ADDIS ABABA UNIVERSITY

GRADUATE STUDIES PROGRAM

CENTER FOR FOOD SCIENCE AND NUTRTION

Nutritional Composition, Anti-nutritional Factors, Anti-oxidant Activities, Functional Properties, Nutritional quality and Sensory Evaluation of Ambasha made from Wheat and Cactus Pear (*Opunta ficus indica*) Seed flour Grown in Hatset Kebele, Hawzen Woreda, Eastern Zone of Tigray, Ethiopia.

By

Tewelde Hailemichael

Advisor: Mr. Kelbesa Urga (Asso.prof.)

A Thesis Submitted to the School of Graduate Studies of Addis Ababa University in Partial Fulfillment of the Requirement for the Degree of Masters in Food Science and Nutrition

June, 2016

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ACKNOWLEDGMENTS

First and foremost I would like to express my sincerely gratitude to the almighty God for his endless help in every activity of my life. Secondly, I would like to thank my advisor Mr. Kelbessa Urga for his continuous guidance and supervision starting from the beginning up to the end. Indeed, Mr. Kelbessa for me is not only my advisor but also he deserves a place of wise father for his generous personality. I have no words to express my great respect I have for him than wishing him all the success in his future career. I also need to appreciate Dr. Ashagrie Zewdu for his support and advising in every academic issue that had been challenging me.

Thirdly, I wish to thank laboratory technicians of the center Ms. Woineshet Abera, Mr. Debebe Hailu and Mr. Yemane H/mariam for their invaluable assistance throughout the whole activity in the laboratory. From the beginning, I know almost nothing about technical laboratory I was deeply thought about how I could finish my thesis. But by now I have had what I need and I hope I will scale up for the future. All these things I have got are definitely the fruitful of these laboratory technicians. I think they would be happy when they see their efforts on me and other students.

My parents are always behind my success and thanks to God once again for his infinite power to accomplish the pray of my parents. They usually encourage me to be strong and patient in every activity of my life.

My special thanks also directly go to all my classmates for their brotherly and sisterly collaboration and friendly approach. Here, I would like to appreciate my friend Mr. Mulubrhan Kahsay for his constructive and tirelessness moral supports and comments in every issue of my life. Finally need to say thanks for all Phd. Candidates for their endless promotion in some academic concerns.
Finally, I would like to say thanks to Ethiopian Police University Collage for the opportunity given to me to learn Msc.degree and the same heart full gratitude goes to Addis Ababa University

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AA	Ascorbic Acid
AAS	Atomic Absorption spectroscopy
ANOVA	Analysis of Variance
AOAC	Association of Official Analytical Chemists
CAM	Crassulacean Acid Metabolism
CAE	Catechine Equivalence
DNA	Deoxyribonucleic Acid
DPPH	Di Phenyl Picryl Hydrazyl
GAE	Gallic Acid Equivalent
OAC	Oil Absorption Capacity
PUFA	Polyunsaturated Fatty Acids
RNA	Ribonucleic Acid
SAERT	Sustainable Agriculture and Environmental Rehabilitation in Tigray
TFC	Total Flavonoid Content
TPC	Total Phenol Content
WAC	Water absorption capacity
WSI	Water Solubility Index

ABSTRACT

Cactus pear (Opuntia ficus-indica) seed is one of the main components of fruit crops which are tightly packed together in a mucilaginous structure inside the endocarp of the fruit. Nutritional composition, anti-nutritional factors, anti-oxidant activities, functional properties and sensory attributes of cactus pear seeds collected from Hatest kebele Hawzen Woreda, Eastern Zone of Tigray region were determined. The moisture, crude protein, crude fat, crude fiber, total ash, carbohydrate and gross energy contents of cactus pear seed averaged as 4.17g/100g, 10g/100g, 10.50g/100g, 18.23g/100g, 1.63g/100g, 55.47g/100g and 392.84cal in dry weight basis, respectively. The dietary Ca, K, P, Fe, and Zn content of the sample accounted 390.14mg/100g, 446.46mg/100g, 206.18mg/100g, 4.37mg/100g, and 2.01mg/100g, respectively. High phytate content (259.20mg/100g) and low contents of tannin (0.13mg/100g) and oxalate (0.11mg/100g) were obtained. The presence of high content of total phenols and total flavoniods in the sample produce an appreciable amount of anti-oxidant capacity which ranged from 43 to 95% of inhibition. When the functional properties of cactus pear seed flour was compared with each other, high value of water solubility index (5.6g/100g) and low value of bulk density (0.80g/ml) in fresh weight basis were obtained. The sensory evaluation of cactus pear seed revealed that consumption of 'Ambasha' (traditional bread) formulated with wheat flour was more preferable up to the ratio of 85:15% (wheat/seed) and the aroma of all sample ratios was highly attractive than the other sensory attributes. The overall acceptability of the bread showed that there was no significant differences ($p > 0.05$) among the samples prepared in wheat to cactus pear seed flour ratios of 100:0%, 95:5% and 90:10%, but these were differed significantly ($p < 0.05$) from the remaining sample fractions (85:15%, 80:20% and 75:25 %). In conclusion, the present study demonstrated that cactus pear seeds can be used as food supplementation in arid and semi-arid areas.

Key words: Succulents, Seeds, Functional foods, Free radicals

1. INTRODUCTION

1.1. Background of the study

Cactus pear (*Opuntia ficus-indica*) is commonly known as "prickly pear" and belongs to the Cactaceae family (Singh, 2003). There are nearly 1500 species of cactus belonging to the Opuntia genus (Cactaceae) among which cactus pear is the most well-known species. Cactus pear is well adapted to grow wild in arid and semi arid regions, where the production of more succulent food plants is severely limited. Because cactus pear can withstand prolonged drought, it is considered as a potential alternative crop for drier regions (Barbera *et al*., 1995). The reason that enables the plant to adapt in arid conditions is associated with the shape of its several organs. Nobel (1988) suggested that the shallow and extensive root system promote the plant to exploit scarce moisture in such environments. More essentially, the successful introduction of *Opuntia* in these areas has been attributed to its Crassulacean acid metabolism (CAM). The CAM plants take up CO_2 primarily at night, leading to high water-use efficiency and high drought resistance, which are important features for arid regions with limited rainfall (Nobel, 1988). Most Cactus pears are widely distributed in Europe, Southwestern United States, Northern Mexico, much of Latin America, South Africa and the Mediterranean countries (Russell and Felker, 1987). In Latin America it is considered as an important nutritional source (bread of the poor people) (Lee *et al.,* 2002). It was introduced into North Africa in the 16^{th} century (Griffith, 2004).

Cactus pear is locally known by the vernacular name "Belles" was introduced between 1848 and 1870 by Catholic Missionaries to Eastern Zone of Tigray, Ethiopia, by a priest called "Abune Yakob" who visited Erob, Northern part of Tigray. He introduced cactus pear with the realization of unsuitability of the area for cropping and other agricultural activities due to recurrent drought, erratic rainfall, rocky and mountainous topography. As a result, the priest brought three spineless cladodes from Mexico, its country of origin (Griffith, 2004) and planted one cladode in Alitena (Erob), the second cladode planted in Golea (Ganta afeshum) and the third cladode planted in Lehe (Eritrea). Then after, the plant was distributed throughout the region and beyond (Kibra, 1992). Cactus pear is also found in eastern and southern parts of Ethiopia, such as in Daro Lebu Woreda and West Hararghe. Currently cactus pear is widely spread throughout Tigray region and is believed to cover more than 379,338 hectares of land, i.e., 7.4% of the total land of Tigray region

(SAERT, 1994) and becomes the integral part of the people's economy (Tesfay *et al.,* 2011). In Tigray region alone, uncultivated cactus covers about 32,000 ha of land.

In Ethiopia, it is considered as a 'Bridge of life' because the stems and fruit store large quantities of water and provides an important food source to both humans and animals (as forage for livestock). In many parts of Tigray region, the fruits are even sold on local markets, such as in Mekele town. Konso people also eat the fruits in times of hardship (El Kossori *et al*., 1998). The fruit is also mentioned in traditional song which is translated from the local language (Tigrigna) as "A farmer without Beles is like a stream without water" (Brutsch, 1997). It grows purposively in the region and has adapted perfectly to the arid zones that is characterized by droughty conditions. Cactus pear in Tigray is generally used as a source of food, forage, fuel wood, cash income, as live fences and soil conservation purposes (Brutsch, 1997). Moreover, Cactus pear is an important part of the cultural heritage and a food source for people in Tigray region (SAERT, 1994). During summer season, many people especially shepherds don't need to eat their lunch once they have already eaten their breakfast; instead they are more interested to consume sweet cactus pears.

Total soluble solids are an indication of sugar content and indeed, it is highly influenced by crop management and environment since cactus pear fruit grown in dry areas are sweeter than those grown in humid areas. Likewise, it has been observed in out-of season production. Which means late fruits growing in cool temperature with cloudy days are less tasty than those produced in hot, sunny days of summer (Monderagon *et al*., 2001). The fairly high sugar content and low acidity in the pulp render the fruits to be delicious and sweet. The sugar pattern in the fruit pulp is very simple and consists of glucose and fructose in virtually equal amounts while the organic acid pattern is dominated by citric acid (Stintzing, 2001). Sweet and tasty fruits of cactus pears, ripening between the end of July and October, may be easily found as spontaneous vegetation, but products from cultivations are now usually marketed (Monderagon *et al*., 2001). However, it is difficult to transport all matured cactus fruits from cultivated and uncultivated areas to the market may be due to inappropriate transportation conditions, so that they are usually discarded. Even their cost is very cheap as compared with other fruit types.

Cactus pear fruit contains three main components namely: skin, pulp and seed. Previous studies showed that the fruit size and shape are affected by the seed number and weight (Barbera *et al*.,

1995). A strong relationship would be expected between the seed and pulp content because the edible tissue (pulp) develops from funiculi and the funicular envelops of the seed (Pemienta, 1990). The cactus pears can be eaten as fresh fruit or after processing in many other ways (Nobel, 1998). As they have a short post-harvest shelf life, it would be useful to prepare in the form of seed flour, juice and other products from cactus fruits that could be used throughout the year (Felker and Guevara, 2001). The production of seed flours is a simple process (by sun drying or dehydration at low temperature) that changes the discarded seed to useful and nutritive sources which are acceptable as food and nutritional supplements (Akpata and Akubor, 1999). The seeds are made of two different tissues, the endosperm and the pericarp in the relative proportion of 1:9, respectively (Habibi *et al*., 2002). The number of seed for each cactus pear fruits collected from the study area ranged between 290-414 or 3.20-4.60 gram per fruit. The seeds of *Opuntia* species which are tightly packed together in a mucilaginous structure inside the endocarp contain high amounts of polyunsaturated fatty acids, especially linoleic and linolenic acids which are known as having a wide variety of health benefits.

1.2. Statement of problem

The Northern part of Ethiopia, 'Tigray region,' including the study area is endowed with the natural plant 'cactus' which is characterized by seasonally growing and drought resistant fruit. This fruit is mutually correlated with the climatic condition and topography of the region. However, there is still no sufficient utilization of these fruits being made in the region in general and in the study area in particular. Though several authors (El Kossori *et al*., 1998) who conduct their studies in different countries showed that cactus pear seed is nutritionally important, it is unusual in the study area to preserve and use of these seeds as a source of food, especially surplus cultivated cactus pears remain stagnated together with the pulp. This is because people know nothing about the nutritional and medicinal values of the seed. However, according to the report of several studies, it is a good opportunity for the future to reuse these cactus pear seeds from industrial wastes such as from wine processing by-products (Ku and Mun, 2008) and cactus fruit seeds (Chang *et al.*, 2008). There are also extra non-cultivated cactus fruit products from which the farmers cannot fully consume or preserve for future uses. This is due to the reason that the fruits have a short–post harvest time and the farmers have no any means of extending the shelf life of these fruits. Once the ripening period of cactus pear is passed, cultivars tend to buy their food from

the market since the product they harvest is not fully enough for consumption throughout the year. Therefore, this study is intended to determine the nutritional composition of cactus pear seeds, evaluate the ant- nutritional factors, investigate the antioxidant capacity, analyze the functional properties and formulate product and evaluate the sensory acceptability of cactus pear seeds.

1.3. Significance of the study

Once this study is effectively completed, it offers a number of contributions such as:-

- Provides a new idea about the nutritional contribution of cactus pear seeds
- Provides the chance of having an alternative food supplementation from cactus pear seed for community
- Reduces the poor management system of cactus pear seeds
- Encourage small-scale enterprises to engage in advanced use of cactus pear seeds
- Attract the attention of researchers who want to study on the cactus pear seed

1.4. Objectives of the study

1.4.1. General Objective

The study was generally intended to determine the nutritional contents, anti-nutritional factors, anti-oxidant activity, functional properties and sensory attributes of cactus pear seeds.

1.4.2. Specific objectives

The specific objectives of this study were:

- To analyze the proximate composition and mineral content of cactus pear seeds
- To determine the anti- nutritional factors of cactus pear seeds
- To measure the anti-oxidant potential of cactus pear seeds
- To assess the functional properties of flours from the seeds and
- To formulate product from cactus pear seeds flour and to evaluate its sensory acceptability.

2. LITERATURE REVIEW

2.1. Cactus pears and nutritional composition of their seeds

Cactus pears have been ignored by the scientific community until the beginning of the 1980s when several studies and reports were published on their biological functions. However, more recent investigations on the chemical components and the nutritional value of *Opuntia spp.* have attracted attention of many authors (Feugang *et al*., 2006). Cactus pears, like other plant species, play a vital role on nutritional, ecological and medicinal aspects (Nobel *et al*., 1988). Northern Ethiopia, particularly Tigray, is known for its crop failures due to shortage and poorly distributed rain. So, identifying crops that utilized water efficiently and produce acceptable yield is a priority for the area (Fetien Abay, 1997). Some *opuntia* species produce colored fruit which is an additional attraction for consumers. Likewise, cactus pear also produce yellow, red, and white fruits, due to the combination of two betalain pigments, the purple-red betanin and the yellow-orange indicaxanthin (Felker and Guevara, 2001). Moreover, the development of the betalain pigment can give a clue to the degree of maturity of the cactus-pear fruits. These pigments also play a vital role in producing natural food colorants (Duru and Turker, 2005). Furthermore, these pigments have shown antioxidant properties being higher than for ascorbic acid (Stintzing *et al*., 2005). Cactus pears do not need much water and accordingly they exhibit unusual physiological and morphological features (Akpata and Akubor, 1999). The composition of the fruit varies with ripening as well as their stage of maturity (Duru and Turker, 2005).

Literatures contain much information concerning the cactus pear, describing the chemical composition of the pulp, peel and seeds (SAERT, 1994). This is due to the reason that the prickly pear is one of the plants that nothing is to throw (Mouden *et al*., 2016). However, among all these literature sources, seed has been the relatively less studied part. Because of this, millions of pounds of fruit seeds are discarded yearly, resulting in disposal problems while proper utilization of these waste products could lead to an important new source of oil and meal (Kamel and Kakuda, 2000). Especially, they are crucial for their high nutritional value, mainly as source of polyunsaturated fatty acids (PUFAs), and dietary fiber, instead of being discarded, as they currently occur (Prieto-García et *al.*, 2006). It is obvious that the edible part of the fruit contains relatively large number of seeds, which represent an important percentage of total fruit mass, on a dry weight basis (Habibi *et*

al., 2008). Several authors have reported that a great variation existed in the number of cactus pear seeds, which ranges from 80 to more than 300 per fruit (Barbera *et al.*, 1991). This means from 2.8 to 7.5 grams of seeds per fruit according to the fruit size and the cultivar (Mondragón-Jacobo and Fernández, 1995). Every single seed has a capacity to form pulp even if it is not completely developed (Mondragon-Jacobo and Bordelon,1996). When the maturity stage increases, the weight of the pulp increases while the proportion of the seed decreases (El-Gharras *et al.*, 2006).

Literature reported that cactus pear seeds contain the nutritional compositions which comprise moisture, carbohydrates, crude fiber, lipids, protein, and ash (Joubert,1993). From these nutritional components, cactus pear seed predominantly contains highest amount of carbohydrate, protein, fat and fiber with a value of 51.11, 13.62, 10.43 and 9.23g/100g, respectively (Nassar, 2008). Hence, the high content of lipid in seeds means a potential source of oil (Piga, 2004). The fatty acid composition of prickly pear seed oil is similar to sunflower and grape seed oils (Tan *et al*., 2000). On the other hand, the protein and lipid composition of cactus pear seed recorded by Salim *et al*. (2009) was about 4.48% and 3.66%, respectively. Cactus pear seed is a good source of fiber and beneficial to human health. Insoluble fiber from the seeds could be extracted in order to use for human consumption. Furthermore, Özcan, *et al*. (2011) clearly stipulated the proximate composition of cactus pear seed as the moisture (6.1%), crude protein (4.78%), crude fiber (12.47%), crude lipid (5.0%) and ash (1.27%).

Seed content is correlated positively with fruit size. In this regard, the ideal fruit should have a large number of seeds to attain good size (Mondragon-Jacobo and Bordelon, 1996). Nevertheless, late fruits have less seeds per unit core weight than summer fruits (Lawes *et al*., 1987). Pimienta and Engleman (1985) have suggested that the pulp develops from the seeds, and completely seedless varieties may be theoretically impossible. Caplan *et al.* (1995) has also stated that the presence of seeds in cactus pears is the major deterrent to first time consumers.

Cactus pear seeds are good sources of the macro and micro minerals and can be consumed as a food ingredient to provide nutrition. Moreover, cactus pear seeds are rich in minerals, with a predominant content of Potassium, (280.28 mg/100g) and followed by Calcium (58.50 mg/100g), Phosphorus (24.93 mg/100g), Iron (13.41 mg/100g) and Zinc (1.36 mg/100g) (El-Safy *et al.,* 2012*)*. But there are differences in the values of these minerals among many authors. A study

conducted in Turkey by El- Kossori *et al*. (1998) implied that the mineral content of O*punta ficus indica* seeds comprises of phosphorus (110), calcium (258), potassium (275), iron (12.1) and zinc (4.16) all described in mg/100g.

2.2. Anti-nutritional factors of cactus pear seeds

Cactus pear seed like other fruit seeds possess different anti-nutritional factors such as phytate, tannin, and oxalate. Phytate content was relatively more distributed in cactus pear seeds. The amount of each anti-nutritional factors accounts 13.22mg/100g (Phytic acid), 2.56mg/100g (Tannin) and 4.54mg/100g (Oxalate) (El-Safy *et al.*, 2012*)* in dry weight basis.

Tannin in fruits imports an astringent taste that affects palatability, reduce food intake and consequently body growth. Tannins are known to inhibit the activities of digestive enzymes and nutritional effects of tannin are mainly related to their interaction with protein. Tannin-protein complexes are insoluble and the protein digestibility is decreased (Carnovale *et al.,* 1991). Different studies also revealed that high tannin in diet adversely affects digestibility of proteins and carbohydrates, thereby reducing growth, feeding efficiency, metabolizable energy and bioavailability of amino acids (Aletor, 1993). The problem with phytic acid in foods is that it can bind some essential mineral nutrients in the digestive tract and can result in mineral deficiencies. Phytic acid also binds to phosphorus and converts it to phytate, while other mineral elements like calcium, zinc manganese, iron and magnesium are converted to the phytic complexes, which are indigestible substance, thereby decreasing the bioavailability of these elements for absorption. Especially the inhibition capacity of phytic acid on the absorption of Ca, Fe, and Zn has been reported by Norhaizan and Norfaizadatul (2009).

Phytic acids also have a negative effect on amino acid digestibility, thereby posing problem to non-ruminant animals due to insufficient amount of intrinsic phytase necessary to hydrolyze the phytic acid complex, but its presence is also beneficiary because it may have a positive nutritional role as an anti oxidant and anti cancer agent (Turner *et al*., 2002). As far as the negative effect on mineral availability is concerned, oxalate is placed at a front line in producing health problems and diet with high oxalate content can increase the risk of renal calcium absorption and has been implicated as a source of kidney stones (Chai and Liebman, 2004).

2.3. Anti-oxidant compound and antioxidant activities of cactus pear seeds

2.3.1. Total phenolic content

Phenol compounds are a large group of ubiquitous molecules synthesized by plants under different environmental factors and stress conditions (Boudet, 2007). They form a diverse group that includes phenolic acids, flavonoids and tannins The interest in plant materials containing phenolic compounds are increasing due to their high antioxidant potency, which may offer protection against cancer through the inhibition of oxidative damage, known to be a potential cause of mutation (Khomdram and Singh, 2011). The anti-oxidative properties of phenolic compounds are a predominant feature of their radical-scavenging capacity (Cotelle, 2001). This activity is attributed to their ability to scavenge free-radical and to chelate metal ions involved in their production. Phenolic compounds are receiving increasing attention because of their health promoting effects, attributed to their antioxidant activity (Tlili *et al*., 2010). The seeds of cactus pear fruit are known to be rich in phenol contents. The high level of phenolic compounds makes seeds of *O. ficus indica* an excellent natural source of these compounds with a possible dietary, industrial and pharmaceutics utility. Nevertheless, the available information regarding the phenolic compound is scarce, since most studies are limited to global phenolic measurements, and only a few identified individual phenolic acids and flavonoids in Opuntia seeds (Tounsi-Saidani *et al*., 2011). The content of total phenols in cactus pear seed accounts about 268 mg/100 g on dry weight basis. Tlili *et al*. (2011) and El-Mostafa *et al*. (2014) also obtained total phenol content in the seed part of cactus pear between the ranges of 48-89 mg/100g on dry basis. On the other hand, results obtained from the study conducted in Algeria shows that the total phenolic acid content, measured by the Folin–Ciocalteu method, is about 74 mg GAE/100g. The results of different cactus pear seed analysis indicated that there is significant correlation between the contents of antioxidants and the activities tested. The samples containing highest antioxidant activities have also a highest phenolics and other anti oxidant compounds (Chougui, *et al.,* 2013).

2.3.2. Total flavonoids

The seed of cactus pear is prominent with its total flavonoid content next to the total phenols. It plays a pivotal role in scavenging the free radicals that could be generating oxidative-based stresses. Based on the data of some literatures, Cactus pear seeds contain a total flavonoid which ranged between 1.5–2.6 mg/100 g (Chougui, *et al.,* 2013).

2.3.3. Antioxidant activity

Antioxidant activity is defined as the capacity of a chemical component to capture and/or inhibit the formation of free radicals (Halliwell, 1996). A free radical is a chemical species that contains a despaired electron in the last atomic orbital, causing it to be highly reactive and to have a very short life time (Young and Woodside, 2001). This characteristic confers the capacity of altering the structure and function of several cellular components as lipids, proteins, RNA, DNA and other bio-molecules, making them able to stimulate the appearance of cancer, diabetes, and cardiovascular diseases (Rolo and Palmeira, 2006).

The nutritional and health benefits of cactus fruit are associated with their antioxidant properties related to ascorbic acid, phenolic compounds and betalains pigments (Feugang *et al*., 2006). Although *O. ficus indica* is widely consumed, its chemical analysis and antioxidant activities have received little attention. It is known that hydroxyl radical is a powerful oxidant that can react with all biological molecules and that oxidative stress can mediate a wide variety of degenerative processes and diseases (Dhalla *et al*., 2000). Natural antioxidants obtained from plant-based extracts are currently a subject of intensive research, and are of interest to both food scientists and health professionals. This has been derived from a number of studies revealing a positive correlation between a diet rich in plant-based food and a reduced risk of diseases associated with oxidative stress, such as cancer, cardiovascular and neurodegenerative diseases (Manach *et al*., 2004). Natural antioxidants from dietary sources include phenolic and polyphenolic compounds, chelators, antioxidant vitamins and enzymes, as well as carotenoids. Their presence in the plants may be for the sake of protecting tissues from injurious damage (Zulueta *et al*., 2009). Indeed, seeds from Opuntia sp. were shown to be rich in polyphenols, flavonoids and tannins, the concentrations of those molecules in fruit seed is always higher than in the fruit pulp (Fernández-López *et al*., 2010).

With regard to literatures, most authors have evaluated the antiradical activity of the seed extract using the DPPH (2, 2-diphenyl-1-picrylhydrazyl) free radical. DPPH is a stable free radical and accepts an electron or hydrogen to become a stable molecule. It was found that antioxidant molecules such as ascorbic acid, tocopherol, flavonoids, and tannins reduce and decolorize DPPH due to their hydrogen donating ability (Kumaran and Karunakaran, 2007). The results of previous studies showed that cactus seed extracts had better scavenging effect with IC_{50} value of 185.85 µg/ml (Toure.*et al*., 2015).

2.4. Functional properties of cactus pear seeds

Functional properties are basic sources of food attributes contribute an important aspect in determining its competitiveness in the market, as they can impact the sensory, physical and chemical properties of a food, which includes texture and organoleptic characteristics (Saskatchewan, 2015). Plant protein is used in foods as functional ingredient to improve stability and texture as well as nutritional quality of the product (Makri *et al*., 2005). Solubility of protein under varying conditions is one of its important functional properties. Because this greatly influences other properties such as foaming and emulsification .Thus, the protein may possess satisfactory properties, e. g. nutritional value, acceptable flavor, odor and texture (Kinsella, 1982). Functional properties of food protein are also important in food processing and product formulation. Some of these properties are bulk density, water/oil absorption capacity, foam capacity and stability, and protein solubility. Water absorption capacity (WAC) represented the ability of a substance to associate with water under a limited water condition (Singh, 2001). Both protein and carbohydrates improved WAC in particularly protein concentrate; this may be due to smaller lipid content in its content compared with cactus seed flour (Jitngarmkusol *et al*., 2008). The Water absorption capacity of prickly pear flour accounts about 3.71 ml/g sample. Moreover, Oil absorption capacity (OAC) was another important functional property, since it plays an important role in enhancing the mouth feel and retaining the flavor (Kinsella, 1976) and its absorption capacity in cactus pear seed flour is about 2.48 ml/g sample (Nassar,2008). According to other literatures, cactus pear seed exhibited Water absorption capacity, Foam expansion, Foam stability and water solubility index with a result of 2.16, 14.21, 63.69 and 22.13 %, respectively (El-Safy *et al*., 2012).

2.5. Sensory evaluation of cactus pear seed

Cactus pear is a promising fruit with large potential for breeders, agriculturists and food technologists. It is possible to scale up the production of different foods from cactus pear fruits and it is desirable to develop and increase the processing technologies applicable to this crop (Saenz, 1996). Since cactus pears are high in dietary fiber, minerals, phenol contents, it shows greater technological potential for water binding capacity and fat absorption. All characteristics reflected by the baked bread determined by the entire contents of the blending ingredients especially fats enhance the flavors of other ingredients as well as contributing to its own flavor (Lauterbach and Albrecht, 1994). The evaluation of sensory attributes from the blended product of cactus cladode and wheat flours confirmed that aroma had the highest numerical values amongst the attributes tested, for all the samples, except for the 17% cactus cladode sample, which had a higher score for taste (6.36) and overall acceptability (6.38). Moreno-Alvarez *et al.* (2009) reported that the inclusion of cactus flour in bread affected the sensory properties. In spite of the incorporation of cactus cladode up to 17% was still acceptable, the levels of 5-10% was quite preferable.

3. MATERIAL AND METHODS

3.1. Description of study area

The study was conducted in Hatset Kebele, Hawzien Woreda, and Eastern Zone of Tigray region. This site is known as one of the naturally endowed areas of the region with cactus pear cultivation and consumption. The potential cultivation of cactus pear and socio-economical linkage of cultivars during ripening season lead to the selection of this area, Hatset Kebele . The study site is actually located at a distance of 73km far from Mekell; capital city of Tigray regional state. It is found at $13^0$58' N latitude and $39^0$25 E longitudes and its altitude ranges from 2000-3000 meters above sea level. The area receives an annual rainfall of 400-600mm and the highest and lowest rain fall is recorded from June to August and often from March to April, respectively. The annual minimum and maximum temperature is 6^0C and 21.8^0C, respectively. According to the Woreda Finance and Economic Development Office, the total population of Hawzien Woreda is estimated to be about 72,631 (Alembrhan and Haylegebriel, 2014).

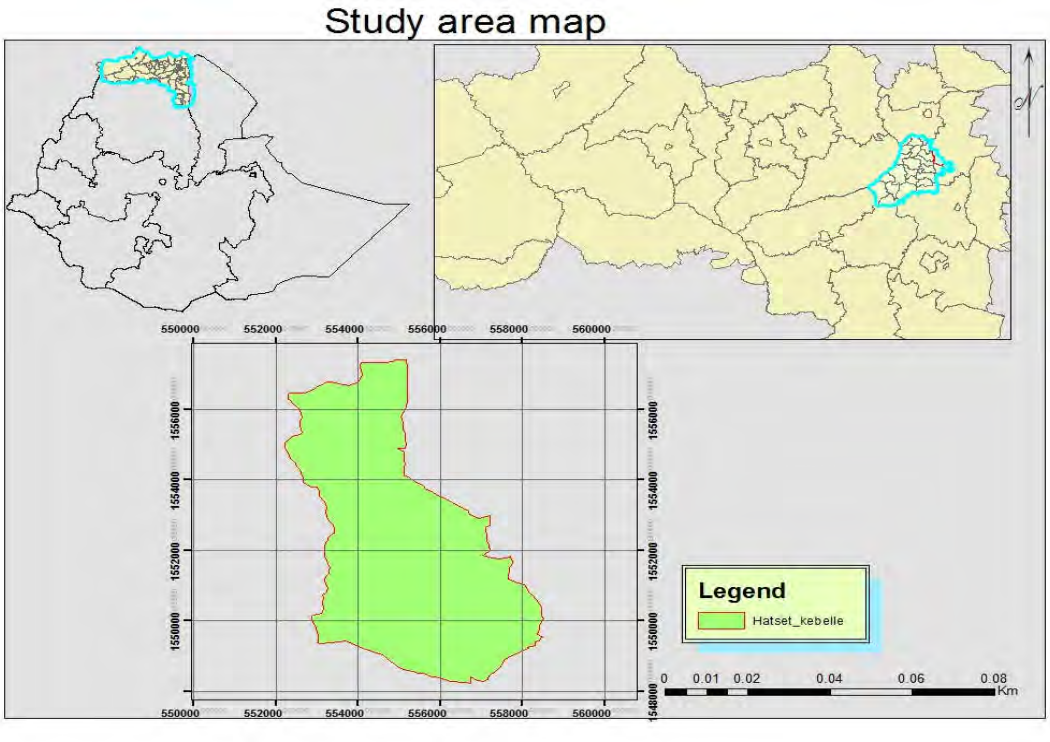

Figure 1.The map of the study area (Hatset), Tigray region, Ethiopia

3.2. Fruit collection

Before the sample collection work started, a critical cross-check of the taxonomical identification of the fruit (cactus pear) has been made. This was confirmed in Addis Ababa University National herbarium with the scientific name of 'O*punta ficus indica'* and a local name of 'Beles.'

After identification, 25 kg of matured (yellowish color) cactus pears fruit were randomly collected in October from the study site. During collection, ripened cactus pears were picked up using a stick plant wisely. Then the fruits were washed by fresh water to release their spines and other foreign materials. The outer part (skin) of the cactus fruit is hand peeled using a stainless knife. The seeds were isolated by pressing the whole edible pulp (fleshy part) while repeatedly washing and finally the seeds were sun dried for twenty seven hours and then brought to the laboratory for analysis.

3.3. Sample preparation

Dried seed of cactus pear fruits were well decorticated using mortar and pistil not only to remove the sticky-remnant pulps but also the seed coat, then ground to powder and passed through 0.45mm sieve. The powder of cactus pear seeds was stored in polyethylene bags at room temperature until analysis. The preparation and analysis of sample is summarized in the flow-chart below.

Figure 2. ***Opinta ficus indica*** **fruits and seeds**

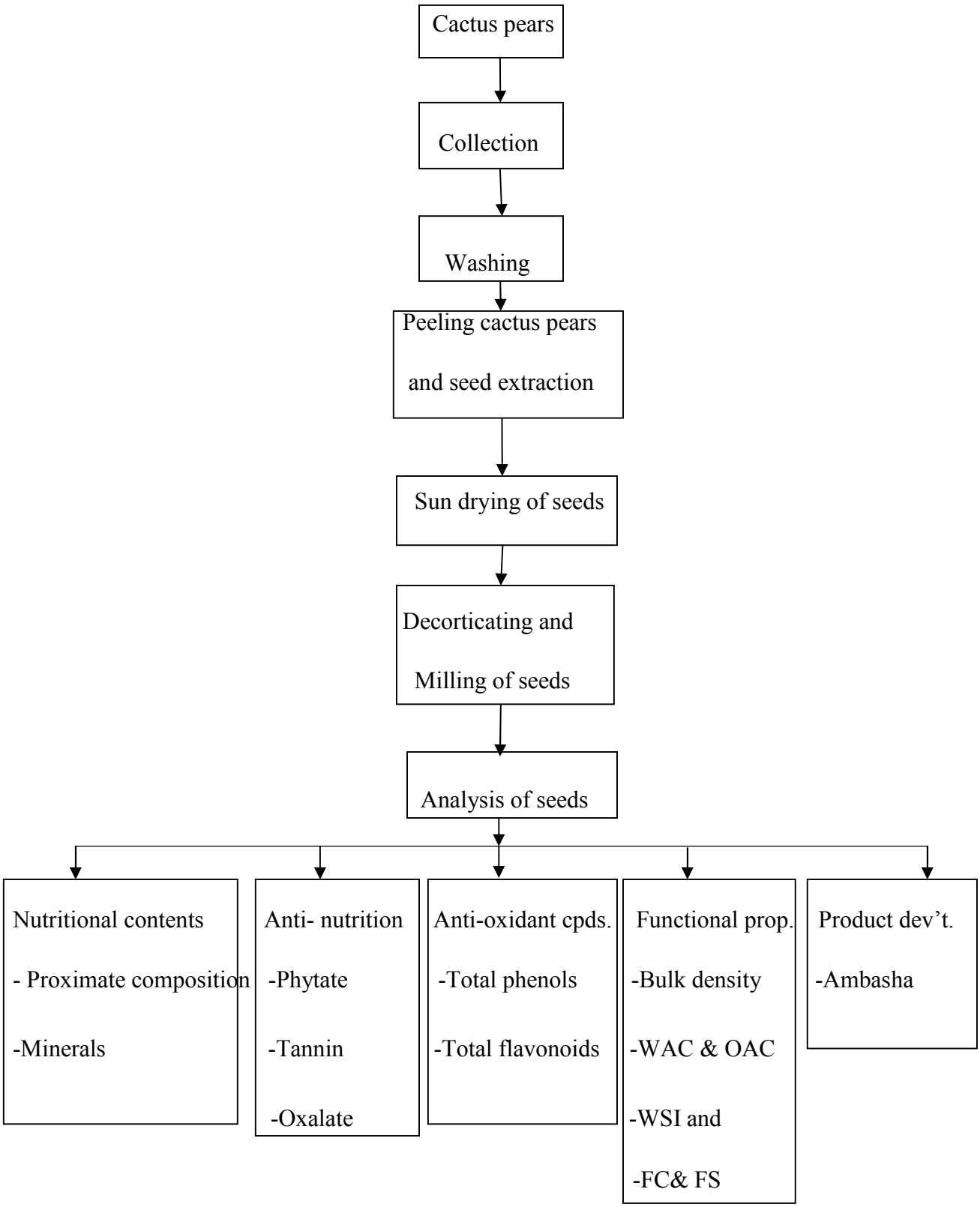

Figure 3. Cactus seed sample preparation and analysis

3.4. Analysis of proximate compositions

3.4.1. Moisture analysis

The moisture content of cactus pear seed flour was determined by hot air drying method according to the official method of 925.09 of AOAC (2000). Steel crucibles were properly cleaned and dried in drying oven at 105^0C for 1 hour. The crucibles were taken out from the oven, cooled in desiccator for 30 minute and then their weights measured (W_1). Five gram of cactus pear seed flour was weighed in each crucible and dried in drying oven at 105^0C for 3 hours. Then the weight of the crucible containing the sample was measured (W_2). After drying, the crucibles containing the dried sample were cooled in desiccator at room temperature, weighing of the sample was continued until a constant weight is obtained (W_3). The last constant measurement was taken to calculate the moisture content of cactus pear seed using the following formula:-

$$\text{Moisture } \% = \frac{W2-W3}{W2-W1} \text{ x}100$$

Where, W_1= Weight of the crucible, W_2 = Weight of the sample and the crucible before drying W_3 = Weight of the crucible and the sample after drying.

3.4.2. Crude protein

The analysis of protein content was determined by the micro kjeldahl method according to the official method 979.09 of the AOAC (2000). The complete analysis of protein involves the following three steps:-

0.5g of cactus pear seed flour was weighed in a clean tecator tube and placed in tecator rack. 6ml of concentrated sulfuric acid was added in to the tubes containing the sample for degestion facilitation purpose using a pipette and then mixed carefully. 3.5ml of Hydrogen peroxide was added in a step wise manner to each sample tube. Here as soon as the most violate reaction has ceased, the tube was shacked for few minutes by hand and put it back in to the rack. 3 g catalytic mixture of copper sulfate and potassium sulfate was added in to the sample tubes and the tubes were let to stand for 15 minutes before digestion.

A. Digestion- The sample tubes were placed in a digester after the working temperature has reached at 370^0C and the digestion process has continued until clear solution was observed. The sample tubes were taken out, placed in the rack and allowed to cool in fume hood. Later on, 50ml of distilled water was added into the sample tubes in order to avoid precipitation of sulfate.

B. Distillation- At this step 25ml of 35% NaOH was added to neutralize sulfuric acid and this enables for the release of ammonia. A 250ml Erlenmeyer flask containing 25 ml of 4% H_3BO_3, 25 ml of distilled water and 3 drops of methyl red indicator solution was placed as receiver on the distillation unit. The distillation process was continued until the volume of the distillate reached between 200ml and 250ml.

C. Titration-the distillate was finally titrated with standardized 0.1N of HCI until the appearance of the first pink color. At this point the amount of consumed HCI was immediately recorded. Furthermore the blank reagent was run to subtract the reagent Nitrogen from the sample Nitrogen. The amount of protein was calculated by using the following formula:-

$$\text{Crude protein } \% = \frac{(V2-V1)\ X\ N\ X\ 14.01\ X\ 6.25}{10\ X\ W}$$

Where, V1 = Volume (ml) of HCI solution required for the blank test.

V2 = Volume (ml) of HCI solution required for the test sample.

N = Normality of HCI

W= weight of sample

3.4.3. Crude fat

The crude fat was extracted using Soxhlet apparatus according to AOAC (2000) official method 45.01. At the beginning, an extraction cylinder was washed with hot water to remove any impurity and put it in to an oven for about 1 hour at a temperature of 105^0C. Then after, take out them and put in to a desiccator, after cooling weighed (W1) and turn out them again in to the desiccators. The bottom of the extraction thimbles was covered with a layer of fat free cotton. About 2g of cactus pear seed flour was measured in the thimbles and was covered with a layer of fat free cotton (W).The thimbles were put in the extraction chamber and extraction cylinders were taken out of the desiccator and put on the bracket. 50ml of petroleum ether with boiling point range between

40-60^{0}C was added into the cylinders and the thimble was immersed in the cylinder containing petroleum ether and digested at 55^{0}C for 2 hours. Fat extraction was continued for additional 2 hours. Once the extraction process completed, the cylinders containing extracted fat were disconnected and were put in a drying oven at 70^{0}C for about 30 minutes. The cylinders were taken out of the oven, cooled in a desiccator for 30 minutes and weighed (W_2) immediately after they taken out of the desiccator. The amount of extractable fat was calculated using the following formula:-

$$\text{Crude fat \%} = \left\{ \frac{W2-W1}{W} \right\} x100$$

Where, W1= Weight of extraction cylinder

W2 = Weight of the extraction cylinder plus the dried crud fat

W = Weight of sample

3.4.4. Crude fiber

Crude fiber content of cactus pear seed sample was determined according to the official method of 979.09 of AOAC (2005). The following steps were used during the complete analysis of the sample:-

Step 1. Extraction: One g of sample was weighed and placed in a 600 ml beaker. After adding 200ml of 1.25% H_2SO_4 and boiling for 30 minute the watch glass was placed over the beaker. It was then gently heated on a hot plate and keeping the level constant with distilled water. 30 minutes later, 20 ml of 28% KOH was added and Boil gently for further 30 minutes and stir wisely.

Step 2. Filtration: The bottom of a sintered glass crucible was covered with 10 mm sand and the layer of sand was with a little distilled water. Solution from beaker poured into sintered glass crucible and turns on vacuum pump. Beaker walls were rinsed with hot, distilled water several times, washings were transferring to crucible and then filtered residue was washed in crucible with hot distilled water and filtered. After wards repeat the step once again. The residue was continuously washed with 1% H_2SO_4, hot distilled water, 1% NaOH, hot, distilled water, 1%

H_2SO_4 and Washed twice more with hot distilled water and it was filtered in each washed intervals, consecutively.

Step 3. Drying and Combustion: in this step crucible was dried for 2 hours in the electric oven at 130^0C and Cooled for 30 minutes in a desiccator and then weighed (W1). Crucible was Transfer to muffle furnace and stay for 30 minute at 550-600^0C, Cooled in a desiccator and weighed (W2).The crude fiber of the sample was finally calculated according to the following formula:

$$\text{Crude fiber in \%} = \frac{(W1-W2)\,x100}{W3}$$

Where, W1 = Crucible weight before drying, W_2 = Crucible weight after drying, W3 = Sample dry weight

3.4.5. Total carbohydrate content

Total Carbohydrate content of the cactus pear seed flour was determined by the difference method (i.e.by subtracting the sum of percentage of moisture, crude protein, crude fat, crude fiber and ash content from 100%).

3.4.6. Gross energy

The gross energy of the seed flour was determined in such a way that the total caloric value of crude fat, crude protein and carbohydrate was calculated using the Atwater's conversion factors; 1g of fat = 9cal (37.4Kj), 1g of protein = 4cal(16.7kJ), 1g of carbohydrate = 4cal (16.7kJ) and 1g of fiber=2cal (8.35KJ) (Tontisirin, 2002).

3.4.7. Total ash content

The ash content of cactus pear flour was determined according to the official method of 923.03of the AOAC (2000). Porcelain crucibles were cleaned and dried in muffle furnace for 30 minutes at 550^0C. After the crucible cooled in a deciccator for 30 minutes at room temperature; they were weighed (W_1) using analytical balance. About 2.5 g of cactus pear seed sample was weighed in each cleaned crucibles (W_2). Next, the samples were charred on a hot plate under a fume hood until the smoke ceased down. The charred sample was incinerated in a muffle furnace at 550^0C

for 5 hours till the residue becomes white in color after that they were cooled in a desiccator and their weigh was recorded (M_3). The amount of total ash presented in a cactus pear seed sample was calculated using the following formula:-

$$\text{Ash } \% = \frac{W3-W1}{W2-W1} \text{ x}100$$

Where, W_1 = Weight of crucible, W2 = Weight of crucible plus sample Weight W3 = Weight of crucible plus ash Weight.

3.5. Mineral analysis

3.5.1. Determination of Calcium, Zinc and Iron

The analysis of Ca, Zn, and Fe was done based on the official methods of 999.11 of AOAC (2005). The analysis process of these minerals follows two successive steps:-

Step 1. Ashing: At the beginning of this step all crucibles were washed with 6N HCl and glass wares with 10% nitric acid, then the crucibles were placed in an oven for about 30 minutes at 100^0C. After the crucible thoroughly cooled in a desiccators for 30 minutes, 2.5 g of sample was accurately weighed. The crucible containing the sample was charred at hot plate starting from low temperature under a hood. Later on, samples were ashed in a muffle furnace at 550^0C for 1 hour and the crucibles were take out from the furnace, cool, and moisten with a few drops of deionized water and evaporated on hot plate. Samples were ashed once again, cooled and some drops of deionized water and 5 drops of concentrated HNO_3 were added then evaporated on hot plate as described above. Sample was finally ashed for 30 minutes at the same temperature as previously described. Crucible was cooled in desiccators for 45-60 minutes and then weighed.

Step 2. Dissolution: At this step ash was treated with 10 ml of 6N HCl to wet it completely and was carefully taken to dryness on a low temperature hot plate. In Addition 15 ml of 3N HCl was added and crucible was heated on the hot plate until the solution just boils. After cooling the crucible, a filtration was carried on through a filter paper into a graduated flask. 10 ml of 3N HCI was again added to the crucible containing the filtrate and heated until the solution just boiled, Cool and continue to filter into the graduated flask. Furthermore, crucibles were washed at least

three times with deionized water and washings were filtered into the flask. In the same manner, the filter paper was washed thoroughly and washings were collected in the flask. In this case, 5 ml of lanthanum chloride solution per 100 ml of solution was added to determined calcium. The contents of the flask (sample solution) was cooled and diluted to the mark with deionized water. Blank was prepared by taking the same amount of reagents used to prepare the sample. Sample solutions were transferred to polyethylene bottles. AAS was calibrated with standards until the curve is fitted. Hence, a series of standard solutions of the minerals were prepared from stock solution of 20 ppm. for Ca (0, 0.5, 2, 4, 6, and 8 ppm.), Fe (0, 0.5, 1, 2, 3, and 4 ppm.) Zn (0, 0.5, 1, 2, 3, and 4 ppm.) Na and K (2, 4, 6, and 8 ppm). The blank, control and samples were run, respectively. The content of each mineral was calculated using the following formula:-

$$\text{Metal content (mg/100g)} = \frac{[(Cs-Cb)xV]}{[10xW]}$$

Where, C_s: concentration of sample in ppm

C_b: concentration of blank in ppm

V : volume (ml) of extract

W: weight (g) of samples

3.5.2. Determination of potassium

Analysis of potassium was done using flame photometer method. Two g of the sample was weighed on a filter paper and the filter paper was fold up and transferred into a 250 ml conical flask. 20 ml of diluted nitric acid was added and boiled gently for about 10 minutes and cooled to room temperature. The digested solution was filtered through a filter paper into a 100 ml volumetric flask, then the conical flask and the filter paper were washed three times each with 10 ml deionized water and the solution was made up to 100 ml and mixed properly (solution A).

Blank was prepared in the same way as stated above excluding the sample (solution B). 5 ml of solution A & B were pipetted into a 100 ml graduated flask, make up to the mark, and mixed (solution C and D).The flame photometer calibrated with 3.15 ppm. of potassium solution which

gives 100 absorbance using potassium filter and the obtained values was Correct for the zero concentration standard the solutions C & D were finally Measured to determine the potassium content and the following formula was primarily used to calculate its value.

$$\text{Potassium content (mg/100 g)} = \frac{(C-D) \times 2000}{W}$$

Where, W = Weight (g) of sample

C & D = [0.033 x A]/10

A = Absorbance

3.5.3. Determination of Phosphorus

Official method of 206.18 of AOAC (1984) was used to analyze the phosphorus content spectrophotometrically. The total ash of cactus pear seed flour was dissolved and digested with 20% HCl which is free from iron and it was washed continuously with deionized water into 100 ml volumetric flask. One ml of clear extract was taken and it diluted to 100 ml with deionized water. Then after adding 0.5 ml molybdate into test tubes containing 5 ml duplicate of the sample dilutions and the solution was vortex, and 0.2 ml of Aminonaphtholesulphonic acid was added and mixed. At this step a standard solution was prepared simultaneously with sample solution preparation by adding 5 ml duplicate of the series standards in test tubes and it was stand for about 10 minutes. Finally, the absorbance of standard, blank and samples was read at 660 nm and after the curve absorbance vs concentration accurately plotted the slope of the curve was immediately found. The following formula was used to calculate the phosphorus content:-

$$\text{Phosphorus content (mg/100g)} = \frac{(As-Ab)\text{x dilution factor x extracted volume x } 100}{\text{Slope x weight of sample x } 1000}$$

Where, A_s= absorbance of sample

A_b= absorbance of blank

Slope = obtaining from the calibration curve.

3.6. Anti-nutritional factors

3.6.1. Phytate content

The phytate contents of cactus pear seed flour was determined according to the method stipulated by Wheeler and Ferrel (1971). 0.03g of seed flour sample was extracted with 10 ml 0.2 N of HCI for 1 hour at ambient temperature. After the mixture centrifuged at 3000 rpm for 30 minutes, 3 ml of the supernatant was mixed with 2ml of wade reagent (The mixture of 0.03% $FeCl_3.6H_2O$ and 0.3% sulfosulcilic acid diluted in distilled water) and the mixture was shocked by vortex mixer. The absorbance which is read by 500nm was measured using UV-VIS spectrophotometer and the phytate content was calculated from the difference between the blank (3ml of 0.2N HCI and 2 ml of wade reagent) and that of assayed sample. The concentration of phytate was calculated using phytic acid standard curve (5, 9, 18, 27 and 36 ppm) and results were expressed as milligram of phytic acid per 100g dry weight. The following formula was used to calculate the phytic acid content of the samples.

$$\text{Phytic acid (mg/100g)} = \frac{[(Absorbance\ of\ blank - absorbance\ of\ sample) - Intercept]}{\text{slop x Weight of sample x3}} \text{x10}$$

3.6.2. Tannin content

Tannin content of cactus pear seed flour was analyzed using the method of Burns (1971) which is modified by Maxson and Roony (1972). About 1g cactus pear seed flour was weighed and 10 ml of 1% HCI in methanol was added to each test tube containing the sample. After that, the test tubes were shacked for about 24 hours at room temperature using mechanical shaker. The shacked sample was then centrifuged at 1000 x g for 5 minutes. 1 ml of the clear supernatant was diluted with 3ml of 1% HCI and 1ml of the diluted solution was mixed with 5 ml vanillin–HCI reagent in another test tube. The mixture was then allowed to wait at a dark place for about 20 minutes so as to complete the reaction. 20 minutes later the absorbance was read at 500nm using UV-VIS spectrophotometer. The concentration of tannin was actually calculated using the D-catechin standard curve (0.12, 0.24, 0.36, 0.48 and 0.6 mg/ml). So that the result was expressed as catechin equivalent of tannin in milligram per 100g in dry weight base. The following formula was used to calculate the tannin content of the sample:-

$$\text{Tannin}\left(\frac{mg}{100g}\right) = \frac{[(\text{Absorbance 0f sample} - \text{Absorbance of blank}) - \text{Intercept}]x4}{\text{Slope x Density x Weight of sample}}$$

3.6.3. Oxalate content

Oxalate content of the cactus pear seed flour was determined using the method of Iwuoha and Kalu (1994). The procedure involves three steps digestion, precipitation and permanganate titration.

Digestion: Digestion: At this step, 2 g (db) of flour was suspended in 190 ml of distilled water contained in a 250-ml volumetric flask; 10 ml of 6M HCl was added and the suspension digested at 100°C for 1 hour, followed by cooling, and then made up to 250 ml before filtration.

Oxalate precipitation: Duplicate portions of 125 ml of the filtrate were measured into a beaker and four drops of methyl red indicator added, followed by the addition of concentrated NH_40H solution (drop wise) until the test solution changed from its salmon pink color to a faint yellow color. Each portion was then heated to 90^0C, cooled and filtered to remove precipitate containing ferrous ion. The filtrate was again heated to 90^0C and 10 ml of 5% $CaCl_2$ solution was added while being stirred constantly. After heating, it was cooled and left overnight at 5^0C. The solution was then centrifuged at a speed of 2500 rev/min for 5 min. The supernatant was decanted and the precipitate completely dissolved in 10 ml of 20% (v/v) H_2SO_4, solution.

Permanganate titration: At this point, the total filtrate resulting from digestion of 2 g of flour was made up to 300 ml. Aliquots of 125 ml of the filtrate were heated until near-boiling, and then titrated against 0.5M standardized $KMnO_4$, solution to a faint pink color which persisted for 30 second. The calcium oxalate content was calculated using the formula.

$$\text{Oxalate (mg/100g)} = \frac{T\times(Vme)(DF)\times 105}{(ME)\times wf}$$

Where T = the titre of $KMnO_4$ (ml) *Vme* = the volume - mass equivalent (i.e. that 1 cm^3 of 0.05 M KMnO, solution is equivalent to 0.00225 g anhydrous oxalic acid)

DF = the dilution factor *VTA (2.4,* where *VT* is the total volume of filtrate (300ml)

A = the aliquot used (125 ml)),

ME = the molar equivalent of $KMnO_4$ in oxalate ($KMnO_4$, redox reaction. (5)) and

wf = the mass of seed flour used.

3.7. Determination of molar ratio of phytae/mineral

The mole of phytate and minerals was determined by dividing the weight of phytate and minerals with its atomic weight (phytate: 660g/mol; Ca: 40 g/mol; Fe: 56g/mol; Zn: 65g/ mol). The molar ratio between phytate and mineral was obtained after dividing the mole of phytate with the mole of minerals (Morris and Ellis, 1989).

3.8. Antioxidant compound and antioxidant capacity of cactus pear seed flour

3.8.1. Phenolic compounds

3.8.1.1. Sample extraction

Seed flour was extracted in accordance to the method of Mau *et al*. (2004). From the beginning, 2.5 g of cactus pear seed flour (duplicate) was weighed, mixed with 25 ml of methanol and put in incubator shaker at 25^0C overnight. The supernatant was decanted in to another conical flask and the extraction process was immediately repeated once again for about 2 hours. The supernatant solution was then poured in to a weighed rotary evaporator flask for the sake of evaporation using rotary evaporator at a speed 300 revolution per minute and at 40^0C. After the complete evaporation of the flask containing an extract was put in to drying oven at 70 ^{0}C for further elimination of some vapor and droplet of methanol. The dried flask was weighed again and the net difference in weight was used to remark how much methanol is going to be needed to mix with the extract which is in fact required for the analysis purpose.

3.8.1.2. Determination of total phenolic contents

The total content of phenol compound was determined calorimetrically, based on procedures described by (Singleton and Rossi, 1965). The sample extraction method was the same for total phenolic and antioxidant activity determination. One ml of cactus seed extracts or Gallic acid standard solutions was mixed with 1ml of folin-ciocalteu reagent in each test tube, followed by Swire and incubated for 3 min at room temperature. After 3 minutes, 1ml of saturated Na_2CO_3 solutions was added and adjusted the solution to 10 ml with distilled water or (7ml distilled water added), mix and incubate at room temperature. The solution was allowed to keep in the dark place for 90 minutes. Finally the absorbance was read at 725nm using UV-VIS spectrophotometer. The

concentration of total phenolic was actually determined using the standard calibration curve of gallic acid at a linearity range of 20-160 μg/ml of the curve and values were expressed as milligrams of gallic acid equivalents (mg of GAE/g of dried extract) using galic acid standard curve.

The calibration curve for galic acid was calculated according to the following formula

$$Y= mx + b$$

Where, Y= Absorbance (nm)

a = slop of galic acid

b = intercept

X = concentration

3.8.2. Flavonoid compounds

3.8.2.1. Sample extraction

Sample extraction was done according to the method described by Saura-Calixto *et al.* (2007). Briefly 0.4g of dried sample was mixed with 12 ml of acidified methanol water solution (50:50 v/v.pH 2) and extracted for 3 hours. The mixture was centrifuged at 2500g for 10 minutes and the supernatant was transferred to another test tube. To the residue 12 ml of aceton water (70:30 v/v) was added and extracted for another 3 hours, centrifugation takes place and the supernatant was mixed with the first extract and stored at 4^0C until analysis.

3.8.2.2. Determination of total flavonoid content

Total flavonoid content was determined using a colorimetric method described previously (Heimler et al., 2005). Briefly, a dose of 0.25 ml of the seed extract or (+)-catechin standard solution was mixed with 1.25 ml of distilled water in a test tube, followed by adding 75μL of a 5% $NaNO_2$ solution. After 6 min, 150μL of a 10% $AlCl_3.6H_2O$ solution was added and allowed to stand for another 5 min before adding 0.5 ml of 1 M NaOH. The mixture was brought to 2.5 ml

with distilled water and mixed well. The absorbance was measured at 510 nm using a UV-Visible Spectrophotometer (UV 160, Shimadzu, Japan). The total phenolic content was determining using the standard calibration curve of (+)-catechin at a linearity range of 100-1000 μg/ml of the curve and values were expressed as milligrams of (+)-catechin equivalents (mg of CAE/g of dried extract)

The calibration curve for catechin was calculated according to the following formula

$$Y = mx + b$$

Where, Y= Absorbance (nm)

a = slop of catechin

b = intercept

x = concentration

The percentage yield extracts were calculated as:

$$\text{Yield (\%)} = \frac{W1}{W2} \text{X100}$$

Where, W_1 = Weight of extract after solvent evaporation, W_2 = Weight of the cactus seed flour

3.8.3. Determination of anti-oxidant activity

The anti-radical DPPH of the seed extract was determined using the method of Kirby Schemidt, (1997). The sample extract that was used for total phenol analysis as described above (3.8.1.1) was again employed here in determining anti-oxidant capacity. Four ml of 0.004% solution of DPPH radical solution in methanol was mixed with 1 ml of various concentrations (20-240 µl/ml) of the extract in methanol and was mixed using a vortex mixer. The test tube containing the sample solution was shacked using a vortex mixer and incubate them in a dark place for 30 minute at a room temperature. Scavenging capacity was finally read spectrophotometrically by monitoring the decrease in absorbance at 517nm using U-V and ascorbic acid was used as standard.

The inhibition capacity of free radical DPPH in percent (I%) was calculated as follows:-

$$I\% = \frac{A\,c - A\,s}{Ac} \times 100$$

Where, I= Inhibition capacity

Ac = Absorbance of a control (blank)

As = Absorbance of a sample

The extract concentration providing 50% of radicals scavenging activity (EC_{50}) was calculated from the graph of DPPH inhibition percentage against extract concentration.

3.9. Functional properties of cactus pear seed flour

3.9.1. Bulk density

The bulk density of cactus pear seed flour was determined using the method of Narayana and Narasinga-Rao (1984). A 10g of sample flour was weighed and continuously tapped in a measuring cylinder until a constant volume is attained. The bulk density was determined from the mass of the flour and the volume it occupied in the measuring cylinder.

$$\text{Bulk density} = \frac{mass\ of\ sample}{volume\ of\ sample}$$

3.9.2. Water absorption capacity (WAC)

WAC was determined with the method reported by Sosulski (1987). A 10.5 ml of distilled water was added to a sample of 1.25 g flour (W_1) in a weighed centrifuge tube (W_2) and stirred six times for one minute at 10 minute intervals. The mixture was centrifuged at 3000 rpm for 25 minute and the clear supernatant was decanted and used for the purpose of determining water solubility index(WSI).pellets were dried at 50^0C for 25 minutes. The adhering drops of water were removed and then reweighed (W3). The amount of water retained in the sample was recorded as weighed gain and was taken as water absorbed. Water absorption capacity was expressed as the weight of water bound by 100 g dried four.

$$\text{Water absorption capacity (g\g)} = \frac{W3-(W1+W2)}{W1} \text{ X } 100$$

Where, W_1 = Weight of a sample, W_2 = weight of a centrifuge tube, W_3 = the weight of sample after partially drying.

3.9.3. Oil absorption capacity (OAC)

The method used by Adeleke *et al*. (2010) was used for oil absorption capacity. Ten ml (V_1) of refined soya bean oil was added to 1g of flour in a 15 ml centrifuge tube. The centrifuge tube containing the sample was stirred for 2 minutes and subsequently centrifuged at 4000 rpm for 20 minutes. The amount of oil separated as supernatant decanted and measured using 10 ml cylinder (V_2). The difference in volume was taken as the oil absorbed by the sample. Oil absorption capacity was expressed as ml of oil bound by 100g dried flour.

$$\text{Oil (fat) absorption capacity (ml)} = (V_1\text{-}V_2)$$

Where, V1 = volume of refined oil (ml), V2 = volume of supernatant

3.9.4. Water solubility index (WSI)

The supernatant preserved from WAC measurement was evaporated at 105^0C for overnight**.** The WSI was calculated as a ratio of dry residue to the original mass (1.25g) which was used to estimate WAI and the result was expressed as percentage (Sosulski, 1987).

$$\text{WSI} = \frac{Wr}{WO} \text{ x } 100$$

Where, Wr = Weight of dry residue, Wo = weight of original mass (sample)

3.9.5. Foaming capacity and stability

The foam capacity was determined using the method of Mittal and Kumar (2000). The flour (2g) was suspended in distilled water (100ml) and stirred at room temperature for 5 minutes using a magnetic stirrer at 1000 rpm speed. The content along with the foam was critically observed and immediately recorded. The volume of foam (ml) after stirring was expressed as the foam capacity and the volume after 60 minute as foam stability.

3.10. Product formulation and sensory evaluation of cactus pear seed

Formulation of product was takes place between wheat flour; a cereal which is served as a common food sources in many countries of the world including Ethiopian and cactus pear seed flour. In order to evaluate the dynamic change in sensory quality of the product, six different formulation ratios that contain cactus pear seed flour with the range between 0 to 25% and wheat flour were prepared. To each formulation an equal amount of salt and backing yeast was added for the sake of baking bread. The dough of each formulation was allowed to stay until floating is started and such procedure is actually used for preparation of the local bread 'Ambasha'. The bread was then evaluated for its sensory attributes like color, taste, aroma, texture and over all acceptability by ten Food Science and Nutrition students who have taken the course of sensory evaluation of food. Seven hedonic scales were used to remark the sensory level of each attributes (Saenz *et al.,* 2002).

3.11. Data analysis

Sample analysis was conducted in triplicate for proximate analysis, ant nutritional factors and for anti-oxidant compound and activity and in duplicate for minerals and functional properties. The data were analyzed using SPSS (version16) statistical software package and results were expressed in mean±standard deviations of the replicate determinations. One way analysis of variance (ANOVA) was used to examine the significant differences among the samples with respect to the studied parameters. Duncan at $p< 0.05$ was used to determine which means were significantly different.

4. RESULTS

4.1. Proximate composition analysis

Table1. Proximate composition and caloric value of cactus pear seed flour (g/100g) (db).

Parameters	Contents
Moisture	4.17 ± 0.00
Crude protein	10.00 ± 0.17
Crude fat	10.50 ± 0.50
Crude fiber	18.23 ± 0.00
Total Ash	1.63 ± 00
Carbohydrate	55.47 ±0.44
Total energy	392.84 ± 2.15

∗Values are represented as mean ± SD. of triplicate analysis.

4.2. Minerals analysis

The macro-and micro-minerals of cactus pear seed flour is given in Table 2.

4.3. Anti-nutritional factors

The amount of anti-nutritional factors particularly phytate, tannin and oxalate in cactus pear seed flour is analyzed and presented in Table 2.

Table. 2. Mineral contents and Anti nutritional factors of cactus pear seed (mg/100g, dwb)

Components	Values
Ca	390.14 ± 0.01
K	446.46 ± 0.01
P	206.18 ± 0.03
Fe	4.37 ± 0.00
Zn	2.01 ± 0.01
Phytate	259.20±3.700
Tannin	0.13 ± 0.004
Oxalate	0.11 ± 0.09

∗Values are represented as means ± SD. in duplicate determinations.

4.4. Molar ratio of phyatae to minerals (Ca, Fe and Zn)

Table 3 shows values for the molar ratio between phytate and divalent cations, Ca, Fe, and Zn.

Table 3. Molar ratio between phytate and minerals of cactus pear seed

Molar ratio	Contents
Phytate/Ca	0.04 ± 0.00
Phytate/Fe	4.99 ± 0.05
Phytate/Zn	12.78 ± 0.43

∗Values are represented as mean±SD (n=2).

4.5. Determination of anti-oxidant compound and anti-oxidant capacity

4.5.1. Extraction yield and Anti-oxidant compounds

Two anti-oxidant components; total phenol and total flavonoid were analyzed in this study and the amount of both components and extract yield in cactus pear seeds are presented in Table 4.

Table 4: Percent yield extracts, total phenols and flavonoids content of cactus pear seed flour.

Anti-oxidant compounds	Means ± SD	Yield (g/100g)
TPC (mg of GAE/100g of extract)	90.2 ± 5.37	7.4
TFC (mg of CAE/100g of extract)	0.19 ± 0.02	5.8

∗Values are represented as means ± SD., n=3

TPC = Total Phenol Content, TFC = Total Flavonoid Content

4.5.2. Anti-oxidant activity

Cactus pear seed flour was tested for its anti-oxidant capacity using DPPH and the result of the analysis shows that the sample and ascorbic acid standard oxidation inhibition capacity ranging from 43% to 95% and 20% to 94.86%, respectively. The IC_{50} of the sample is 1.32 mg/ml and ascorbic acid is 2.4mg/ml. The co-relational relationship between cactus pear seed and ascorbic acid standard in regard to their anti-oxidant capacity is depicted in Figure 4.

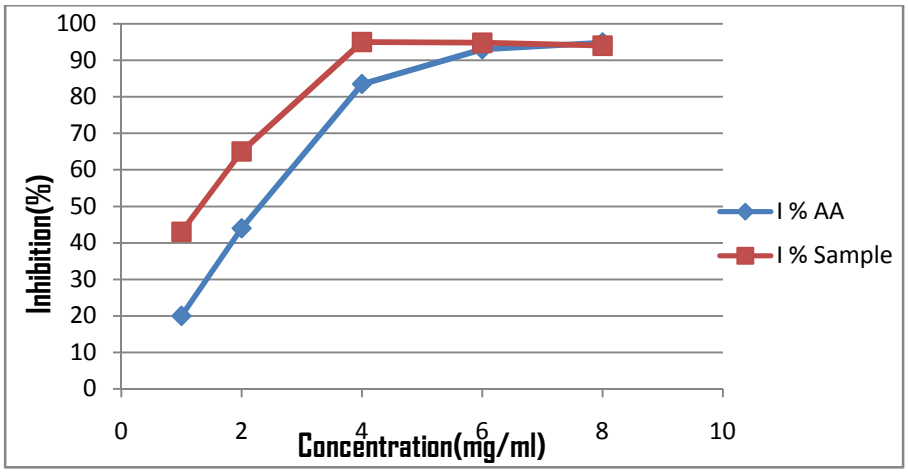

Figure 4. Antioxidant activity of cactus pear seed and standard ascorbic acid

4.6. Functional properties of cactus pear seed flour

Bulk density, Water absorption capacity, Oil absorption capacity, Water solubility index, Foaming capacity and stability were analyzed in this study so as to evaluate the functional properties of cactus pear seed flour. Accordingly, high amount of water solubility index (5.6g/100g) and low bulk density (0.80g/ml) were recorded (Table 5).

Table 5. Functional properties of cactus pear seed flour.

Properties	Values
Bulk density(g/ml)	0.80 ± 0.00
Water absorption capacity (%)	1.64.00 ± 0.1
Oil absorption capacity (ml)	1.45 ± 0.07
Water solubility index (%)	5.6 ± 0.00
Foaming capacity (ml)	4.75 ± 0.35
Foaming stability (ml)	3.75 ± 0.35

∗Values are presented as mean ± SD. (n=2)

4.7. Evaluation of sensory attributes of cactus pear seed flour supplemented Ambasha.

In the present study the sensory characteristics of cactus pear seed particularly its color, taste, aroma, texture and over all acceptability were evaluated. Thus, the results from Table 6 show that there was significant difference ($p<0.05$) in the consumers' preferences among the attributes of each formulation. It was also noticed that the control (only wheat bread) was the most preferable in terms of its color, taste, aroma, texture and overall acceptability as compared with the flour formulations. In contrast, the formulation ratio of 75:25 scored the least sensory attributes than the other samples. With regard to the overall acceptability, there was no significant difference ($p < 0.05$) among the samples (100:0%, 95:5% and 90:10%), but these ratios differed significantly ($p > 0.05$) from the remaining samples (85:15%, 80:20% and 75:25%). The overall acceptability of the control sample scored a value of 8.3 and subsequently decreased (4.3) as the proportion of the cactus pear seed flour increases in the formulation

Table 6. Sensory attributes of cactus pear seed and wheat flour Ambasha.

	Sensory attributes				
Flour ratios	Color	Taste	Aroma	Texture	Overall Accep.
100:0% (control)	8.6±0.97^{a}	8.1±1.45^{a}	8.1±0.88^{a}	8.3±1.25^{a}	8.3±0.94^{a}
95:5%	7.7±2.4^{ab}	7.3±2.01^{ab}	8.1±0.88^{a}	6.6±2.59^{b}	7.1±2.37^{a}
90:10%	7.1±1.52^{b}	7.5±1.96^{ab}	7.9±0.74^{ab}	6.7±1.40^{b}	7.3±2.00^{a}
85:15%	5.8±2.42^{c}	6.5±2.17^{ab}	7±1.33^{bc}	6±2.7b^{c}	5.5±2.79^{b}
80:20%	4.4±2.22^{d}	5.8±1.93^{bc}	7.1±1.44^{abc}	5.3±2.45^{cd}	4.9±1.05^{b}
75:25%	3.6±2.22^{d}	4.6±1.78^{c}	6.7±1.42^{c}	4.4±2.22^{d}	4.3±2.49^{b}

∗Values are represented as mean±SD.

∗Means followed by the same superscript letter in the same column are not significantly different ($p < 0.05$)

Moreover, the sensory results implies that there was virtually similar maximum (8.23 ± 0.11) and minimum (4.43 ± 0.15) values of all evaluated organoleptic characteristics except aroma which shows narrow difference (8.1-6.7) between the control and 75:25 formulation ratio.

A **B** **C**

Figure 5. Representative samples of Ambasha formulated from cactus seed and wheat flours with a ratio of 100% wheat (A), 5:95 % (B) and 10:90% (C)

5. DISCUSSION

To the best of author's knowledge, there were no published studies on the nutritional composition and other properties of cactus pear seed grown in Ethiopia although it was intensively shown in other parts of the world. In Ethiopia, most of the previous studies were largely emphasized on the pulp and cladode part of the cactus pear while the cactus pear seed has been neglected. The present study has attempted to evaluate the major nutritional composition of cactus pear seed particularly macro and micro nutrients, anti-nutritional factors, antioxidant activity, functional properties and its overall sensory attributes.

It has been reported that cactus pear seeds can be used as a source of food since it has high content of protein, fiber, lipids, minerals and carbohydrates (Joubert, 1993). Of these nutritional components, it has been confirmed in this study that Cactus pear seed predominantly contains highest amount of carbohydrate followed by fiber and this is in agreement with the studies conducted by Nassar (2008). These dietary fibers are important components which may help to prevent a variety of diseases (Roehrig, 1988). Soluble fiber produces viscous solutions that lower the absorption and the clearing of the small intestine, delays the increment of glucose and cholesterol in the blood and induces the intestinal transit time (Dulce, 2014). The present study also showed that cactus pear seed has a fat content (10.50g/100g) which is in harmonious with the findings (10.43g/100g) reported by Nassar (2008). The high content of fat in seeds means a potential source of oil (Piga, 2004). The protein and ash content is higher as compared with the result found by Özcan and Al Juhaimi (2011). On the other side the fat and protein contents obtained in the present study were highly greater than those values reported by Salim *et al*. (2009). The difference in results between the present study and other literature sources may be due to the variations in growth conditions, varieties, genetic factors, harvesting time, soil properties of cactus pear plants.

With regard to the macro and micro elements of cactus pear seeds, appreciable amounts of each mineral were obtained in this study as compared to other literature sources (El-Safy, 2012 and El-kossori *et al*., 1998). In the present study potassium content was very high (446.46mg/100g) where as the Zink content was relatively low (2.01 mg/100g) as compared with the other minerals and this was in good agreement with the findings of El-Safy (2012) and El-kossori *et al*. (1998). The

differences in minerals content reported by various studies could be attributed to the location of plants, the application of fertilizers and irrigation use, climate; and genetic differences between the varieties (Muñoz *et al*., 1995).

Ant-nutritional factors are generally toxic and may negatively affect the nutritional value of cactus pear seeds by impairing protein digestibility and mineral availability. Three ant-nutritional factors namely phytate, tannin and oxalate were examined in this study (Table 2). The results showed that phytate content was considerably higher (259 mg/100g) than the other factors which was similarly reported by El-Safy (2012) while the oxalate content was lower than the two ant-nutritional factors. The presence of appreciable amounts of condensed tannin compounds are of great importance in the health promotion like the antioxidant components (Kunyanga *et al*., 2014). The anti nutritional factors obtained in this study can be minimized or eliminated using some processing methods such as soaking (*El-Safy et al., 2012*), cooking (Ndidi *et al*., 2014) and fermentation (Wyss *et al*., 1998).

The molar ratio between phytate and minerals indicates the impact on the bioavailability of dietary minerals. The critical molar ratio, above which mineral absorption may be inhibited, has been determined as PA:Ca >1.56, PA:Fe >14 and PA:Zn >10 (Saha *et al*., 1994) and in this study these limits were employed to predict the bioavailabity of minerals. Accordingly, the values pointed out in Table 2 revealed that the molar ratio of both phytate:Ca (0.04) and phytate:Fe (4.99) was found to be below the critical limit. This implies that the bioavailability of calcium and Iron is not inhibited by the concentration of phytate present in the cactus pear seed flour. However, the molar ratio of phytate to Zn exhibited a high ratio (12.78) which was beyond the stated critical limit. Thus, such value indicates that the bioavailability of Zn in the seeds is inhibited by phytate. Therefore, the risk of Zn inhibition in this regard requires a preferable mode for minimization of the concentration of phytate in the cactus pear seeds.

Antioxidant compound and antioxidant activity of natural plants are more prominent with their functional properties that contribute an indispensable role on human health. Thus, cactus pear seed as a crop plant needs to investigate its antioxidant compound and antioxidant activity. In the present study, the anti-oxidant compound particularly total phenol and total flavonoid (Table 4) were account 90.2 mg/100g and 0.19mg/100g, respectively. Meanwhile, the results of this study

showed that total phenol content was comparable with the findings (48-89 mg/100g) suggested by (El-Mostafa *et al*., 2014). However, it was not similar with the values reported by Tlili (2011) and Chougui *et al*. (2013) whose report accounts about 268 mg/100g and 74 mg GAE/100 g respectively. Such differences could probably be created due to the variation in extraction time, incubation period, types of standard and reading wavelength among literatures. The climatic conditions also have a remarkable effect on the production of total phenol content. Indeed, summer crop displayed much higher polyphenol content than those collected in November and a rise in total phenolic content was generally found in plants grown in sunny situations relative to shady ones. It also seems that rainfall scarcity and long light exposure may be involved in the activation of phenol biosynthesis (Naczk and Shahidi, 2006). Concerning to the total flavonoid content, the values obtained in this study was in line with the result recorded by Chougui *et al*. (2013). However, differences in total flavonoids may be created due to the variation in geographical origin of the fruits, degree of maturity, extraction protocols and analytic assays.

It has been recognized that total phenol content (TPC) of plant extract is associated with their antioxidant activity due to their redox properties. In the present study, the antioxidant activity of cactus pear seed was analyzed (Figure 4). Hence, the minimum and maximum capacity of cactus pear seed for scavenging free radicals was ranged between 43%-95%. It was also noticed from the same figure that the percentage inhibition activity was greater than the ascorbic acid standard (20%-94.86%). The extract concentration providing 50% of radicals scavenging activity (IC_{50}) was calculated from the graph of DPPH inhibition percentage against the extract concentration. The lower the IC_{50} value, the higher is the scavenging potential. The concentration of the cactus seed flour extract required for the formation of IC_{50} was more likely closer to the ascorbic acid standards but lower than the results recorded by Toure.*et al*. (2015). The differences in antioxidant activity might be associated with the levels of phenolic compounds since the influence of an extract phenolic composition in the antioxidant capacity is a well-known fact (Lien *et al*., 1999).

Functional properties contribute an important role in determining the competitiveness of ingredients or products in the market, as they can impact the sensory, physical and chemical properties of a food. Some representative attributes such as bulk density, water/oil absorption capacity, foam capacity and stability, and water solubility index were analyzed to evaluate the

functional properties of cactus pear seed flour (Table 5). It was observed from the table that the bulk density which influences the amount and strength of packaging materials, energy density, texture, and mouth feel (Udensi, 2006) was lower as compared to the other properties. All investigated properties except bulk density were lower than the results reported by El-Safy *et al.* (2012). It was also noticed that the water and oil absorption capacity of cactus pear seed was comparable to each other and this is in line with the findings of El-Safy *et al.* (2012) which was Conducted on cactus pear cladode. The similarity in water and oil absorption properties may be associated with the proportional balance of hydrophobic (López-Cervantes *et al*., 2011) and hydrophilic (Crawford *et al*., 2010) nature of the particles of the sample. However, the water solubility, foaming capacity and foaming stability obtained in this study were not in agreement with those reported by El-Safy *et al.* (2012). The functional attributes of products may vary considerably due to the differences in the raw material, processing, extraction methods and environmental conditions used during testing.

The sensory attributes of Ambasha which is composed of cactus pear seed and wheat flours at different proportional ratios were evaluated using 7-point hedonic scale by semi trained panelists of first year M.Sc. program students of Food Science and Nutrition. Results from Table 6 show the mean scores of panelists on each sensory attributes and it was noted that there was no significant difference ($p>0.05$) between 0% sample replacement (control) and 5% sample replacements in all sensory attributes except texture. Based on the sensory scores marked by the panelist, incorporation of cactus pear seed up to 15% was appeared to be acceptable with the approximate preference level of ”moderately” or “slightly like” on the given hedonic scales in all over the attributes. This was directly agreed with the idea of (Moreno-Álvarez *et al*., 2009) that was conducted on cactus pear cladode. From the same table, it was observed that the level of sensory value was decreased as the replacement ratio of cactus pear seed increased which is in accordance with the suggestions forwarded by Saenz (2000). Surprisingly, Aroma had the highest score values for all attributes and sample ratios, with a representative preference hedonic range of “like” to “moderately like” and this was in good agreement with previous studies (Moreno-Alvarez *et al.,* 2009). The appreciable aroma level might be due to the availability of the volatile organic matters perhaps the fatty or oily components of the cactus pear seeds.

6. CONCLUSION AND RECOMMENDATION

6.1. Conclusion

The present study demonstrated that cactus pear seeds can be used as food supplementation in arid and semiarid areas. It contains high amount of carbohydrate and fiber which is followed by lipid and protein. In addition, cactus pear seed has an appreciable content of potassium and calcium and low level of ant-nutritionals (tannin and oxalate) except phytate. The remarkable capacity of scavenging free radicals which resulted from high value of total phenol in the cactus pear seed is the promising aspects for health promotion role. Moderate levels of functional properties were exhibited in the cactus pear seed flour. Moreover, *Opunta ficus indica* seed flours can be consumed by formulating with wheat flours up to a limited ratio.

6.2. Recommendation

Based on this study, the following recommendations were forwarded:

- Consumers should get awareness about the nutritional advantage of cactus pear seeds.
- Fatty acid profiles of the cactus pear seed have to be studied.
- A study also needs to be conducted on processing methods that could be used to minimize the anti-nutritional factors that might affect the palatability of cactus pear seeds.
- The bioactive component of cactus pear seed that cause constipation has to be studied.
- Methods that would be used to extend the shelf life of cactus pear fruit should be assessed.
- Government should adopt and motivate small scale enterprises so as to engage on the production of new cactus pear seed products.

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APPENDICES

Appendix 1. Questionnaire for sensory evaluation of cactus pear seeds

Personal information

Code……..

Sex……

Date……..

Instruction

You have been provided with five coded samples. please indicate by ticking the level of response which best describes the attributes of the samples. So try to examine the samples and indicate how much you like or dislike based on its color, taste, aroma, texture and overall acceptability. Please take water after evaluating each sample to rinse your palates**.**

Attributes	**Level of responses**						
	Extremely dislike	Dislike	Slightly dislike	Neither like nor dislike	Slightly like	like	Extremely like
Color							
Taste							
Aroma							
Overallacceptabilty							

Comments

……

……

……

Appendix 2. Photos taken in the laboratory

(A). Total Phenol analysis

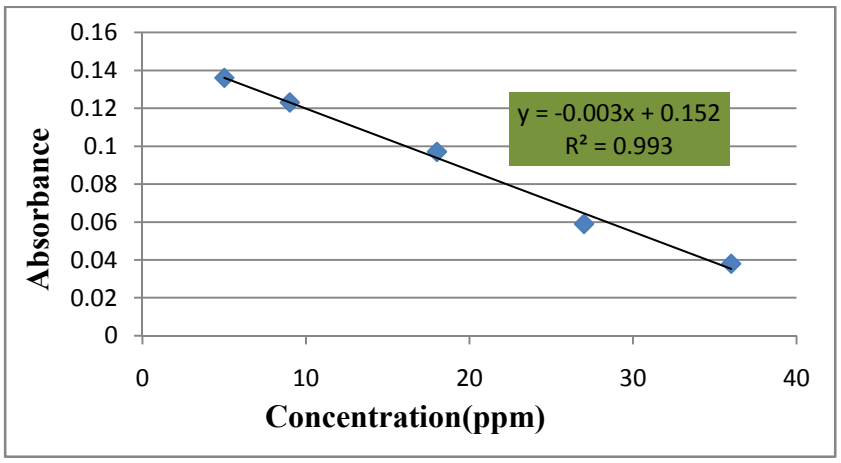
(B). UV-VIS.spectrometer reading

(C). Cactus pear seed

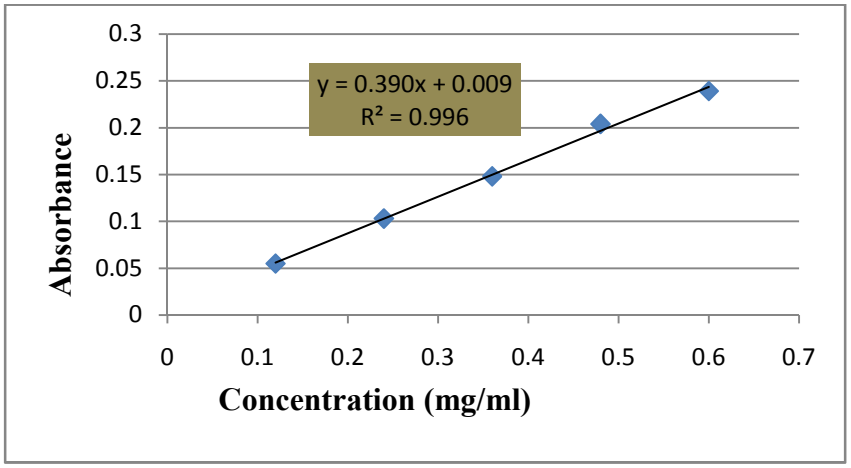
(D). Cactus pear seed flour

(E). Sensory evaluation of Ambasha with semi-trained panelists

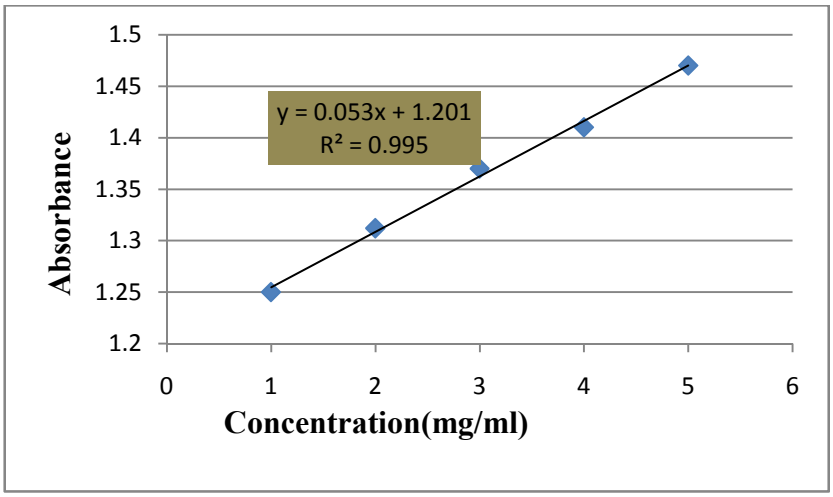
Appendix 3. Calibration curve for phytate



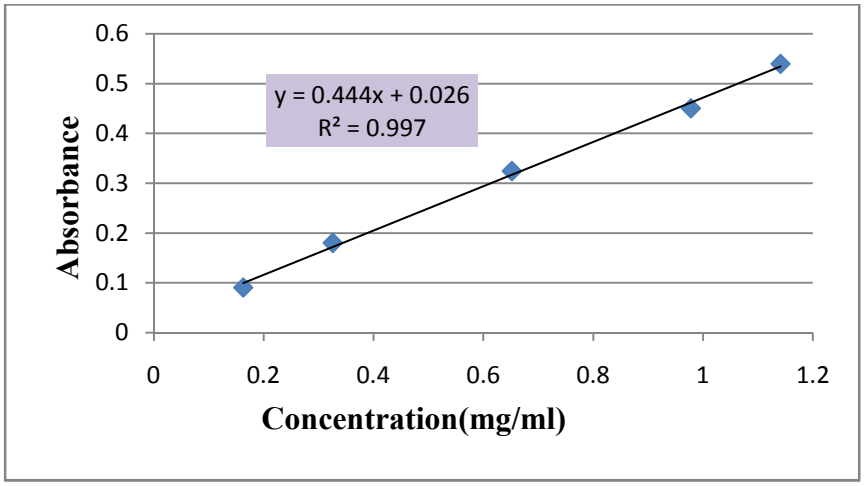
Appendix 4. Calibration curve for tannin



Appendix 5. Calibration curve for total phenol



Appendix 6. Calibration curve for total flavonoid



DECLARATION

I, the under signed, declare that this thesis is my original work and has not be presented for any degree in this or any other institution and that all sources of materials used in this thesis have been duly acknowledged.

Candidate: **Tewelde H/michael** **Signature**_____.

This thesis has been submitted for examination with my approval as a University advisor. In addition, I declare that this thesis is the original work of my student and has been done under m supervision.

Advisor: Mr. Kelbessa Urga (Asso.pro.) **Signature**_____

APPROVAL

This thesis has been approved by the examining board:

Name	**Signature**	**Date**
1. _____	_____	_____
2. _____	_____	_____
3. _____	_____	_____

Place of Submission: Addis Ababa University School of Graduated studies
Center for food Science and Nutrition
Addis Ababa, Ethiopia
June, 2016