14.0 2.5 3.2				

Crude fibre (%)	3.0	0.6	0.6-1.0	2.0
Ash (%)	2.8	1.6	1.4	1.6
Minerals (mg/100g)				
Calcium	165.2	50	6.9	39.5
Copper	2.6	0.4	0.2	0.2
Iron	15.7	6.0	0.57	3.5
Magnesium	181.0	180.0	16.9	103.5
Manganese	3.8		0.4	1.0
Phosphorus	425.4	263.3	61.7	
Potassium	380.0	225.2	181.7	
Sodium	15.9	6.2	0.5	
Zinc	4.8	2.0	2.0	1.94

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

2.5. Use of tef as human food

Because of its small size, tef grains are milled into whole-grain flour (bran and germ included). This results in a much higher content of fibres and other nutrients such as minerals, vitamins and bioactive phenolic compounds than most other cereals (Gebremariam, M. M., Zarnkow, M., & Becker, T., 2014). Also, the nutritional value of tef is similar or even higher than that of wheat (Spaenij-Dekking, L., Kooy-Winkelaar, Y., & Koning, F, 2005).The energy value of tef is 1406 kJ per 100 g of flour (Wrigley, C., Corke, H., Seetharaman, K., & Faubion, J, 2016).

Cereals are composed of five main components namely carbohydrates, proteins, fat, fibre and ash (Wrigley, C., Corke, H., Seetharaman, K., & Faubion, J, 2016).

In Ethiopia, tef flour is mainly used to make injera or flat bread. This fermented-circular soft bread is very popular in Ethiopia and forms the traditional basic diet (Tatham, A. S., Fido, R. J., Moore, C. M., Kasarda, D. D., Kuzmicky, D. D., Keen, J. N., & Shewry, P., 1996). A flowchart for the preparation of injera is given in figure 2.1. There are two main steps during the process. The first step involves a fermentation. The fermentation process starts after adding the back slope. The fermentation step takes about 24-72 hours at 25°C. The second step involves a baking step which is normally performed for 2 to 3 minutes at about 200 to 250oC (Wrigley et al., 2016).

Injera is different from other types of bread because of its high moisture content and its chewy and elastic properties. The flour can also be used to prepare porridge, gruel (muk), homemade beverages and several gluten-free food preparations like cakes (The National Academies, 1996).

The dietary requirement for a micronutrient is defined as an intake level which meets a specified criteria for adequacy, thereby minimizing risk of nutrient deficit or excess. These criteria cover a gradient of biological effects related to a range of nutrient intakes which, at the extremes, include the intake required to prevent death associated with nutrient deficit or excess.

2.6. Injera making procedure

The traditional method of *injera* preparation varies from household to household and from region to region. However, in general *injera* preparation involves two fermentation stages. The first takes 24-48 hr (depending on the sourness desired) from mixing the flour with water and adding the back -slopped culture. Then a portion of the fermented dough is cooked and added back to the fermented dough to initiate the second fermentation. The mixture is brought to a batter consistency and allowed to ferment for about 2-3 hr. After gas bubbles have formed and subsided the batter is poured on a hot clay griddle and baked covered. By cooking part of the dough to gelatinize the starch, the carbon dioxide produced by the fermentation is trapped and leavens the *injera* on baking.

Table 2.2. Processing of injera from tef (Source: Fellow, 1997)

Process	Notes
Raw materials ↓	Tef (*Eragrostis tef*) is an indigenous cereal for making injera. Other cereals which may be used are sorghum, millet, barely, wheat or a combination of cereals.
Clean ↓	All impurities are removed by hand and winnowed in the case of sorghum, millet, barely, and wheat. Tef is simply winnowed and sifted through a fine sieve.
Hull ↓	Sorghum, barley and wheat are usually dampened and pounded traditionally in a wooden mortar and pestle to remove the bran.

	Mechanical hullers are also available.
Grind ↓	The sifted tef is ground through a stone mill.
Mixing and first fermentation ↓	Mix one part of flour, two parts of water and about 16 percent ersho (a starter saved from previously fermented dough) by weight of the flour. Mix very well and leave it to ferment for three days.
Thin and heat ↓	Discard the surface water formed on the top of the dough. For every 1kg of original flour, take about 200ml of the fermented mixture and add twice as much water, mix and bring to a boil (traditionally known as 'absit' making). It should be cooled to about 46^0c/115^0F before it is mixed into the main part of the dough. Thin the main dough by adding water equal to the original weight of the flour.
Batter making a02nd second fermentation ↓	Add the absit to the thinned dough and mix very well (known as batter making). Leave the batter for about 30 minute to rise (the second fermentation), before baking commences. A small portion or the batter is saved to serve as a starter (ersho) for the next batch.
Griddle ↓	Injera is griddled by pouring about two-thirds of a litre of the batter onto the hot greased electrical 'metad' (injera griddle made of clay) using circular motion from the outside towards the center. It is cooked in about 2-3 minutes at a temperature of the metad reaching 90-95^0c.Rapeseed oil is used to grease the metad between each one.
Store	Several layers of injera can be stored in a 'messhob' (traditional straw basket) with a tight cover for three days in a cool, dry, ventilated place.

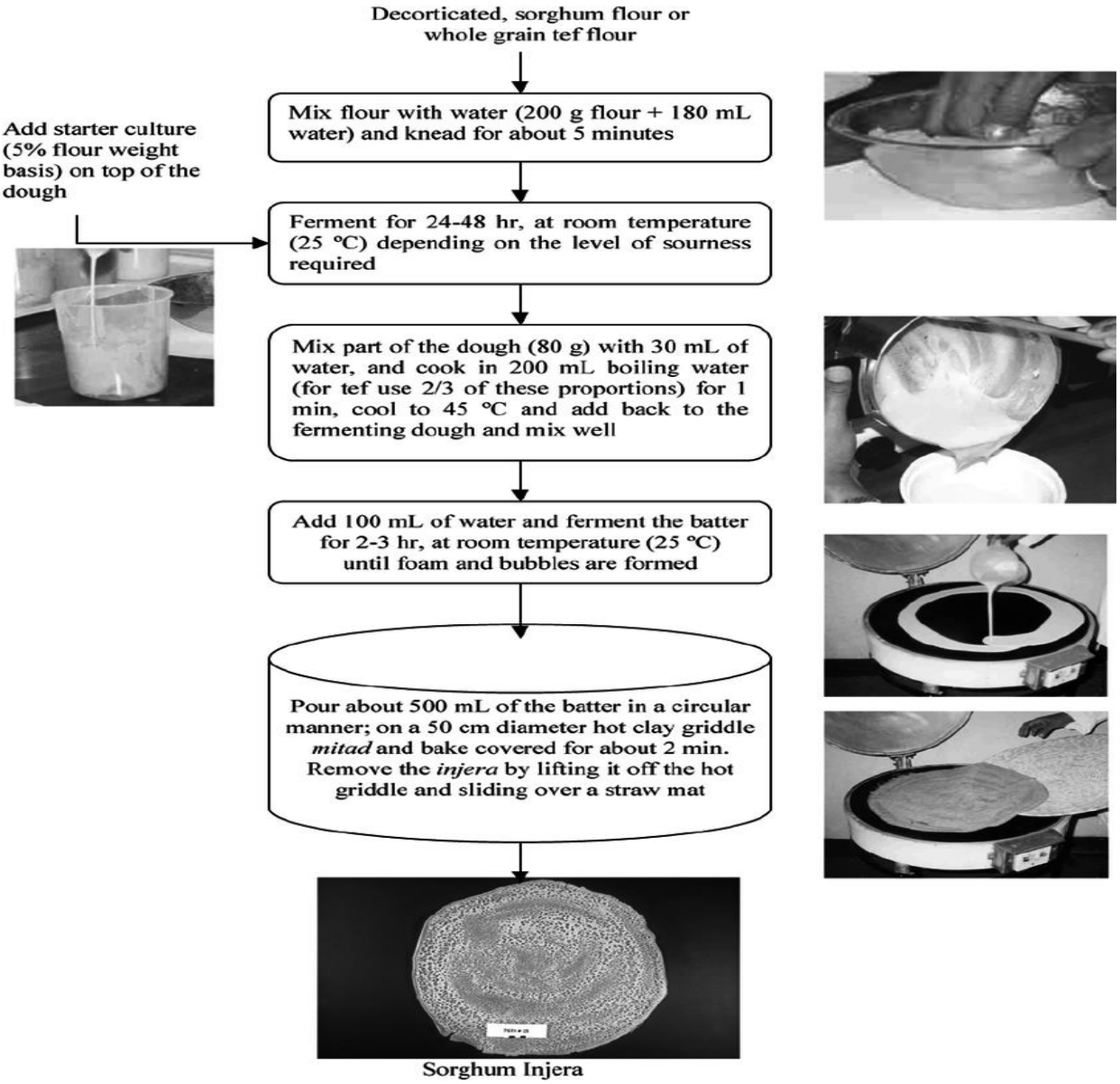

Fig 2.1: flow diagram of injera production

source:https://www.researchgate.net/publication/228550617_Effects_of_Sorghum_Cultivar_on_Injera_Quality

2.7. Health benefits of tef grain food product

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

confirms the advantage of tef in this regard (Umeta, 2005). It is known that celiac patients suffer from an immune mediated disease, triggered by the ingestion of gluten proteins from grain wheat, barley, rye, triticale and also from oats because of the possibility of contamination. The estimated number of celiac patients range 0.6-1% of the world population (Gujral, 2012). The increasing numbers of diagnosed cases and growing awareness makes the availability of gluten-free foods an important socioeconomic issue. The only effective treatment is the complete avoidance of this protein, i.e. the adherence to a gluten-free diet (Vavilov, 1951.). Hence, products of tef grain are becoming popular globally mainly due to the absence of gluten (Bultosa, 2017)) and because it is always consumed as whole grain and in many respects tef grain provides more nutritious than common cereal grains. A systematic review and dose-response meta-anlaysis of cohort studies by (Aune, 2013) suggests that a high intake of whole grains, but not refined grains, is associated with reduced type 2 diabetes risk. The higher total and soluble dietary fiber, content in tef grain described earlier may indicate its potential in prevention and control of diabetes mellitus. Carbohydrate type and its digestibility are also critical in determining the glucose levels after eating and thereby diabetes risks. In this regard also tef grain can serve as an option as it has a lower glycemic index when compared to common cereal grains like wheat and rice (Wolter, A., Hager, A.-S., Zannini E., Arendt, E.K., 2013). Phytates and phenols available in tef grain could also make the grain beneficial for antioxidant activity which could contribute in prevention of cardiovascular diseases and cancers (Boka, 2014).

2.8. FERMENTATION PROCESS

2.8.1. Introduction

The most common meaning of fermentation is the conversion of a sugar into an organic acid or an alcohol. Fermentation occurs naturally in many foods and humans have intentionally used it since ancient times to improve both the preservation and organoleptic properties of food (Paulová, 2016). However, the term “fermentation” is also used in a broader sense for the intentional use of microorganisms such as bacteria, yeast, and fungi to make products useful to humans (biomass, enzymes, primary and secondary metabolites, recombinant products, and products of biotransformation) on an industrial scale (Paulová, 2016).

2.8.2. Microbial activity

Microorganisms in the process of self-replication, produce numerous complex macromolecules from about 100 different monomer units. In the biochemical pathways to achieve this a bacterial cell uses well over 1000 different enzymes and a eukaryotic cell may employ twice as many. The biochemical metabolism can be divided into two broad classes: the anabolic pathways (anabolism) synthesize the complex molecules and their intermediate precursors, and the catabolic pathways (catabolism) supply the energy needed for the anabolic processes. These two divergent activities are closely linked.

Microorganisms that carry out their metabolism using oxygen are referred to as aerobic microorganisms. Some microorganisms can substitute nitrate, others sulfate or ferric ion, for oxygen and thus grow in the absence of oxygen. These microorganisms are referred to as anaerobic. Microorganisms can be classified according to the lowest temperatures at which significant growth occurs. Growth of yeast is optimal in the region of 20-30°C for mesophiles species (Pumphrey, 1996).

In general a shift in the incubation temperature from the optimum to a lower temperature results in a temperature-dependent reduction of metabolic activity. An increase in the incubation temperature can cause a reduction in both the biomass concentration and cell viability due to a temperature- and exposure-dependent decrease in enzyme activity. Many microorganisms display an optimum pH for growth at around 7, with the majority favoring the pH range 5-8. However, there are exceptions including acetic acid bacteria, thiobacilli and urea decomposing bacteria. In addition numerous algae live in natural waters above pH 10 (Pumphrey, 1996).

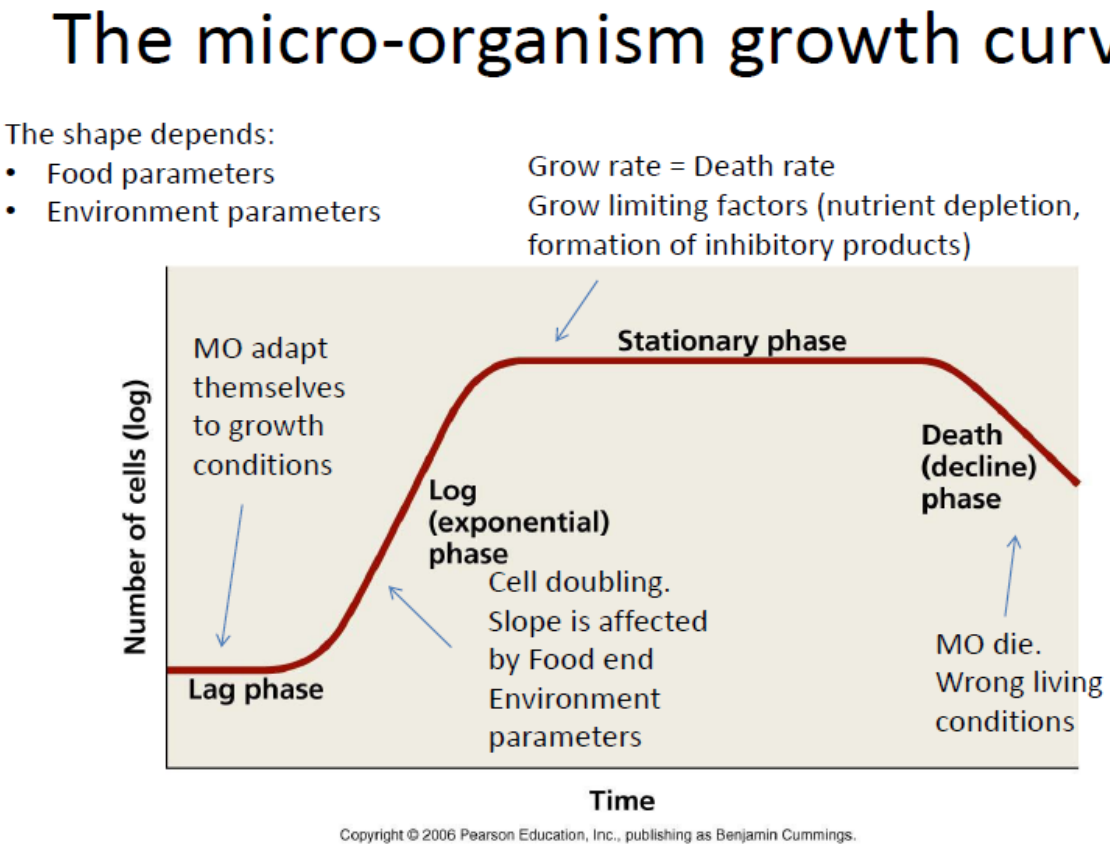

Fig 2.2: Microbial growth curve of fermentation process

2.8.3. Fermentation of injera dough

Fermentation to produce foods such as *injera* involves the controlled souring by naturally occurring lactic acid bacteria. Like many other traditional fermented foods, the fermentation in *injera* making is originally spontaneous and dependent upon the load and flora of microorganisms naturally present in the flour, mixing water and air borne contaminants. However, households are generally able to carry out consistently successful fermentations through practicing a system of back-slopping, whereby a 'portion of liquid from a successful fermentation is used to inoculate freshly prepared dough of sorghum flour.

Over a number of such cycles, rapidly fermenting lactic acid bacteria with a high acid tolerance are probably selected, as reported by Taylor (1996) for *ting* a lactic acid fermented firm sorghum porridge. Showed that by back-slopping each day, the normally slow fermentation process (2-3 days) was accelerated by enrichment with acid producing strains of lactic acid bacteria. This

simple method of carrying out predictable lactic acid fermentation is also practiced in Ethiopian households for *injera* production. Due to destruction and reduction of antinutrients such as phytic acid and tannins during fermentation, the bioavailability of micronutrients in fermented foods is higher than those of unfermented foods (Urga et al 1997). Fermentation also improves *in vitro* carbohydrate availability (Kazanas and Fields 1981), starch digestibility (Hassan and EI Tinay 1995) and protein digestibility (Taylor and Taylor 2002). Additionally, the low pH generated protects fermented foods against the growth of pathogenic microorganisms (Svanberg et al 1992).

The fermentation of tef dough for *injera* making involves several groups of microorganisms, viz: Gram-negative rods, lactic acid bacteria and yeasts, growmg m succession (Gashe et al 1982). The dominating yeast flora at the peak of the fermentation consist of Torulopsis and Saccharomyces species (Gifawesen and Bisrat 1982). An amylase-producing bacteria (Bacillus sp. A-001) has been isolated from fermenting tef dough (Lealem and Gashe 1994), which might be involved in the breakdown of the starch in the dough. Lealem and Gashe (1994) reported that about 9% of the tef dough starch was utilized after fermenting the dough for 72 hr. In tef *injera* fermentation there was a reduction in the pH of the dough from about pH 5.8 to pH 3.8 (Umeta and Faulks 1989). These authors observed that lactic acid and acetic acid were the major organic acids produced during dough fermentation. The carbon dioxide produced during fermentation plays a fundamental role in the formation of the cellular structure of leavened breads (Bloksma 1990), including is *injera*.

2.8.4. Factors affecting fermentation

Fermentation can be influenced by numerous factors, including temperature, pH, nature and composition of medium, dissolved O2, dissolved CO2, operational system (e.g. batch, continuous, fed-batch), feeding condition, mixing and shear rates in the fermenter (Chisti, 1999). Variation of this factors may affect: the rate of fermentation; the product spectrum and yield; the organoleptic properties of the product (appearance, taste, smell and texture); the generation of toxins the nutritional quality: and other physico-chemical properties.

The formulation of the fermentation medium affect the yield, rate and product profile. The medium must provide the necessary amount of carbon, nitrogen trace elements and micro nutrients (Chisti, 1999).

2.9. FOOD SUPPLEMENT

2.9.1. Definition

The European Food Safety Authority (EFSA) defines food supplements as concentrated sources of nutrients or other substances with a nutritional or physiological effect, whose purpose is to supplement a normal diet (European Food Safety Authority, 2019). These substances are produced as pills, capsules, or liquids, and are similar to formulations typical of medicines. They contain concentrated vitamins, minerals, or other substances in specific dosages.
Food supplements are concentrated sources of nutrients or other substances with a nutritional or physiological effect aimed to supplement the normal diet. Food supplements can be marketed in "dose" form, such as pills, tablets, and capsules.

Food supplements either contain nutrients (vitamins and minerals), botanicals (i.e. plant-based products) or other substances (such as amino acids). Botanicals are plant parts, concentrated sources of plants or their extracts or derivatives with a physiological effect. Some well-used botanical supplement products include St. John's Wort, Ginkgo biloba (ginkgo), Valeriana officinalis (valerian), garlic, Echinacea purpurea (echinacea), Panax ginseng (ginseng), Aloe vera (aloe) and Vaccinium myrtillus (blueberry) (Garcia-Alvarez A, 2014).

The use of both nutrients and botanicals in food supplements may pose risks for human health. For minerals or vitamins the risk lies with potential overdose, while not all botanicals are safe for use in food supplements.
In particular, the term 'botanical' may have several confusing meanings and/or synonyms. The same botanical may be used simultaneously in a food supplement and as a medicinal product, depending on the product's intended use. However, both food supplements and medicinal products may be available in the same form i.e. herbs, powders, pills or tablets. All these complexities make it difficult for consumers to distinguish between the thousands of products labelled as 'herbal', 'botanical', 'natural supplement', 'plant food supplement', 'herbal medicinal product' or even 'medical device'.
The common motivations for the consumption of food supplements are to prevent disease, to enhance mental and general health, to enhance sport performance, and to compensate for dietary deficiencies (Frey A, Hoffmann I, Heuer T., 2019).

2.9.2. Dietary fiber as a food supplement

Dietary fiber, also known as roughage or bulk, includes the parts of plant foods your body can't digest or absorb. Unlike other food components, such as fats, proteins or carbohydrates — which your body breaks down and absorbs — fiber isn't digested by your body. Instead, it passes relatively intact through your stomach, small intestine and colon and out of your body.

Fiber is commonly classified as soluble, which dissolves in water, or insoluble, which doesn't dissolve.

A high-fiber diet Normalizes bowel movements. Dietary fiber increases the weight and size of our stool and softens it. A bulky stool is easier to pass, decreasing your chance of constipation. If you have loose, watery stools, fiber may help to solidify the stool because it absorbs water and adds bulk to stool.

Helps maintain bowel health. A high-fiber diet may lower your risk of developing hemorrhoids and small pouches in your colon (diverticular disease). Studies have also found that a high-fiber diet likely lowers the risk of colorectal cancer. Some fiber is fermented in the colon. Researchers are looking at how this may play a role in preventing diseases of the colon.

Lowers cholesterol levels. Soluble fiber found in beans, oats, flaxseed and oat bran may help lower total blood cholesterol levels by lowering low-density lipoprotein, or "bad," cholesterol levels. Studies also have shown that high-fiber foods may have other heart-health benefits, such as reducing blood pressure and inflammation.

Helps control blood sugar levels. In people with diabetes, fiber — particularly soluble fiber — can slow the absorption of sugar and help improve blood sugar levels. A healthy diet that includes insoluble fiber may also reduce the risk of developing type 2 diabetes.

Aids in achieving healthy weight. High-fiber foods tend to be more filling than low-fiber foods, so you're likely to eat less and stay satisfied longer. And high-fiber foods tend to take longer to eat and to be less "energy dense," which means they have fewer calories for the same volume of food.

Helps you live longer. Studies suggest that increasing your dietary fiber intake — especially cereal fiber — is associated with a reduced risk of dying from cardiovascular disease and all cancers.

How much fiber do you need? The Institute of Medicine, which provides science-based advice on matters of medicine and health, gives the following daily fiber recommendations for adults:

Refined or processed foods — such as canned fruits and vegetables, pulp-free juices, white breads and pastas, and non-whole-grain cereals — are lower in fiber. The grain-refining process removes the outer coat (bran) from the grain, which lowers its fiber content. Enriched foods have some of the B vitamins and iron added back after processing, but not the fiber.

2.9.3. Fiber supplements and fortified foods

Whole foods rather than fiber supplements are generally better. Fiber supplements — such as Metamucil, Citrucel and FiberCon — don't provide the variety of fibers, vitamins, minerals and other beneficial nutrients that foods do.

Another way to get more fiber is to eat foods, such as cereal, granola bars, yogurt and ice cream, with fiber added. The added fiber usually is labeled as "inulin" or "chicory root." Some people complain of gassiness after eating foods with added fiber.

However, some people may still need a fiber supplement if dietary changes aren't sufficient or if they have certain medical conditions, such as constipation, diarrhea or irritable bowel syndrome. Check with your doctor before taking fiber supplements.

The crude fiber of the baked injera varied from 3.91 to 5.21% (Abate Z, & Ashagrie D, 2012). Injera made from 100% tef had the highest fiber content (5.21%) and injera containing 85% tef, and 15% maize with no rice has the least crude fiber content (3.91%). However, Ashen (Ashenafi, 2006) reported that the crude fiber of injera made from tef and maize were 1.00% DM and 0.8% DM, respectively.

2.9.4. Zinc as a food supplement

Zinc has an atomic weight of 65.37 and is classified as a group IIB post-transition metal. In biological systems, zinc exists as Zn^{2+} and is present in all tissue and fluids in the body. Total body content of zinc is between 2 and 4 g and plasma concentration is between 11 and 18 μM (approximately 0.1% of total body content) (Albert Flynn, 2005). Urinary zinc excretion is

between 300 to 700 µg/day. Zinc is also present in foods and supplements as salts of the divalent cation. Under European legislation the following salts of zinc: acetate, chloride, citrate, gluconate, lactate, oxide, carbonate and sulphate are included in the list of substances that can be used in the manufacture of foods for particular nutritional uses and in food supplements (the legal measure on food supplements is expected to be adopted in the immediate future). Zinc content in the most common single nutrient supplements on the market is 30 mg per capsule, range 15-50 mg and in the most common multiple nutrient supplements is 10-15 mg, range 2-20 mg (Albert Flynn, 2005).

It is likely that identifying and correcting borderline nutritional zinc deficiency will offer widespread health benefits. Pharmacological doses of zinc may also be beneficial in certain circumstances and harmful in others. In the public health arena, the positive results of zinc supplementation trials on childhood morbidity and mortality in developing countries have been remarkable and offer promise as a low cost solution to specific health problems (Rebecca B. Costello, 1998).

2.9.5. Iron as a food supplement

Iron is a mineral in the human body. It is one of the parts of hemoglobin, the substance in red blood cells that helps blood carry oxygen throughout the body. If you do not have enough iron, your body cannot make hemoglobin, and you may get anemia, a health problem that occurs when there is not enough hemoglobin in the blood. When you get anemia, you are said to be "anemic".

The major signs of anemia are: Feeling tired, Problems breathing, Dizziness, Headache, and Feeling cold (C. Cole, 2014).

Beyond proper nutrition as important in maintaining your body’s normal functions and overall general health, adequate iron intake and balance is important in maintaining our body’s normal function of manufacturing blood cells. At the Blood Center, a fingerstick blood count determination (sometimes called iron level) is used as a screening test to qualify you for blood donation. A minimum acceptable result in this test is set in order to avoid temporarily lowering your blood count through blood donation to below normal levels. If your blood count was below this minimum acceptable level for blood donors today, we have to consider increased dietary iron intake i.e. red tef injera in order to stimulate increased blood cell production in anticipation of future blood donations.

The amount of iron intake for different age group is reported in Food Factsheet of the British Dietetic Association as follows table (Breese, 2017).

Table 2.7.1 per day iron intake

Group	Age (years)	Iron (mg) per day
Infants	0-3 months 4-6 months 7-12 months	1.7 4.3 7.8
Children	1-3 years 4-6 years 7-10 years	6.9 6.1 8.7
Adolescents	11-18 years	14.8(girls) 11.3 (boys)
Adults	19-50 years 19-50 years 50+ years	8.7 (males) 14.8

2.9.6. Sodium as a food supplement

Sodium (Na) is a metal with an atomic mass of 23. It is found widely in nature and as a normal constituent of foods. It is added to foods, most frequently as sodium chloride (NaCl), common known as salt (1 mmol is equivalent to 23 mg sodium and approximates 58 mg sodium chloride), but also as other salts, e.g.nitrate, nitrite, phosphates, glutamate. In drinking water, the guide level of sodium is 20 mg/L (Council Directive 80/778/EEC). Sodium is an essential nutrient and a dietary inadequacy may lead to serious consequences. Sodium is present in biological systems as the main cation in the extracellular space, acting to maintain extracellular volume and plasma osmolality It is sometimes difficult to differentiate the effects of the sodium moiety from sodium salts such as NaCl on physiology and metabolism. Therefore this Opinion should be read in conjunction with the Panel's Opinion on the tolerable upper intake level of chloride (NDA, 2005).

Sodium is found in plant and animal based food and also in drinking water. Sodium is added to foods, commonly as sodium chloride, during processing, cooking and immediately prior to consumption, but also in other forms, for example as sodium nitrate, sodium phosphate or sodium glutamate. The main reasons for the addition of salt during the processing of foods are for flavour, texture and preservation. The sodium content of natural foods varies from around 0.1 to 3 mmol/100g, with fruit containing 0.1 mmol/100g, vegetables 0.3 mmol/100g, and meat, fish or eggs 3.0 mmol/100g. The content of sodium as sodium chloride in processed foods may be much higher; bread 20 mmol/100g; cheese, 30 mmol/ 100g; salted butter, 40 mmol/100g; and lean raw bacon, 80 mmol/100g. It is difficult to obtain reliable information on the sodium chloride content of foods as consumed, because of variable practices in terms of processing, food preparation and personal preferences.

2.9.7. Calcium as a food supplement

Calcium (Ca) belongs to group II of the third period of the Periodic Table of Elements. It has an atomic weight of 40.08; its atomic number is 20, its valency is 2. It is the fifth most abundant element in the human body. The calcium content of the human body is 25 to 30 g at birth (0.8% of the body weight) and between 900 and 1300 g in adult men (up to 1.7% of body weight) (Weaver *et al*, 1996). Over 99% of the total calcium of the body is located in the bones, where it accounts for 39% of the total body bone mineral content (Weaver, 2001), and in the teeth, mostly as hydroxyapatite. Bone mineral provides structure and strength to the body and, very important, a reservoir of calcium that helps to maintain a constant concentration of blood calcium. Less than 1% of total body calcium is found in soft tissues (~7 g) and body fluids (~1 g). Calcium in the extracellular fluid and the blood are kept constant at 2.5 mmol/L (10 mg/dL) (between 2.25 and 2.75 mmol/L) via cell surface calcium-sensing receptors in parathyroid, kidney, intestine, lung, brain, skin, bone marrow, osteoblasts and other organs. Calcium is present in blood in three different forms: as free Ca2+ ions, bound to protein (about 45%), and complexed to citrate, phosphate, sulphate and carbonate (about 10%). Ionised calcium is kept within narrow limits (Worth *et al*, 1981) by the action of three hormones, parathyroid hormone, dihydroxycholecalciferol, and calcitonin. Extracellular calcium serves as a source for the skeleton and participates in blood clotting and intercellular adhesion. Intracellular calcium varies widely betweentissues and is predominantly bound to intracellular membrane structures of the nucleus, mitochondria, endoplasmatic reticulum or contained in special storage vesicles. Free Ca2+ is only

0.1 μmol/L in the cytosol, which is 10,000 times lower than in the extracellular fluid (1 mmol/L). Intracellular calcium rises in response to stimuli interacting with the cell surface receptor. The increase of intracellular calcium comes from influx of extracellular calcium or from release of intracellular calcium stores. This activates specific responses like hormone or neurotransmitter release, muscle contraction, cellular differentiation and many others. Calcium must be ingested with the diet in sufficient amounts to allow for calcium deposition during bone growth and modeling and to compensate for obligatory intestinal, faecal and dermal losses during the life-time.

Foods vary widely in calcium content. The best sources are milk (120 mg/100 g) and milk products (up to 1100 mg/100 g), from which about 32% is absorbable (Weaver, 2001). In European diets about 45 to 70% of the dietary calcium intake is provided by dairy products (Guéguen and Pointillart, 2000; IUNA, 2001). Some plants are good sources of well-absorbable calcium, e.g. brassica, almonds, dried apricots. However, some vegetables contain considerable amounts of calcium, which is poorly absorbed because of a high content in oxalate (rhubarb, spinach) and which forms sparingly soluble calcium oxalate. Drinking water and mineral waters (>150 mg calcium/L) can also be good sources of absorbable calcium.

Dietary intake

Total calcium intake of men and women who consumed calcium supplements more than once per week was significantly higher (men: 1275-1394; women: 1146-1221 mg/day) than in those never taking calcium supplements (men: 1190-1242; women: 1081-1117 mg/day) (Mensink and Ströbel, 1999). Men consume in absolute amounts about 10% more calcium than women. In Germany the highest calcium intake was observed in young men between 15 to 24 years: 2100 mg/day without supplements (Heseker *et al*, 1994). A longitudinal observational study (*DONALD study*), which started in 1985 and follows children from the age of 3 months to 18 years (sample size 400 to 500 subjects) showed that the mean calcium intake values in these healthy children were below the recommended intake values beyond the age of 3 years. Less than 10% of adolescents (13 to 18 years) consumed more than the recommended calcium intake (Alexy and Kersting, 1999). In this group calcium from fortified food amounted to maximal 5% of the total daily intake between 2 and 14 years of age (Sichert-Hellert *et al*, 2001).

2.9.8. Potassium As a food supplement

Potassium is widely distributed in the earth's crust, seawater as a mono-valent cation. It occurs naturally in the form of several mineral salts but does not occur as metallic potassium. Potassium in foods is associated with salts of weak organic acids. Various potassium salts, e.g. KCl, are used in many applications, amongst others as ingredients in foods (e.g. additives), food supplements and drugs, household chemicals etc. In this opinion, the term potassium refers to ionic potassium, except where specific potassium compounds are stated. One mmol potassium is equivalent to 39.1 mg. Important potassium sources include potatoes, fruit and berries, vegetables, milk products (excl. cheese) and nuts. Potassium occurs in foods mainly associated with weak organic acids. Potassium is also found in mineral, spring, and table waters, but the content varies considerably. Some mineral waters available on the market can, when consumed in large quantities, contribute significantly to the daily intake. The average dietary intake of potassium according to European food consumption studies is in the range of 3000 to 4000 mg/day. . The 95th to 97th percentile intake is in the range of 4000-5500 mg/day.
A number of food additives also contain potassium as the cation. The level of potassium added to foods as additives generally contribute only to a minor degree to the daily intake. Salt substitutes, in which part of the sodium chloride has been substituted with potassium salts (usually KCl), can contribute to the potassium intake. Food supplements can contribute significant amounts of potassium (usually as KCl), but according to recent food consumption surveys average reported contributions were only up to 5 % of the total potassium intake Männistö *et al* (2003).

2.10. Spray dryer technology

Spray drying is a 140 years young and flourishing drying technique. Throughout all these years, this perpetual process has exhibited an ebullient growth, imbibing innumerable innovations in terms of its operational design and widely varied applications. (Anandharamakrishnan and Padma Ishwarya S, 2015).

Spray drying has its origin in the United States, since the first patented design was registered there in 1872. World War II was a significant chronological (Anandharamakrishnan and Padma Ishwarya S, 2015).

By definition, “Spray drying is the transformation of feed from a fluid state into a dried particulate form by spraying the feed into a hot drying medium.” (Masters, Spray Drying Handbook, 1991)

A spray dryer operates on convection mode. The principle of working is moisture removal by application of heat to the feed product and controlling the humidity of the drying medium. Here, the uniqueness is that the evaporation of moisture is promoted by spraying the feed into a heated atmosphere, resulting in improved drying rate. The mechanism can be better understood, when the spray drying process is divided into its constituent unit operations.

A liquid feed entering the spray dryer undergoes a series of transformations before it becomes powder. The changes are due to the influence of each of the four stages (Figure 2.11.1) involved in spray drying, namely:

1. Atomization of the feed solution.
2. Contact of spray with the hot gas.
3. Evaporation of moisture.
4. Particle separation.moisture.

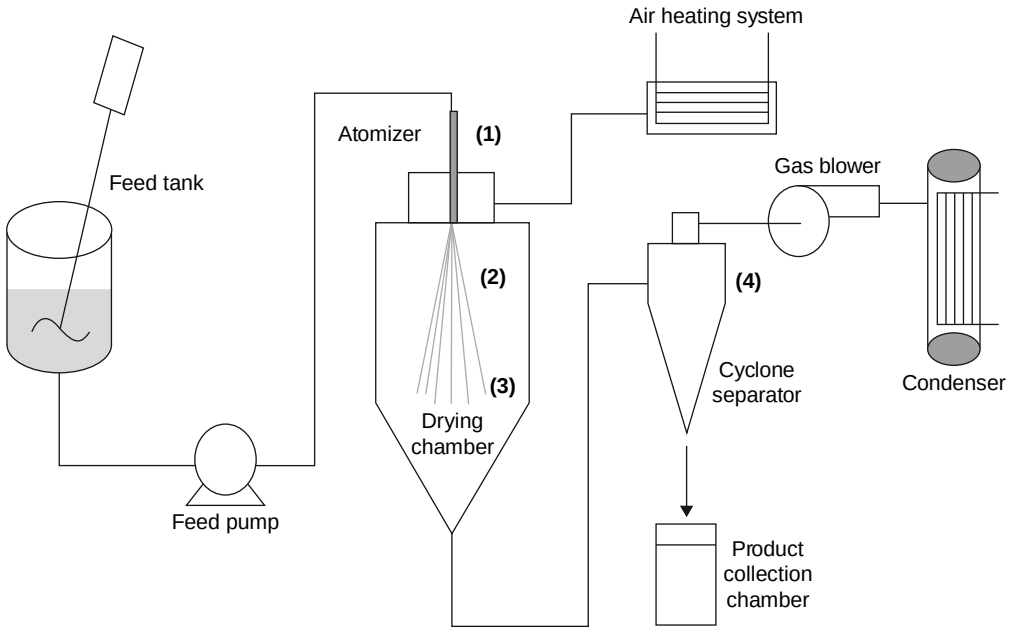

Figure Process steps of spray drying. (1) Atomization. (2) Spray – hot air contact. (3)

Each of the above exerts influence on the final product quality. Understanding the process steps, along with the hardware systems involved in it, will enable visualization of the operation on a glimpse of a reading.

2.10.1. STAGE 1: ATOMIZATION

Atomization is the heart of spray drying, and is the first transformation process that the feed undergoes during spray drying. The breakup of bulk liquid into a large number of droplets drives the rest of the spray drying process by reducing the internal resistances to moisture transfer from the droplet to the surrounding medium. This is because of the enormous increase in surface area of the bulk fluid as the droplet fission proceeds, with its instability increasing in accordance with the intensity of atomization.

Atomization is central to the spray drying process, owing to its influence on shape, structure, velocity and size distribution of the droplets and, in turn, the particle size and nature of the final product. A cubic meter of liquid forms approximately 2×10^{12} uniform 100 micron-sized droplets, offering a total surface area of over 60,000 m^2 (Masters, Spray Drying Handbook, 1991). This greater surface-to-volume ratio enables spray drying to achieve a faster drying rate (as drying time is proportional to the square of the particle dimension). Consequently, there is minimal loss of heat sensitive compounds and, eventually, particles of the desired morphology and physical characteristics are obtained.

2.10.2. PRINCIPLE OF ATOMIZATION

The working principle of the atomizers is governed by the liquid disintegration phenomenon explained by several researchers. It is worth understanding the progression in the concepts on atomization phenomenon across the years. This will also help in appreciating the science of droplet formation from an atomizer. Disintegration of the liquid at the periphery or tip of the atomizer is by virtue of the turbulence in the emerging liquid jet and the action of air forces; the resistance to disintegration is offered by viscosity and surface tension forces in the liquid. The realignment of shear stresses within the liquid, once the droplet is airborne, contributes to the droplet fission during atomization.

2.10.3. STAGE 2: SPRAY-AIR CONTACT

This stage, and the subsequent process steps of spray drying, constitute the particle formation phase. With the bulk feed atomized into tiny droplets, the next step is to bring the droplets into intimate contact with the hot gas. This enables rapid evaporation of moisture from the surface of all the droplets in a uniform manner. Here, the critical requirement is uniform gas flow to all parts of the drying chamber.

During spray-air contact, the droplets usually meet hot air in the spraying chamber, either in co-current flow or counter-current flow. In co-current flow (Figure 2.11.2(a)), the product and drying medium passes through the dryer in the same direction.

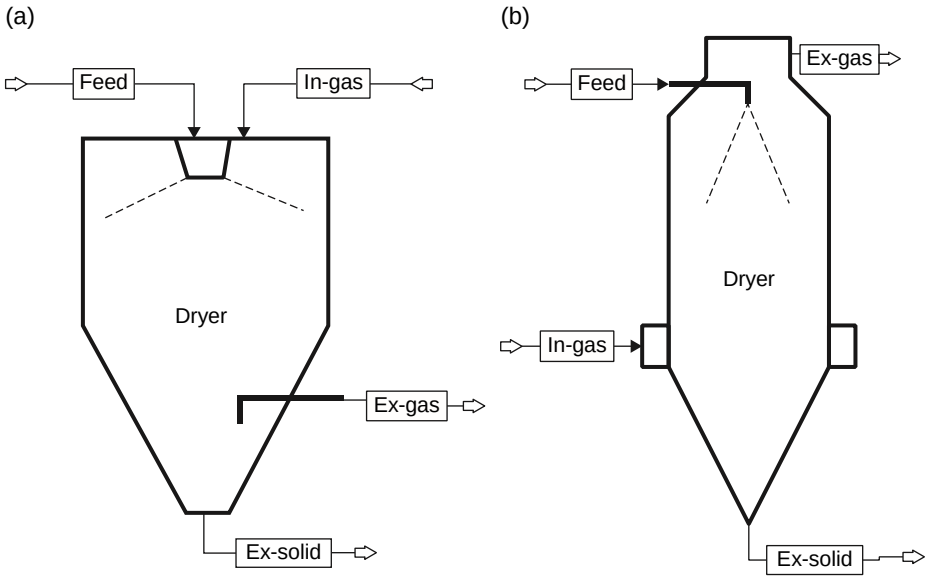

Figure 2.13.2: Spray dryer configurations: (a) co-current (left); (b) counter-current (right) (Oakley,

In this arrangement, the atomized droplets entering the dryer are in contact with the hot inlet air, but their temperature is kept low due to a high rate of evaporation taking place and is approximately at the wet-bulb temperature. Wet-bulb temperature is the thermal energy of hot air used for evaporation (i.e., the removal of latent heat of vaporization from the air that cools it, and this is termed as “evaporative cooling”. This allows the particle to be maintained at a temperature below the outlet temperature of the drying air.) The cold air, in turn, pneumatically conveys the dried particles through the system. The contact time of the hot air with the spray droplets is only a few seconds, during which drying is achieved, and the air temperature drops instantaneously.

This results in advantages of low temperature and low residence time of particles, with the added merit of less thermal degradation of heat sensitive products.

In contrast, in the counter-current configuration (Figure 2.11.2(b)), the product and drying medium enter at the opposite ends of the drying chamber. Here, the outlet product temperature is higher than the exhaust air temperature, and is almost at the feed air temperature, with which it is in contact. This type of arrangement is used only for heat-resistant products.

In another type, called mixed flow, the dryer design incorporates both co-current flow and counter-current flow. This type of arrangement is used for drying coarse free-flowing powder, but the drawback is the higher exit temperature of the product.

2.10.4. STAGE 3: EVAPORATION OF MOISTURE

The most critical step in particle formation, this process step is associated with the morphology of the final product. Evaporation of moisture during spray drying can be visualized as two stages:

i. constant rate period;
ii. Falling rate period.

Examining the drying kinetics of the spray drying process is critical in predicting the heat and mass transfer in the drying material. This can be best explained by a mathematical model for the evaporation of a single droplet which is subjected to convective drying in a spray dryer (Figure 2.11.3). Initially, when the droplet is exposed to hot gas, rapid evaporation takes place. During this exposure, the droplet is heated from its initial temperature (T_0) to the temperature of equilibrium evaporation temperature (T_{eq}) (Figure 2.11.3, AB). During this period, the removal of moisture follows the *constant rate period* of the drying rate curve as the moisture is removed constantly from the surface of the droplet keeping it sufficiently cool. The droplet surface remains saturated with moisture at this stage and its temperature is constant at the wet-bulb temperature (Figure 2.11.3, BC; (Dolinsky, 2001).

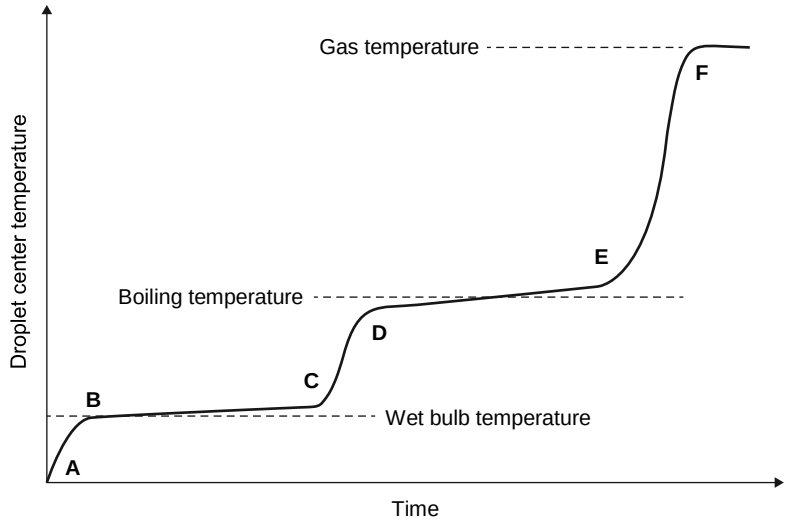

Figure 2.13.3: Temperature history during spray drying of a liquid droplet (Handscomb, C.S., Kraft, M. and Bayly, A.E, 2009)

2.10.5. Spray-drying process parameters and their influence on product quality

It is obvious from the previous sections on the spray drying process steps that spray drying is a method which strongly and equally depends on material properties, equipment design and setting of operation parameters. The above listed factors influence the final product quality, mainly in terms of residual moisture, particle size and morphology. Although the optimization of these variables is usually achieved by a "trial and error" approach, an understanding of the basic guidelines of spray drying would result in intelligent operation of the equipment. The different spray drying process parameters, corresponding to each of the four steps of spray drying, are discussed in the following section.

2.10.6. Atomization parameters

The performance of atomizers depends on three parameters: the atomization pressure, feed flow rate, viscosity and density. These parameters have an influence on the droplet size.

2.10.6.1. Atomization pressure

With the same type of nozzle and feed material, as the atomization pressure is increased, droplet size decreases according to the relationship below (Equation (Masters, Spray Drying Handbook, 1991)):

$$\frac{D_2}{D_1} = \left(\frac{P_2}{P_1}\right)^{-0.3}$$

Where D_1 and D_2 are the initial and final droplet sizes on changing the atomization pressure from P_1 to P_2, respectively. This is due to the requirement of an efficient delivery of the atomization energy to the bulk liquid to drive droplet fission.

2.10.6.2. Feed flow rate

At constant atomization pressure, increasing the flow rate increases the droplet size, since the nozzle's hydraulic energy has to atomize more liquid. As the liquid interaction with the atomization energy is minimal, the droplet fission is insufficient to reduce its size. Feed rate is associated with the peristaltic pump speed which feeds to the spray solution to the rotary or nozzle atomizer.

As the atomization pressure is for the nozzle atomizers, wheel rotation speed and diameter are for the rotary atomizers. The wheel rotation speed and wheel diameter have an inverse relationship to the droplet size.

2.10.6.3. Feed viscosity

The feed viscosity has a direct relationship to droplet size. As the feed viscosity increases, the atomization energy supplied to the nozzle must overcome large viscous forces to achieve smaller droplet sizes. Viscous forces tend to reduce the energy available for breaking the droplets, thus resulting in larger droplets. The equation describing the relationship between droplet size and feed viscosity is given below. The same principle also applies to that of feed density.

$$\frac{D_2}{D_1} = \left(\frac{\mu_2}{\mu_1}\right)^{-0.2}$$

Where D_1 and D_2 are the initial and final droplet sizes on changing the feed viscosity from μ_1 to μ_2, respectively.

2.10.6.4. Feed surface tension

As described in the principle of atomization, surface tension of the liquid plays an important role in the extent of atomization. To achieve atomization, the atomizer should overcome the surface tension of the feed liquid. Hence, a liquid having higher surface tension is difficult to atomize. This necessitates the preparation of an emulsion with the aid of emulsifier addition, and a homogenization step prior to spray drying in certain cases, in order to combat the surface tension of a feed, especially that involving multiple components.

2.10.7. Parameters of spray-air contact and evaporation

An important parameter involved in this process step is the spray angle, which is measured at the nozzle orifice and is related to the nozzle's liquid tangential velocity. The liquid tangential velocity is the speed at which the feed liquid spins inside the nozzle before it is divided into fine droplets and sprayed into the drying chamber. Widening the spray angle increases this velocity to reduce the droplet size. The choice of spray angle is related to the type of air flow, namely co-current or counter-current, with the wider angle being used with the former and the narrow angle with the latter. In the co-current operation, the downward-flowing air narrows down the spray angle. In the counter-current operation, the spray moving downward is widened by the upward-flowing air. The other important parameters in this step are the aspirator rate, air humidity, inlet and outlet air temperature, the glass transition temperature, and the residence time of particles in the spray dryer.

2.10.7.1. Aspirator flow rate (or speed)

The aspirator in the spray dryer is associated with the supply of drying air to the spray chamber by the aspirator motor under pressure conditions. By altering the aspirator flow rate, the amount of heated drying air entering the spray chamber can be regulated.

2.10.7.2. Inlet temperature

Inlet temperature is the temperature of the heated drying air. The inlet temperature is significant in cooling the atomized feed droplets to their wet-bulb temperature. In addition, it has a direct

relationship to the wet-bulb temperature of the surrounding hot air (Oldfield, 2005). Inlet temperature is often associated with dryer evaporative capacity and thermal efficiency. Therefore, higher inlet temperature is favorable in terms of achieving higher throughput of spray dryers. However, a lower value of the inlet temperature reduces the wet-bulb temperature of the surrounding hot air, and prevents the degradative losses of the active compounds during the initia stages of spray drying. The trade-off between the aforementioned circumstances is important in deciding an optimal inlet temperature for the spray drying process.

2.10.7.3. OUTLET TEMPERATURE

The temperature of the air laden with solid particles before entering the cyclone is defined as the outlet temperature. This temperature is a result of the heat and mass balance in the drying cylinder, and therefore cannot be regulated. Striking the optimum temperature difference between the inlet and outlet temperature is most critical in a spray drying process.

Outlet temperature correlates with the final moisture content and surface topography of the final product (Maas *et al.*, 2011). For instance, operation at high outlet temperature is carried out to achieve high moisture content to obtain agglomerated "instant" powdered products. This is because higher outlet air temperature promotes rapid crust formation while the drying of the inner core is still not complete

2.10.7.4. GLASS TRANSITION TEMPERATURE (T_G)

Glass transition is a feature of second-order time temperature dependent transition, which is characterized by a discontinuity in physical, mechanical, electrical, thermal, and other properties of a material (Rahman, 1995). T_g is the temperature above which the matrix shifts from the structurally rigid glassy state to a rubbery state, which is associated with product stickiness on the spray chamber wall in spray drying. Stickiness is considered to be the major process challenge in spray drying. It leads to product agglomeration and poses problems of caking and lumping of the product during packaging of the spray dried products. The T_g of feed depends on the constituent solutes present in the feed.

2.10.7.5. RESIDENCE TIME OF PARTICLES IN THE SPRAY CHAMBER

This parameter is important from two perspectives – namely, with respect to complete drying of feed droplets to achieve optimum product specifications, and in the control of particle

temperature to minimize aroma loss and thermal degradation of heat sensitive materials. The particle residence time (RT) also affects product quality indices such as solubility and bulk density. RT is divided into two parts: primary and secondary residence times. The primary RT is calculated from the time taken for droplets leaving the nozzle to impact on the wall or leave at the outlet. The secondary residence time can be defined as the time taken for a particle to slide along the wall from the impact position to the exit (Anandharamakrishnan and Padma Ishwarya S, 2015). While prediction of RT in a spray chamber is experimentally difficult, recent advancements in modeling and powerful computational simulation techniques aid in efficiently calculating the RT.

2.10.8. Spray-drying applications in the biomedical area

Spray drying medical applications are mainly focused on the production of microparticles designed for encapsulation purposes and drug delivery systems, which can be then administered orally, pulmonary, ophthalmologically, parenterally, nasally or even vaginally (Daniel Santos, Ana Colette Maurício, Vitor Sencadas, José Domingos Santos, Maria H. Fernandes and Pedro S. Gome, 2017). The fact that this technological process enables to dry heat-sensitive components, like enzymes or proteins, without compromising their biological activity makes the production of such systems possible (Daniel Santos, Ana Colette Maurício, Vitor Sencadas, José Domingos Santos, Maria H. Fernandes and Pedro S. Gome, 2017). As a result, within the biomedical field, spray drying is primarily used to produce dry powder aerosols and to tune active pharmaceutical compounds, making them useful and suitable for drug delivery (Daniel Santos, Ana Colette Maurício, Vitor Sencadas, José Domingos Santos, Maria H. Fernandes and Pedro S. Gome, 2017). In that sense, different strategies have been used to tailor the sprayed products according to the desired goals.

Regarding the spray-drying approach aiming drug encapsulation and delivery systems, it usually takes advantage of a complex initial system containing the active drug substance and an aqueous/organic phase (solution, emulsion or suspension) to produce either microspheres or microcapsules (Daniel Santos, Ana Colette Maurício, Vitor Sencadas, José Domingos Santos, Maria H. Fernandes and Pedro S. Gome, 2017).

The fabrication of biodegradable microspheres filled with an active drug is one of the most common strategies of spray drying. Polyesters gather important requirements, such as good biocompatibility, biodegradability and easiness to process, and thus they are usually chosen for the manufacturing of such products. As a result, it is possible to control the drug release over time as the polymer fraction is gradually degraded toward the physiological environment (Daniel Santos, Ana Colette Maurício, Vitor Sencadas, José Domingos Santos, Maria H. Fernandes and Pedro S. Gome, 2017). There are several studies which have reported the production of a wide variety of polymeric microspheres using spray drying.

The incorporation of hydrophilic domains in the spray-dried particles is an alternative way of controlling the drug release behavior. In fact, the hydrophilicity of the polymers used to encapsulate the drug has a direct impact on the behavior it, as a more hydrophilic polymer enables fast gelation, consequently slowing the drug release rate. On the other hand, a low concentration of hydrophilic polymeric coating can enhance the drug release rate of poorly water soluble drugs, since it will improve the wettability of the surrounding fluids. Derivative cellulose polymers such as sodium carboxymethyl cellulose, hydroxypropylmethyl cellulose and methylcellulose are just three examples of hydrophilic polymers which have been purposed for these endings (Daniel Santos, Ana Colette Maurício, Vitor Sencadas, José Domingos Santos, Maria H. Fernandes and Pedro S. Gome, 2017).

A singular method focused on the encapsulation of sensitive bioactive compounds (e.g. peptides, proteins) or other drugs can be also achieved through spray-drying technology associated to sol-gel polymerization process. In other words, sol-gel process is carried under soft conditions, and when combined with spray drying can deal with the production of microspheres with sensitive molecules entrapped on them (Daniel Santos, Ana Colette Maurício, Vitor Sencadas, José Domingos Santos, Maria H. Fernandes and Pedro S. Gome, 2017). Spray-dried silica gel microspheres are reported in the literature as a promising system to be administered in the form of a drug injectable (Daniel Santos, Ana Colette Maurício, Vitor Sencadas, José Domingos Santos, Maria H. Fernandes and Pedro S. Gome, 2017).

CHAPTER THREE

3. MATERIALS & RESEARCH METHODOLOGY

3.1. Sample collection

For this study tef was bought from the local market, which is used by most of people for day to day consumption of injera by the community. 5kg of white tef and 5kg of red tef were bought from the market for this study.

3.2. Dough preparation

The white and red tef dough were prepared at in the same way as done traditionally in every household. Tef flour was mixed with clean water in the ratio 1:2 (w/w) and 16 % of starter (ersho) by the weight of the flour and was kneaded by hand in a bowl in the traditional way. The resultant dough was allowed to ferment for one day (24h), two days (48h) and for 3 days (72h) at ambient temperature (Ashenafi, 2006).

Fig 3.1: Prepared dough

After this primary fermentation, the surface water formed on the top of the dough was discarded. For every 1kg of original flour, 200ml of the fermented mixture was mixed and with 400 ml of water and brought to boil (traditionally known as ‘absit’ making). It was cooled to about 450C before it was added into the main part of the dough.

The main dough was thinned by adding water equal to the original weight of the flour and stirred for 15minutes. The batter was left covered for 2 hours for secondary fermentation. After 2 hours, the absit was added to the thinned dough and mixed very well (known as batter making). The batter was left for about 30 min to rise (the second fermentation), before baking commenced. Some more water was added to thin down and form the right batter consistency. This methodology is taken from the previous research (Abate Z, & Ashagrie D, 2012).

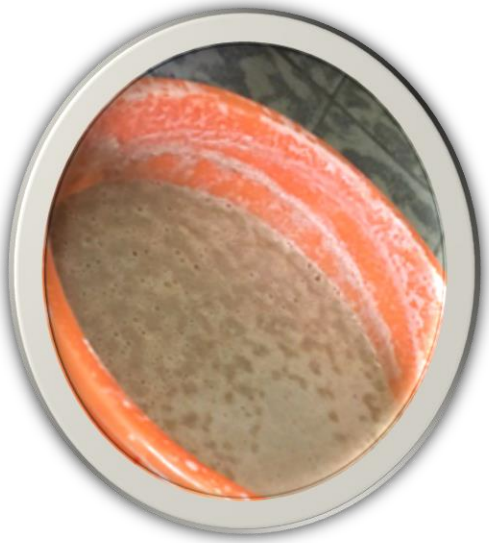

Fig 3.2: Red tef dough

Fig 3.3: White tef dough

3.3. Powder injera production

Tined dough & fermented dough mixed with absite was sieved with 250µm sieve. This will prevent blockage of nozzle tips with large dough particles. 1L of fermented dough mixed with absite was further tinned with 1L of water.

Fig 3.4: Secondary fermented dough and thinning water.

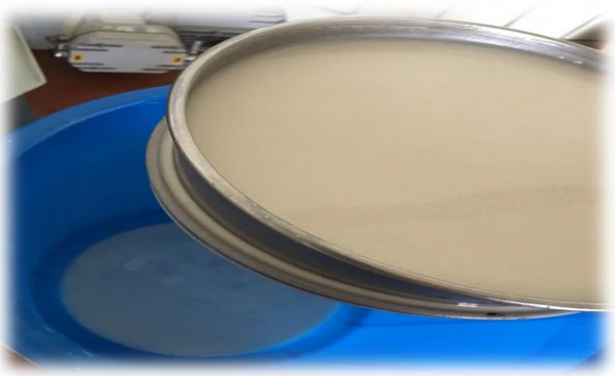

Fig 3.5: Sieving the dough with 250µm sieve.

The thinned dough feed to feed tank of the spray dryer with the operating parameters set at, inlet temperature of 210^0C, outlet temperature of 110^0c and initial relative humidity of 0.

Inlet air is blown into top of the drying chamber via an electric heater. An exhaust fan draws the air through an exhaust pipe towards the bottom of the chamber through the cyclone separator and expels it to the atmosphere. Both inlet and exhaust fans are fully controlled.

The progressing cavity feed pump is used to pump the thinned dough spray to be dried the spray nozzle at the top of the chamber.

Fig 3.6: The Feed tank

Compressed air is also taken to the nozzle via a regulatory valve. The compressed air atomizes the product externally to the nozzle using a 2 fluid nozzle principle and atomized tined dough mixed with additives spray is drawn through the chamber by the air flow. In the hot air flow the atomized tined dough was dried.

Fig 3.7: Lab scale spray dryer used for the research

Table 3.1: Operational parameters of spray dryer

Viscosity of feed	4.8	mPaS
Inlet temp	210	^{o}C
Feed pressure bar	8.10	
Initial relative humidity	<4	
Final relative humidity	19.0	
Outlet temp	100	^{o}C
Compressor pressure	0.05	MPa
Particle size	<250	micro meter
Feed rate	7.5	ml/s
Chamber pressure	8.9	Mbar
Initial mass	2	L
Product mass	56.02	G

The larger particle fall in to the chamber collection vessel but smaller will be carried out of the chamber the airflow. The cyclone separator extracts the powder injera food supplement from the air and deposit the powder injera food supplement in the cyclone collection vessel.

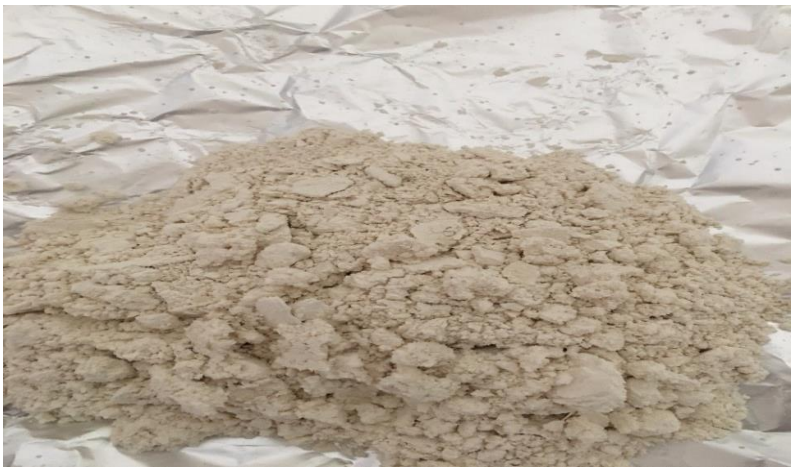

Fig 3.8: Powder injera

3.4. ANALYTICAL METHODS

3.4.1.DETERMINATION OF MOISTURE CONTENT

A clean box was dried on its inverted lid in the drying oven at 92^0C for 1hr. The box was covered with the lid and cooled in the desiccator for 30minutes and weighed. 2g test portion powder injera was transferred. In to each (W1) and shacked until content evenly distributed. The box with the sample was dried at 135^0C +/- 2 for two hours, if the sample is feed material, note; The inverted lid was put under the box before inserting it to the oven.The box took out with lid on and let it cooled in a desiccator for 30 minutes. The loss in weight was weighed and calculated on drying LOD) as estimate of water

3.4.2.DETERMINATION OF ASH

The requisite number of crucible was Place in an oven to drive off the moisture content. The crucible was cooled in desiccators up to reaching room temperature. The crucible was weighed soon after reaching room temperature (W1). Accurately weighed was 3.0g of powder injera sample (W3)The powder injera sample was charred in a muffle furnace at 55^0C for 2 hours until light gray ash result. The crucible was cooled in a desiccator up to reaching room temperature. The samples was transferred in to a muffle furnace at 550^0C for 3 hour. The crucible were took

out from the furnace, cooled in a desiccator up to reaching room temperature. Weigh the crucible soon after reaching room temperature (W2)

3.4.3. DETERMINATION OF FIBER

Fiber content was determined by following the method No. 32-10 as described in AACC, 2000. Sample of 2 g was taken and placed in 1000 ml beaker. 200 ml solution of 1.25 % H2SO4 was added in the beaker. The sample was then digested by boiling for 30 min. Then it was filtered by using suction apparatus. The residue was washed with hot water until it became acid free. The residue was again transferred to 1000 ml beaker and boiled with 200 ml solution of 1.25 % NaOH for 30 min. It was again filtered and the residue was transferred to pre-weighed crucible and dried in an oven with type PF 120 (200) at 100 ºC for 24 h till constant weight was obtained. Then the dried residue was charred on a burner and ignited into muffle furnace with type ELF 11/14. England at 550ºC for 6 hours, cooled in desiccators and weighed. The loss in weight during incineration represents the weight of crude fiber in sample.
The crude fiber % was calculated by using the following formula;

$$\text{Fiber \%\_} = \frac{\text{Weight of residue – Weight of ash)*}}{\text{Weight of sample}} \times 100$$

3.4.4.DETERMINATION OF MINERALS CONTENT

3.4.4.1. DETERMINATION OF IRON, ZINC, & CALCIUM CONTENT

Reagents preparation:

3N HCl: 25 ml of concentrated HCl (sp.gr. 1.14) was added in 100 ml deionized water (v/v). 6NHCl: 50 ml of concentrated HCl (sp.gr. 1.14) was added in 100 ml deionized water (v/v). 10%$LaCl_3$: 10 gm of $LaCl_3$ was dissolved in 100 ml deionized water(w/v). 0%HNO_3: 10 ml of HNO_3 was added in 100 ml ofdeionized water (v/v). 10 ppm Ca: 1 ml of Ca from 1000ppm was added in 100 ml of deionized water. For series standard of Ca preparation 0.5, 1.0, 1.5, 2.5 & 3 ppm take 0.5, 1.0,1.5,2.5 & 3 ml respectively was added in 10 ml volumetric flask containing 2.5 ml 3N HCl,0.5 ml 10%$LaCl_3$& make up with deionized water. 10 ppm Cu: 1 ml of Cu from

1000ppm added in 100 ml of deionized water. For series standard of Cu preparation 0.5, 1.0,1.5 ,2.5 & 3 ppm take 0.5, 1.0,1.5,2.5 & 3 ml respectively was added in 10 ml volumetric flask containing 2.5 ml 3N HCl, & mad up with deionized water. 10 ppm Zn: 1 ml of Zn from 1000ppm was added in 100 ml of deionized water. For series standard of Zn preparation 0.6, 1.0, 1.4 & 2.0 ppm 0.6, 1.0, 1.4 & 2.0 ml taken respectively then added in 10 ml volumetric flask containing 2.5 ml 3N HCl& mad up with deionized water. 20ppm of Fe: 2 ml of Fe from 1000ppm added in 100 ml of deionized water. For series standard of Ca preparation 2,6,10, & 12 ppm 1.0, 3.0, 5.0 & 7.0 ml taken respectively was added in 10 ml volumetric flask containing 2.5 ml 3N HCl, & make up with deionized water.

Procedure

All crucibles were Washed with 6NHCl and glass wares with 10%nitric acid, The requisite number of crucible were placed in an oven for 30 minutes at 100^{o}C. Then the crucibles were cooed in desiccators for 30 minutes or more and Accurately 2.5 g of sample was weighed. Then the sample was charred at hot plate starting from low temperature under a hood. The samples was transferred in to a muffle furnace at 550^{0}C for 1 hour. The crucible were took out from the furnace, cooled, and moisten with a few drops of deionized water. The water was evaporated on a hot plate. The sample were made ash once more for 30 min. at 550^{0}C & the crucibles were Cooled; some drops of deionized water and 5 drops of concentrated HNO_3 was added. Then evaporated on hot plate as above. Finally the sample was ash as above for 30 minutes at the same temperature as previously described. The crucible was cooled in a desiccators for 60 minutes. Then weighed

Action (dissolution) The ash was treated with 10 ml 6N HCl to wet it completely and carefully taken to dryness on a low temperature hot plate 15 ml of 3N HCl was added and the crucible heated on the hot plate until the solution just boils Then the sample solution was Cooled and filtered through a filter paper into a graduated flask. 10 ml of 3N HCl was added to the crucible and heated until the solution just boils. Then Cooled and filtered into the graduated flask. The crucible were washed three times with deionized water: The washings was filtered into the flask The filter paper was washed thoroughly and washing was collected in the flask. 5 ml of lanthanum chloride solution added per 100 ml of solution. Cool and dilute the contents of the flask to the mark with deionized water. A blank was prepared by taking the same amount of

reagents through instruction 1-9. The sample solutions was transferred to polyethylene bottle. AAS was turned on & optimized according to the operation manual. The AAS was calibrated with standards. If the curve is fit, The blank , control and Samples was run respectively. After removal of organic material by dry ashing the residue is dissolved in dilute acid. The solution is sprayed into the FAAS and the absorption of the metal to be analyzed is measured at a specific wavelength.

Determination of sodium & potassium Content

ACTION (DILUTION OF THE SOLUTION FOR SODIUM)

The nearest mg, 2 g of the powder injera sample was weighed on a filter paper (diameter 9 cm) The filter paper was folded up and transferred into a 250 ml conical flask. 20 ml of 1:1 dilute nitric acid was added. Boiled gently for about 10 minutes and cooled to room temperature. The digested solution was filtered through a filter paper (diameter 12.5 cm) into a 100 ml volumetric flask. The conical flask and the filter paper was filtered three times each with 10 ml deionized water. Made up to 100 ml and mixed (solution A). A blank was prepared starting from procedure a. ii (solution B) 50 ml of solution A & B and 5 ml potassium dilution solution pipetted into 100 ml graduated flasks, made up to the mark and mix (solution C & D respectively)

ACTION (DILUTION OF THE SOLUTION FOR POTASIUM DETERMINATION)

5 ml of solution A & B Pipetted into a 100 ml graduated flask, make up to the mark, and mix (solution E and F)

Action *(Measurement)*

The flame photometer calibrate with 3.05 ppm of sodium solution which gives 90 absorbance using sodium filter and with 3.15 ppm of potassium solution which gives 100 absorbance using potassium filter.

The obtained values were corrected for the zero concentration standard

The solutions C & D were measure for sodium and solution E & F were measure for Potassium.

After wet digestion the sodium or potassium content is measured flame photometrically.

Calculation	Sodium content (mg/100 g) = (C-D)*200/W
	Potassium content (mg/100 g) = (E-F)*2000/W
	Where:
	W weight (g) of sample
	C & D = [0.034*A]/10
	E & F = [0.033*A]/10
	A = absorbance
Procedural	preparation of samples was carried out under hood.

3.5. Statistical Data Analysis

In this study, a multivariate statistical technique called RSM of the Design-Expert version 11 software was used. Generation of the model equation, main and interaction effects of the independent variables, and surface plots from the effects of the factors was evaluated. Based on their effect on the composition of the product powder injera, the factors were evaluated for their level of significance.

Two-factor and three level BBD design consisting of 18 runs for the content of powder injera were employed. The experimental design was analyzed and done by Design-Expert version 11 software and Microsoft excel. The quality of fit of the regression model expressed as the coefficients of determination (R^2), the statistical significance determined by ANOVA and interaction graphs was plotted. For each factor, an experimental and analysis result were compared based on the results of literature data.

CHAPTER FOUR

4. RESULTS AND DISCUSSION

4.1. STATISTICAL ANALYSIS

In general the experimental and analysis result found that the average mean values of nutritional composition of red tef powder injera is 6.535535% w/w of moisture content, 2.945067% ash content, 0.678825g/100g of fiber content, 13.9888g/100g of Calcium, 0.496454g/100g of zinc, 4.236726g/100g of iron, 259.4043mg/100g of potassium and 15.29903mg/100g of sodium is observed. And the average mean values of nutritional composition of white tef powder injera is 7.14422% w/w of moisture content, 2.929733% ash content, 0.543356g/100g of fiber content, 13.29113g/100g of Calcium, 0.470325g/100g of zinc, 4.126106g/100g of iron, 358.821mg/100g of potassium and 16.6054mg/100g of sodium is observed.

The researcher observe that red tef has higher calcium, zinc, and iron value than the white tef but the white tef powder injera has sodium and potassium content than the red tef powder injera. And it is observed that at fermentation time of 48h the powder injera has higher mineral content than at fermentation time of 24h and 72h.

4.1.1. Statistical analysis of moisture content of powder injera

Table: 4.1.1: Mean value of moisture content of white and red tef powder injera

Type of Tef	Fermentation time	Moisture content	Mean Average
White	24	7.264585	7.14422
	48	6.401746	
	72	7.766329	
Red	24	5.708659	6.535535
	48	6.913807	
	72	6.984139	

From the table we can see that there is slight difference in moisture content of powder at different fermentation time. And the mean value of moisture content white and red tef powder injera is 7.14422% w/w and 6.535535 %w/w respectively. The moisture content of white tef powder injera has somehow higher value than the red tef powder injera. This show that white tef has higher wettability than the red tef powder injera. But the difference in fermentation time doesn't create clear difference in moisture content.

Thus the amount of moisture content rather depend on the operational parameters of spray dryer.

4.1.2. Statistical analysis of ash content of powder injera

Organic matter is burned off at as low as a low temperature as possible and the in organic materials remaining is cooled and weighed. Heating is carried out in stages, first drive of the water, then to char the product thoroughly and finally to ash at 550^0C in muffle furnace.

Ash is used to quantify the trace materials, as one factor for the calorie and carbohydrate values in food products.

Table: 4.1.2. Mean value of ash content of white and red tef powder injera

Type of tef	Fermentation time	Ash content	Average mean
White	24	2.9464	
	48	2.9296	
	72	2.9592	2.945067
Red	24	2.94365	
	48	2.9228	2.929733
	72	2.92275	

The table show that the mean value of ash content of powder injera of white tef, at fermentation time of 24, 36, & 72 is 2.9464, 2.9296, and 2.9592 respectively. And the mean value of value of ash content of powder injera of red tef, at fermentation time of 2.94365, 2.9228, and 2.92275 Increased fermentation time from 24 hour to 48 hour somewhat showed decreased in the ash content of the powder Injera. But in both type of tef at fermentation time of 72 hour it showed

increase in ash content of powder injera. This finding was partially similar with they reported that there is gradual decrease in the ash contents with the fermentation days (Bultosa, 2017).

4.1.3. Statistical analysis of fiber content of powder injera

Table: 4.1.3. Mean value of fiber content of white and red tef powder injera

Type of Tef	Fermentation time [h]	Fiber content	Mean Average
White	24	0.576705	0.543356
	48	0.644839	
	72	0.408526	
Red	24	0.797163	0.678825
	48	0.828321	
	72	0.686476	

The table show that the mean value of fiber content of powder injera of white tef, at fermentation time of 24h, 36h, & 72h is 0.576705%, 0.644839%, and 0.408526% respectively. And the mean value of fiber content of powder injera of red tef, at fermentation time of 24h, 36h, & 72h is 0.797163 0.828321, and 0.686476.

The mean values of fiber content of powder injera shows that there is increase in fiber content at fermentation time of 48 h when it compared to fermentation time of 24 and 72.

This result show similar result with pervious study (Bultosa, 2017) The result also revealed that long fermentation time reduced the fiber content of the Injera. The expected decrease in fiber content during fermentation could be attributed to the partial solubilization of cellulose and hemi cellulosic type of material by microbial enzymes (Afify MR, 2011).

And also the average mean value White tef powder injera and red tef powder injera is 0.543356% and 0.678825% respectively. This indicate the red tef powder injera has higher fiber content than the white tef powder injera.

4.1.4. Statistical analysis of calcium content of powder injera

Table: 4.1.4. Mean value of calcium content of white and red tef powder injera

Type of Tef	Fermentation time	Calcium content	Mean Average
White	24	14.17829	13.29113
	48	13.37726	
	72	12.31783	
Red	24	14.69509	13.9888
	48	14.0491	
	72	13.22222	

Above result show that the mean value of calicium content of powder injera show decrease in calcium content while increasing the fermentation time. Mean average value show that red tef has higher calcium content than white tef. This result show similar result with pervious study (Arslan, 2018).

But the total amount of composition of normal injera and powdered injera shows a difference. The calcium contents of all composite injera varied from 25.99 to 51.11 mg/100 g. The highest value (51.11 mg/100 g) was obtained when the sample is processed from pure tef and the lowest value (25.99 mg/100 g) was obtained when 80% tef, 10% maize and 10% rice were blended. Calcium content of injera increased when the proportion of tef in the blend increased. The observed high calcium content may be contributed by high calcium content of tef (165.2 mg/100 g) (Bultosa et al., 2002) than maize (7.0 mg/100 g) and rice (9 mg/100 g) (Nuss & Tanumihardjo, 2010).

4.1.5. Statistical analysis of iron and zinc content of powder injera

Table: 4.1.5: Mean value of iron and zinc content of white and red tef powder injera

Type of Tef	Fermentation time	Zinc g/100g	Mean Average	Iron g/100g	Mean Average
White	24	0.391937	0.470325	4.126106	
	48	0.515118		4.435841	4.126106
	72	0.503919		3.816372	
Red	24	0.481523	0.496454	4.65708	
	48	0.604703		4.115044	4.236726
	72	0.403135		3.938053	

The experiment result of the content of powder injera show that there is increase in composition of iron and zinc value as increase in fermentation time from 24 h to 48h, then decrease as fermentation time increase from 48h to 72 h. but the previous studies show that there is decrease in mineral content of injera as increase fermentation time.

From this result we can see that the optimum fermentation time with higher mineral content value of injera powder is 24h.

The average mean value of white tef red powder injera and tef powder injera is 0.470325 and 0.496454 g/100g of powder injera respectively. This indicate that the red injera has higher zinc content than white tef powder injera.

The total iron contents of all composite injera vary from 17.73 to 25.13 mg/100 g. The iron content of injera processed from pure tef is significantly ($p < 0.05$) higher (25.13 mg/100 g) than the injera processed from other composite injera because grain tef has high iron contents (25.53 mg/100 g) (Baye 2014) than maize (2.71 mg/100 g) and rice (0.8 mg/100 g). A minimum value (17.73 mg/100 g) was obtained at 70% tef, 15% maize & 15% rice.

The total zinc content ranged from 1.62 to 2.10 mg/100 g). Injera prepared from pure tef had significantly ($p < 0.05$) higher zinc content (2.10 mg/100 g) than other formulations. This is

explained by the higher content of zinc (2.4–6.8 mg/100 g) in tef grains than maize (2.6–4.6 mg/100 g) and rice (2.2 mg/100 g) (Baye, 2014).

4.1.6. STATISTICAL ANALYSIS OF SODIUM AND POTASSIUM CONTENT OF POWDER INJERA

4.1.6: Mean value of sodium and potassium content of white and red tef powder injera

Type of Tef	Fermentation time	Potasium mg/100g	Mean Average	Sodium mg/100g	Mean Average
White	24	378.6688	358.821	15.95429	16.6054
	48	478.9163		15.9125	
	72	218.8779		17.94941	
Red	24	219.374	259.4043	19.88666	15.29903
	48	339.3134		23.92943	
	72	219.5255		7.98483	

The mean value of white tef powder injera potassium content is 378.6688, 478.9163, and 218.8779mg/g respectively. And the mean value of red tef powder injera potassium content is 219.374, 339.3134, and 219.5255mg/100g respectively. The mean values show that there is increase in potassium content for both red tef powder injera and white tef powder injera at fermentation time of 48h when compared with the potassium content of powder injera at fermentation time 24h and 72h.

The average mean value of white tef powder injera and red tef powder injera is 358.821 and 259.4043mg/100g. This greater mean value of white tef powder injera shows that the white tef powder injera has higher sodium value than red tef powder injera.

The mean value of white tef powder injera sodium content is 15.95429, 17.94941, and 15.9125mg/g respectively. And the mean value of red tef powder injera sodium content is 19.88666, 23.92943, and 7.98483mg/100g respectively. The mean values show that there is increase in sodium content for both red tef powder injera and white tef powder injera at fermentation time of 48h when compared with the sodium content of powder injera at fermentation time 24h and 72h.

The average mean value of sodium content of white tef powder injera and red tef powder injera is 16.6054 and 15.29903mg/100g respectively. This indicate that white tef has slightly higher sodium content than red tef powder injera.

4.2. REGRESSION ANALYSIS POWDER INJERA

4.2.1. Regression analysis of moisture content

Table: 4.2.1. Regression analysis of moisture content of white and red tef powder injera

Source	Sum of Squares	Mean Square	F-value	p-value	
Model	54.36	4.18	0.9123	0.6009	not significant
A-Replicate	12.01	6.00	1.31	0.3652	
B-Type of Tef	9.09	9.09	1.98	0.2318	
C-Fermentation Time	12.28	6.14	1.34	0.3587	
AB	3.61	1.80	0.3936	0.6982	
AC	13.01	3.25	0.7095	0.6262	
BC	4.37	2.19	0.4767	0.6521	

P-values less than 0.0500 indicate model terms are significant. In this case there are no significant model terms. Values greater than 0.1000 indicate the model terms are not significant.

The above table show that fermentation time and type of tef has no significant impact on moisture content of the powder injera. But we can see the mean value the of the moisture content of the produced powder injera is 7.283715% by weight.

4.2.2. Regression analysis of ash content powder Injera

4.2.2.1. Normal Probability plot

The normal probability plot indicates whether the residuals follow a normal distribution, thus follow the straight line. Expect some scatter even with normal data. Thus the figure shows that the date is normally distributed.

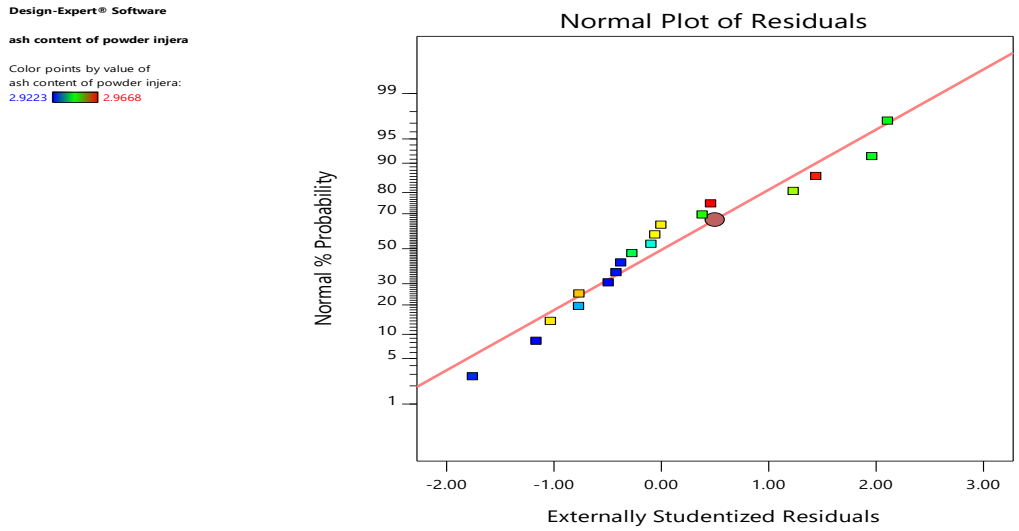

Fig: 4.2.2. Normality plot of ash content

Table: 4.2.2: regression analysis of ash content of white and red tef powder injera

Source	Sum of Squares	Mean Square	F-value	p-value	
Model	0.0033	0.0008	12.43	0.0002	significant
A-A	0.0001	0.0001	1.74	0.2103	
B-Fermentation time	0.0005	0.0005	6.98	0.0203	
AB	0.0019	0.0019	28.80	0.0001	
B^2	0.0008	0.0008	12.19	0.0040	
Residual	0.0009	0.0001			

Factor coding is coded. Sum of squares is Type III – Partial the Model F-value of 12.43 implies the model is significant. There is only a 0.02% chance that an F-value this large could occur due to noise. P-values less than 0.0500 indicate model terms are significant. In this case B, AB, B^2 are significant model terms. Values greater than 0.1000 indicate the model terms are not significant.

Thus the fermentation time has significant effect on ash content of powder injera. The design expert result show that the type of tef has no significant impact on ash content of powder injera. But the type of tef and fermentation time has cumulative effect on ash content of powder injera.

As it is shown in statistics analysis the following 3D plot also show that there is gradual decrease in ash contentment of powder injera as fermentation time increase.

Table 4.2.3: Fit Statistics of ash content

Std. Dev.	0.0081		R^2	0.7927
Mean	2.94		Adjusted R^2	0.7289
C.V. %	0.2766		Predicted R^2	0.6289
			Adeq Precision	8.7740

The Predicted R^2 of 0.6289 is in reasonable agreement with the Adjusted R^2 of 0.7289; i.e. the difference is less than 0.2.

Adeq Precision measures the signal to noise ratio. A ratio greater than 4 is desirable. The resulted ratio of 8.774 indicates an adequate signal. This model can be used to navigate the design space.

Final Equation in Terms of Actual Factors

Ash content of powder injera,

White tef = $+2.97963 - 0.002101\ T + 0.000025\ T^2$

Red tef +3.02501-0.003152 Fermentation time + 0.000025 Fermentation time²

The equation in terms of actual factors can be used to make predictions about the response for given levels of each factor.

4.2.3. Regression analysis of fiber content of powder injera

Table 4.2.4: regression analysis of fiber content

ANOVA for Selected Factorial Model						
Analysis of variance table [Partial sum of squares]						
	Sum of		Mean	F		
Source	Squares	DF	Square	Value	Prob > F	
Model	0.16	5	0.031	1.07	0.4590	not significant
A	*0.099*	*1*	*0.099*	*3.37*	*0.1158*	
B	*0.049*	*2*	*0.024*	*0.83*	*0.4812*	
AB	*9.465E-003*	*2*	*4.733E-003*	*0.16*	*0.8547*	

P-values less than 0.0500 indicate model terms are significant. In this case there are no significant model terms. Values greater than 0.1000 indicate the model terms are not significant.

Table 4.2.5: Fit Statistics fiber content

Std. Dev.	0.1741		R^2	0.0000
Mean	0.5723		Adjusted R^2	0.0000
C.V. %	30.42		Predicted R^2	-0.1901
			Adeq Precision	NA[(1)]

[(1)] Case(s) with leverage of 1.0000: Pred R^2 and PRESS statistic not defined.

A negative Predicted R^2 implies that the overall mean may be a better predictor of your response than the current model. In some cases, a higher order model may also predict better.

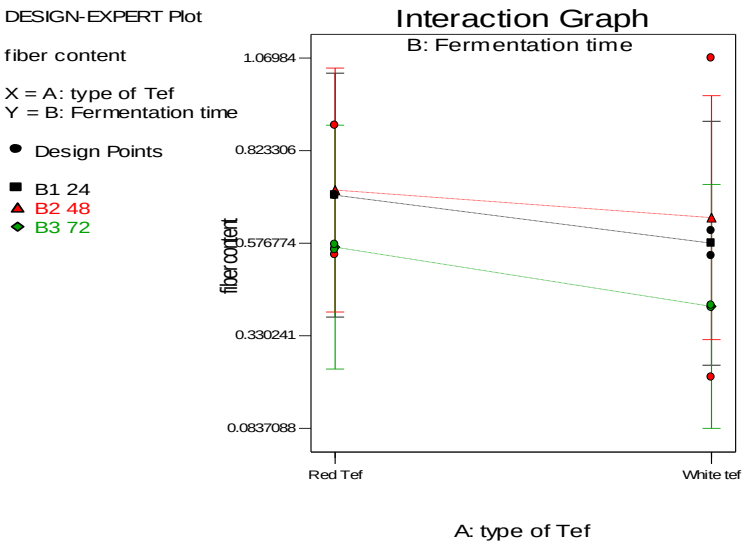

Fig4.2.1: Interaction graph fiber content

The interaction graph show that there is high amount of fiber content is found in fermentation time of 48h when compared to 24h and 72h of fermentation time.

4.2.4. Regression analysis of iron, zinc, and calcium content powder Injera

Table 4.2.4.1: ANOVA for selected factorial model **Response 3: calcium **

Source	Sum of Squares	Mean Square	F-value	p-value	
Model	7.12	1.42	2.850E+06	< 0.0001	significant
A-Type of Tef	1.46	1.46	2.920E+06	< 0.0001	
B-Fermentation Time	0.1073	0.0536	1.073E+05	< 0.0001	
AB	5.56	2.78	5.557E+06	< 0.0001	

The Model F-value of 2849632.88 implies the model is significant. There is only a 0.01% chance that an F-value this large could occur due to noise.

P-values less than 0.0500 indicate model terms are significant. In this case A, B, AB are significant model terms. Values greater than 0.1000 indicate the model terms are not significant.

This indicate the calcium content of powder injera is affected by fermentation time and type of tef.

Table 4.2.4.2: Fit Statistics

Std. Dev.	0.0007		R^2	1.0000
Mean	13.64		Adjusted R^2	1.0000
C.V. %	0.0052		Predicted R^2	1.0000
			Adeq Precision	4754.5220

The Predicted R^2 of 1.0000 is in reasonable agreement with the Adjusted R^2 of 1.0000; i.e. the difference is less than 0.2.

Adeq Precision measures the signal to noise ratio. A ratio greater than 4 is desirable. Your ratio of 4754.522 indicates an adequate signal. This model can be used to navigate the design space.

Table 4.2.4.3: ANOVA for selected factorial model **Response 1: Iron **

Source	Sum of Squares	Mean Square	F-value	p-value	
Model	0.5943	0.2971	2.12	0.1764	not significant
AB	0.5943	0.2971	2.12	0.1764	

The Model F-value of 2.12 implies the model is not significant relative to the noise. There is a 17.64% chance that an F-value this large could occur due to noise.

P-values less than 0.0500 indicate model terms are significant. In this case there are no significant model terms. Values greater than 0.1000 indicate the model terms are not significant.

The p-value shows that fermentation time and type of injera has no significant effect on iron content of powder injera.

Table 4.2.4.4: Fit Statistics

Std. Dev.	0.3747		R^2	0.3199
Mean	3.93		Adjusted R^2	0.1688
C.V. %	9.53		Predicted R^2	-0.2090
			Adeq Precision	3.0708

A negative Predicted R^2 implies that the overall mean may be a better predictor of your response than the current model. In some cases, a higher order model may also predict better.

Adeq Precision measures the signal to noise ratio. A ratio of 3.07 indicates an inadequate signal and you should not use this model to navigate the design space.

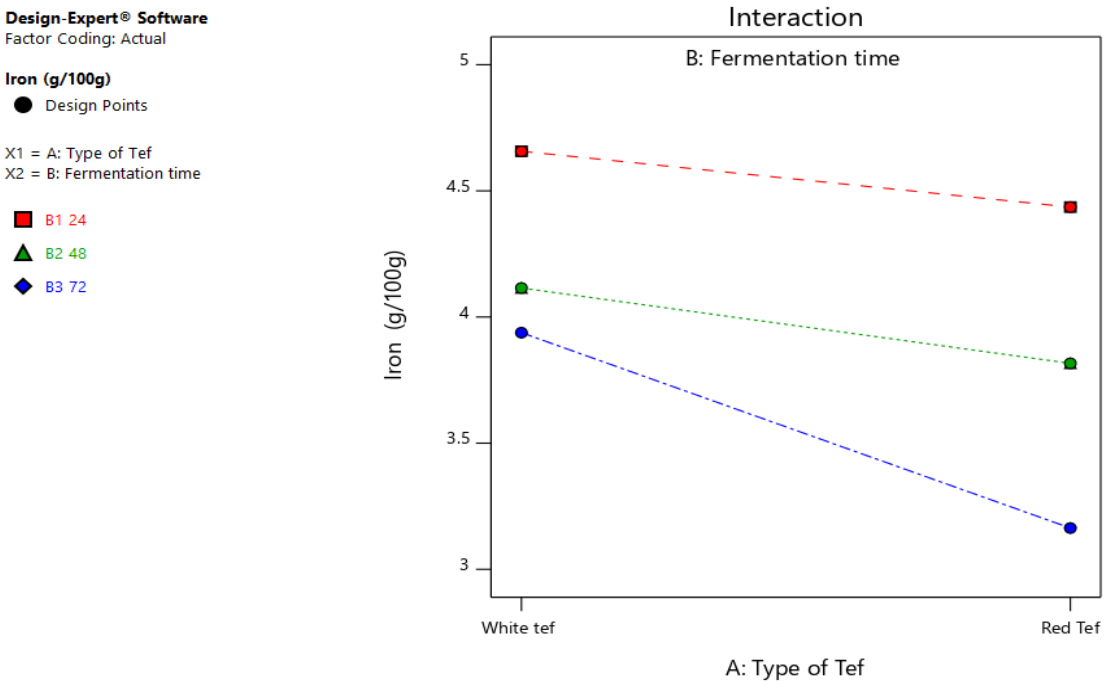

Fig 4.2.2: Interaction graph of iron

From the graph we can see that as the fermentation time increase the amount of iron content is decreasing. And it show higher fall of iron content of red injera than the white tef powder injera. Thus the shorter fermentation time is better to get higher amount of iron content.

4.2.5. Regression analysis of sodium and potassium content powder Injera

Table 4.2.5.1: regression analysis of sodium

Source	Sum of Squares	df	Mean Square	F-value	p-value	
Model	1.176E+05	5	23521.85	1.41	0.3394	not significant
A-Type of Tef	29651.04	1	29651.04	1.78	0.2303	
B-Fermentation time	72745.03	2	36372.52	2.19	0.1936	
AB	15213.20	2	7606.60	0.4572	0.6534	

P-values less than 0.0500 indicate model terms are significant. In this case there are no significant model terms. Values greater than 0.1000 indicate the model terms are not significant. If there are many insignificant model terms (not counting those required to support hierarchy).

This indicate the fermentation time and type of injera has no significant effect on sodium content of powder injera.

Table 4.2.5.1: Fit Statistics

Std. Dev.	128.99		R^2	0.5409
Mean	309.11		Adjusted R^2	0.1583
C.V. %	41.73		Predicted R^2	-0.8364
			Adeq Precision	2.8511

A negative Predicted R^2 implies that the overall mean may be a better predictor of your response than the current model. In some cases, a higher order model may also predict better.

4.2.5.1. Normal Probability plot

The normal probability plot indicates whether the residuals follow a normal distribution, thus follow the straight line. Expect some scatter even with normal data. Thus the figure shows that the date is normally distributed.

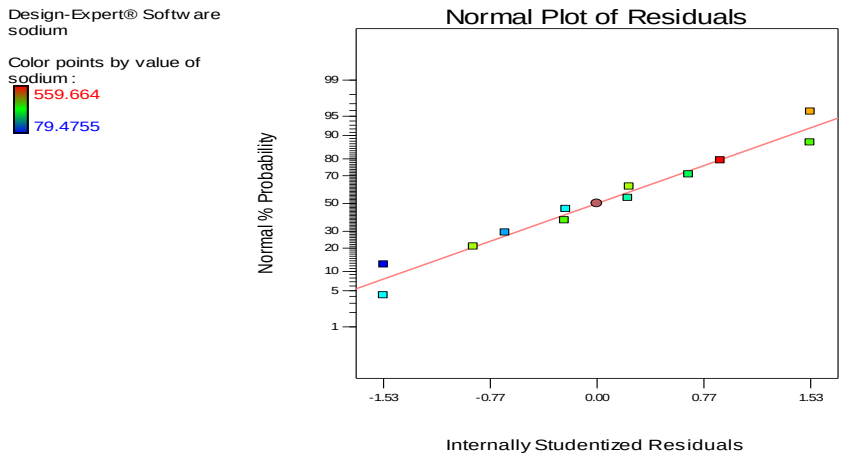

Fig 4.2.5.1: normality plot of sodium

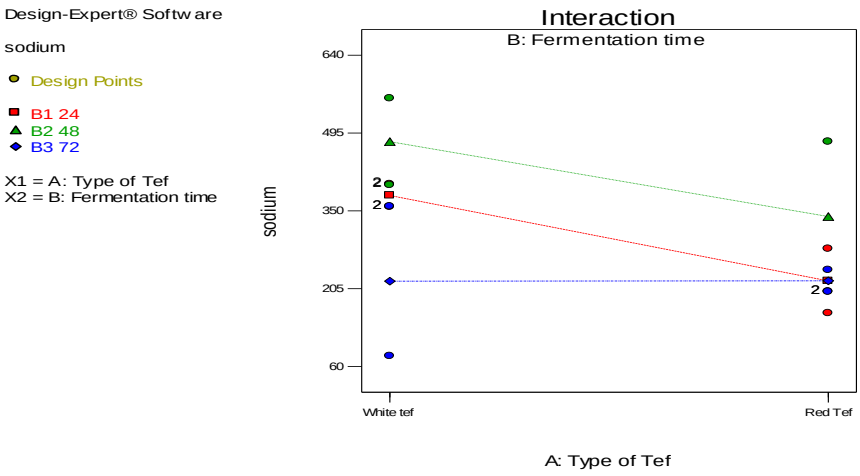

Fig 4.2.5.2: Interaction Graph of sodium

The interaction graph show that there is increase in sodium content for both red tef powder injera and white tef powder injera at fermentation time of 48h when compared with the sodium content of powder injera at fermentation time 24h and 72h.

4.2.6. Regression analysis of potassium content powder Injera

4.2.6.1. ANOVA for selected factorial model**Response 1: potassium **

Source	Sum of Squares	df	Mean Square	F-value	p-value	
Model	148.60	5	29.72	0.6361	0.6819	not significant
A-Type of Tef	1.20	1	1.20	0.0257	0.8779	
B-Fermentation time	74.09	2	37.05	0.7929	0.4948	
AB	73.31	2	36.66	0.7845	0.4981	

P-values less than 0.0500 indicate model terms are significant. In this case there are no significant model terms. Values greater than 0.1000 indicate the model terms are not significant. This show that potassium powder injera content not affected by type of tef and fermentation time.

4.2.6.2. Normal Probability plot

The normal probability plot indicates whether the residuals follow a normal distribution, thus follow the straight line. Expect some scatter even with normal data. Thus the figure shows that the date is normally distributed.

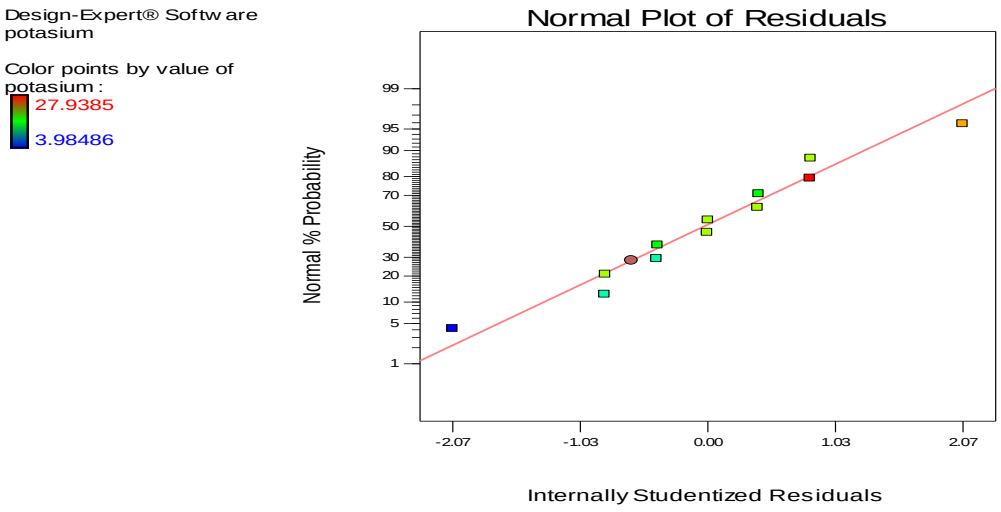

Fig 4.2.5.3: normality plot of potassium

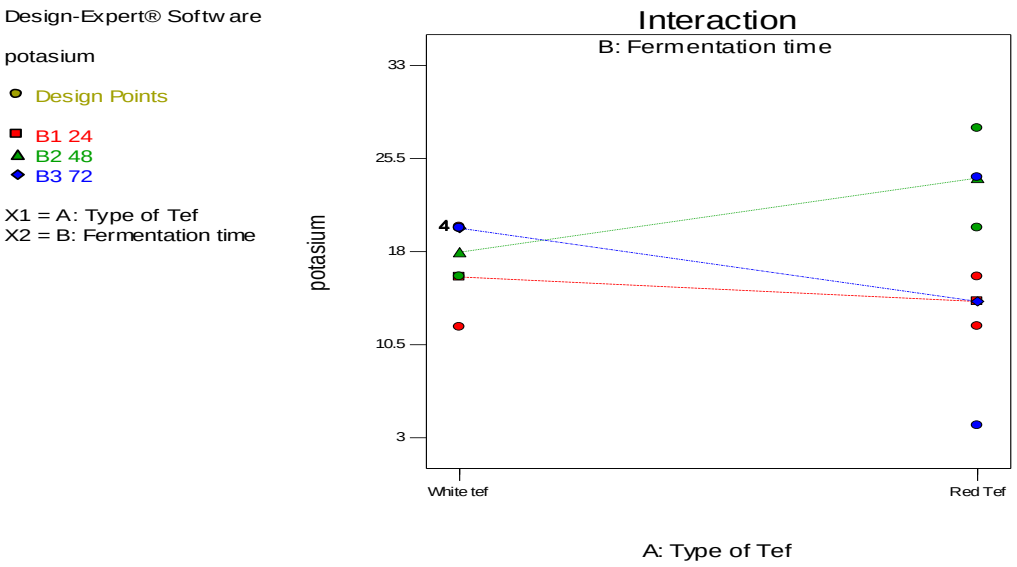

Fig 4.2.5.4: interaction graph of potassium

The above potassium content of powder injera show, for red tef it indicates that there is higher amount of sodium content at fermentation time 48h but for white tef powder injera there is gradual decrease while increasing fermentation time. This finding was somehow similar with they reported that there is gradual decrease in the mineral contents of normal injera with the fermentation days (Bultosa, 2017).

CHAPTER SIX

5. CONCLUSION AND RECOMMENDATION

5.1. CONCLUSION

The researcher found the following conclusion from this research laboratory work, laboratory result analysis, from general observation, and discussion with scholars.

- The laboratory result show that the powder injera can be good source of mineral content such as iron, calcium, zinc, potassium, and sodium. And also good source of fiber content.
- The result of moisture content of powder injera show that there is no clear difference of the moisture content of powder injera with varying the fermentation time. This may be because of the parameters of spray dryer. Moisture content of powder injera mostly depend on operational parameters of spry dryer. But there is slight difference on type of injera thus the moisture content of red tef powder injera has higher than that of white tef powder injera.

  This show that there is wettability difference between red and white tef powder injera produced at the same operational parameters of spray dryer.
- The ash content result show that increased fermentation time from 24 hour to 48 hour somewhat showed decreased in the ash content of the powder Injera. But in both type of tef at fermentation time of 72 hour it showed increase in ash content of powder injera.
- The mean values of fiber content of powder injera shows that there is increase in fiber content at fermentation time of 48 h when it compared to fermentation time of 24 and 72.
- Experimental and analysis result show that the content of calicium content of powder injera show decrease in calcium content while increasing the fermentation time. Mean average value show that red tef has higher calcium content than white tef.
- The experiment result of the content of powder injera show that there is increase in composition of iron and zinc value as increase in fermentation time from 24 h to 48h, then decrease as fermentation time increase from 48h to 72 h. but the previous studies

show that there is gradual decrease in mineral content of injera as increase fermentation time.

- It is observed that there is increase in potassium content for both red tef powder injera and white tef powder injera at fermentation time of 48h when compared with the potassium content of powder injera at fermentation time 24h and 72h.
- There is increase in sodium content for both red tef powder injera and white tef powder injera at fermentation time of 48h when compared with the sodium content of powder injera at fermentation time 24h and 72h. But it is observed that white tef has slightly higher sodium content than red tef powder injera.
- Now the researcher can boldly speak that the best operation time to obtain optimum amount of fiber content, iron and calcium content is fermentation time of 48h when compared to the 24h and 72h. Because the experimental result show that there is increase in most of mineral content at fermentation time of 48h.
- But the inverse is observed in case of sodium content of white tef powder injera and red tef powder injera. Thus red tef powder injera has higher iron calcium and potassium value than the white tef powder injera. But white tef has higher amount of potassium and sodium than red tef powder injera.
- So one can use more of red tef powder injera in desire of iron, calcium, zinc and fiber while other use white tef powder injera in desire of sodium and potassium.

5.2. RECOMMENDATION

- As a citizen and scholar of the country great emphasis has to be given to do a lot of research on tef products and product diversification of injera.
- Further research has to be done process optimization of spry dryer technology and injera fermentation process.
- Deeper study has to be done fermentation process metabolic activity and factors affecting the product quality of powder injera. And On formulation and evaluation of powder injera as a food supplement.
- Since tef provides over two-thirds of the human nutrition in Ethiopia, further researches and research projects has to be done on tef and injera derivatives.
- As scholars process modification and product diversification has to be given great attention to solve problem of the society, and to fight poverty.
- Further and deeper study has to be done using powder injera as a food supplement to protect the society from disease and malnutrition.

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APPENDICES

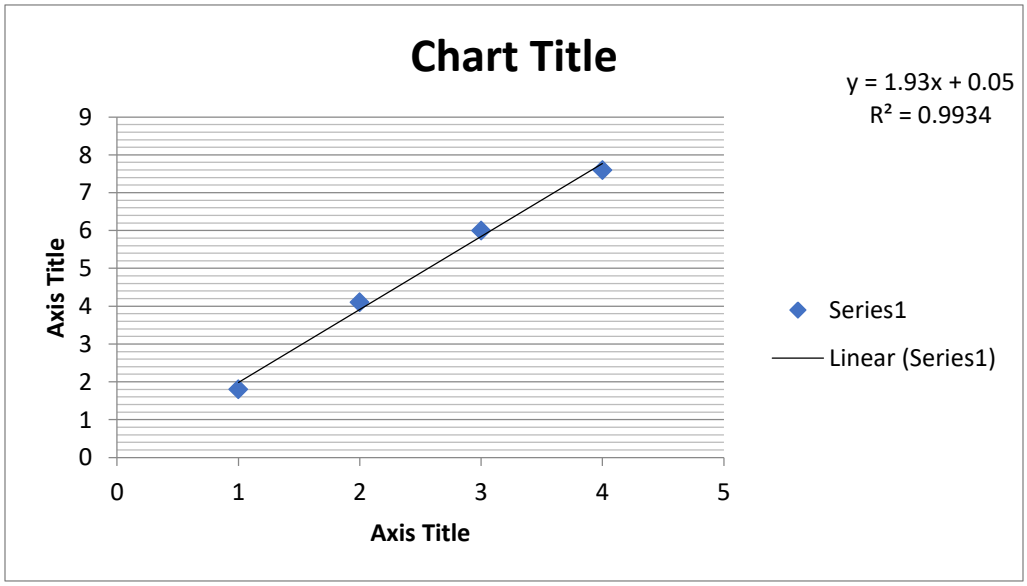


Fig equipment calibration curve for potassium measurement

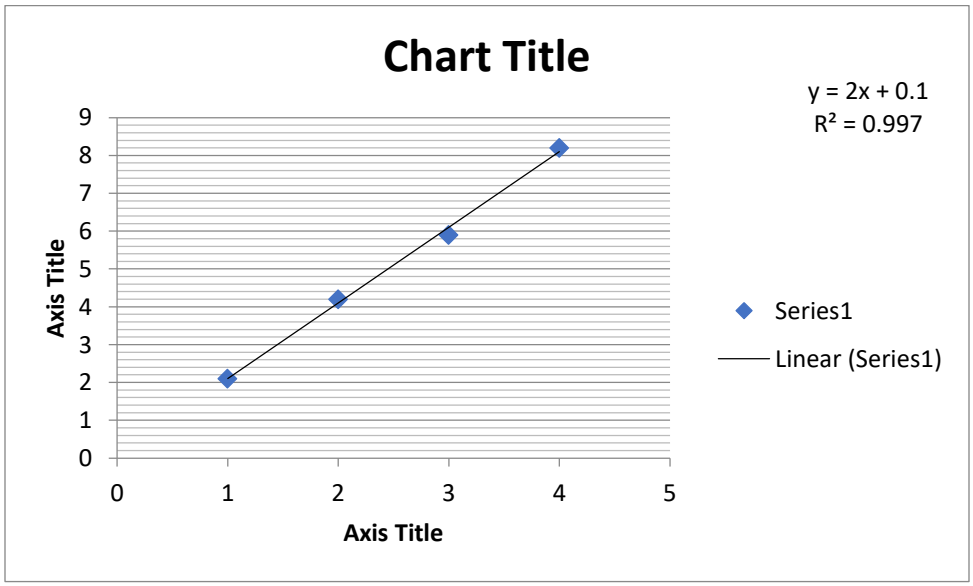


Fig equipment calibration curve for sodium measurement

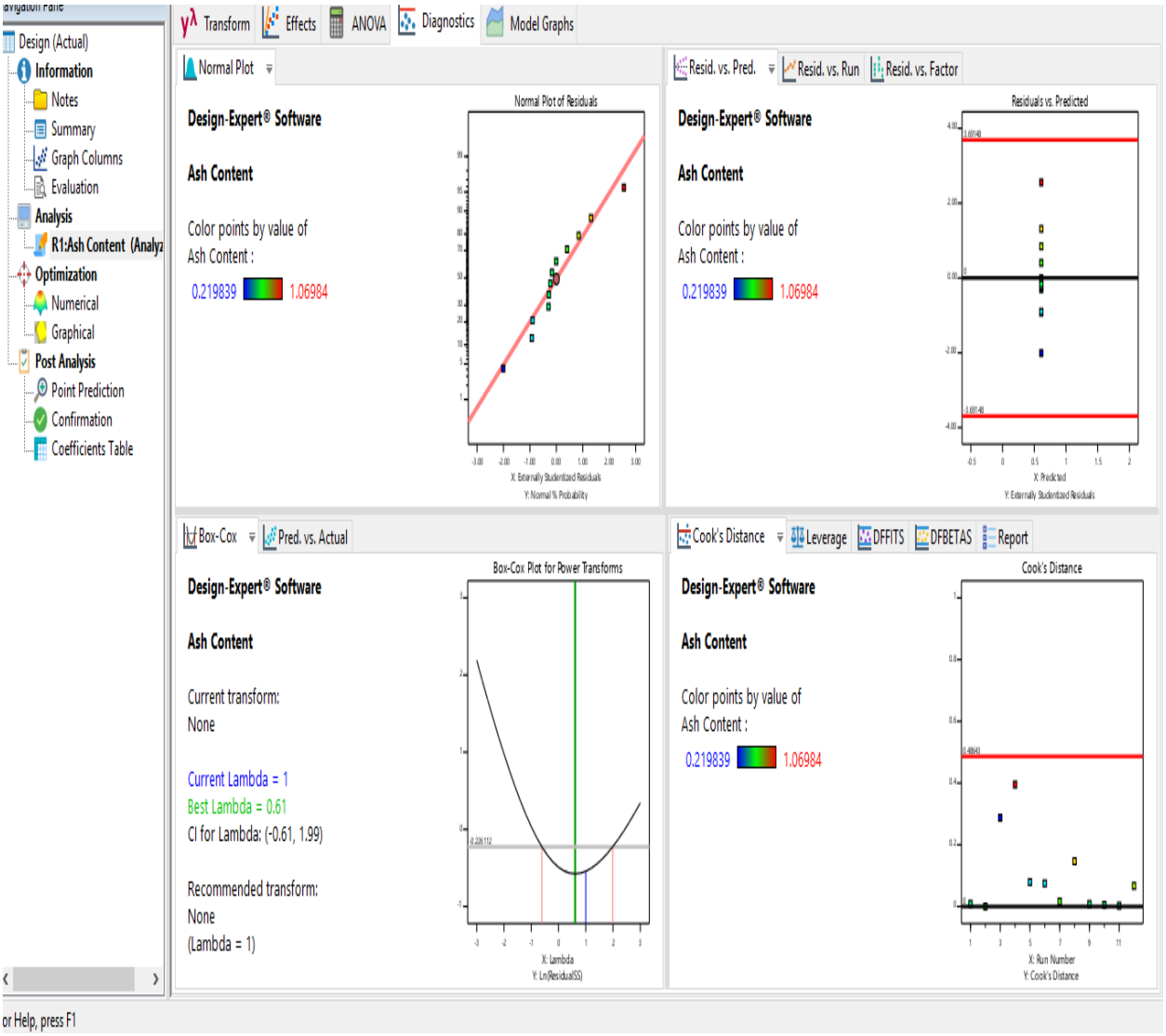
Table: sample raw data 1

moisture Teste data record										
Teste method, oven dry method										
lab No.	box No.	Wt. of box (g)	wt. of fresh sample (W1) (g)	wt.of fresh sample and box (W2) (g)	wt. of dry sample & box(W3)(g)	wt. of moisture (w4) (g)	moisture (%)	Mean	std	rsd
1	72/91	32.2297	2.0295	34.2592	34.1084	0.074304016	7.430402	7.264585	0.261141	3.635601
2	240/77	34.0232	2.0018	36.025	35.8768	0.07403337	7.403337			
3	77/91	32.2836	2.0158	34.2994	34.1591	0.069600159	6.960016			
4	71/71	33.7928	2.0903	35.8831	35.8057	0.037028178	3.702818	6.401746	2.341235	36.57182
5	273/238	33.8114	2.0035	35.8149	35.6569	0.078861992	7.886199			
6	124/274	31.2759	2.022	33.2979	33.1439	0.076162216	7.616222			
7	231/80	33.3073	2.0658	35.3731	35.2111	0.078419983	7.841998	7.766329	0.065612	0.844828
8	76/260	44.9802	2.009	46.9892	46.834	0.077252364	7.725236			
9	128/236	32.8358	2.0086	34.8444	34.6891	0.077317535	7.731753			
10	256/15	45.1072	2.055	47.1622	47.1595	0.0013138	0.13	5.7	5.1	91.

	5					69	1387	08659	97793	05104
11	1/154	46.1072	2.0815	48.1887	48.0518	0.065769877	6.576988			
12	80/76	34.3266	2.0043	36.3309	36.1221	0.104176022	10.4176			
13	279/273	33.2857	2.0022	35.2879	35.1506	0.068574568	6.857457	6.913807	0.133808	1.935368
14	239/74	33.5321	2.0038	35.5359	35.3943	0.070665735	7.066574			
15	160/80	46.5339	2.0125	48.5464	48.4092	0.068173913	6.817391			
16	276/276	33.3492	2.025	35.3742	35.2285	0.071950617	7.195062	6.984139	0.193434	2.769622
17	130/102	30.6894	2.022	32.7114	32.5736	0.068150346	6.815035			
18	239/76	33.3539	2.0267	35.3806	35.2399	0.0694232	6.94232			
19	236/73	33.9769	2.0285	36.0054	35.702	0.149568647	14.95686	9.94674	4.343334	43.66659
20	123/75	32.3332	2.0068	34.34	34.1946	0.072453658	7.245366			
21	235/130	31.5521	2.011	33.5631	33.4095	0.076379991	7.637991			
22	78/238	34.1273	2.0059	36.1332	35.956	0.088339399	8.83394	8.922574	0.125347	1.404835
23	237/275	34.1211	2.0075	36.1286	35.9477	0.090112088	9.011208			

Table 2: raw data ash content

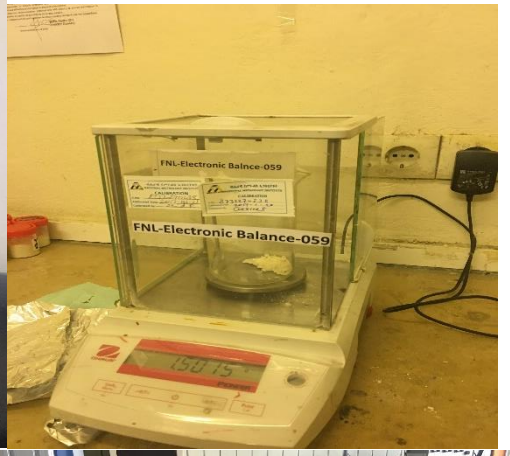
crucible no	crucible weight	sample weight (W1)	saple weight (W3)	Crucibe &sample weight(W2)		ash %W/W
1	12.5012	3.0002	15.5014	12.5553	2.9461	2.9464
2	35.3926	3.0022	38.3948	35.4533	2.9415	
3	27.848	3.0053	30.8533	27.9017	2.9516	
4	11.8046	3.0056	14.8102	11.8753	2.9349	2.9296
5	11.838	3.0036	14.8416	11.9177	2.9239	
6	12.1876	3.0085	15.1961	12.2661	2.93	
7	12.1901	3.0005	15.1906	12.2348	2.9558	2.9592
8	11.694	3.0005	14.6945	11.7383	2.9562	
9	12.0706	3.0018	15.0724	12.1068	2.9656	
10	12.1926	3.0005	15.1931	12.2348	2.9583	2.960567
11	11.6934	3.0015	14.6949	11.7383	2.9566	
12	12.0656	3.008	15.0736	12.1068	2.9668	
14	37.0117	3.0096	40.0213	37.078	2.9433	2.94365
15	12.2326	3.0098	15.2424	12.2984	2.944	
16	11.555	3.0099	14.5649	11.6426	2.9223	2.9228
17	11.289	3.008	14.297	11.3737	2.9233	
19	33.5991	3.0014	36.6005	33.678	2.9225	2.92275
20	36.2453	3.0029	39.2482	36.3252	2.923	
22	11.2121	3.009	14.2211	11.2679	2.9532	2.9528
23	11.0625	3.0075	14.07	11.1176	2.9524	



box pre drying in oven and boxes in desiccator for moisture content left to right

sample in oven for moisture content determination Crucible pre drying for ash determination

Charring boiling the sample


Picture from laboratory work at AAiT and at Ethiopia public health institute.