CHANGES IN NUTRITIONAL COMPOSITION AND ANTI-NUTRITIONAL FACTORS OF SISNU IN HOT WATER AND STEAM BLANCHING AND COOKING METHOD

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Changes in Nutritional Composition and Anti-Nutritional Factors of Sisnu in Hot Water and Steam Blanching and Cooking Method

A dissertation submitted to the department of Nutrition and Dietetics, Central Campus of Technology, Tribhuvan University, in the partial fulfillment of the requirements for the degree of B.Sc. Nutrition & Dietetics.

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Approval Letter

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Abstract

Sisnu is an important traditional food item along with a high nutrient value. *Sisnu* is grown wild, due to which it has been consumed as an inexpensive source of nutrient in Developing countries, like Nepal. There is enough data to support the high nutrient quality of *sisnu*. However, their anti-nutritive values have not been explored, which are either potentially toxic or may limit the availability of nutrient. Different processing techniques are often utilized in order to reduce anti-nutritional factors. However, cooking is a common and easy form of processing in plants that are consumed as a food source. *Sisnu* (*U. plaviflora*), was brought from Bishnupaduka, Dharan-20, Sunsari district, Nepal and the changes in nutritional composition and anti-nutritional factors of sisnu in different household steam and hot water-based cooking practices was studied on the basis of loss of anti-nutrients and retention of vitamin C at different time of cooking.

The mean value of phytate, oxalate and vitamin C contents in the raw *Sisnu* were found to be 2.99, 1471.32 and 33.14 mg/100g (DM) respectively. Both steaming and boiling showed significant reduction ($p<0.05$) in Phytate, oxalate and vitamin C. There was significant reduction in oxalate content on both steaming for 5 and 15 min (1162.713 and 1051.514 mg/100g DM) respectively on dry basis. But boiling for 5 and 15 min showed higher reduction of oxalate (763.49 and 704.94 mg/100g respectively on dry basis). Boiling for 15 min showed reduction of phytates (2.18 mg/100g), oxalates (704.94 mg/100g DM) and vitamin C (11.4 mg/100g DM). Loss of vitamin C in boiling was higher than in steaming. Retention of vitamin C was found to be higher in steaming for 5 min (28.88 mg/100g DM) and 15 min (225.01 mg/100g DM) respectively on dry basis. Reduction of antinutrient was observed high on boiling for 15 min but retention of vitamin C was very low. Hence, steaming was considered as an effective method of cooking.

Contents

Approval Letter ..... **iii**
Acknowledgement ..... **iv**
Abstract ..... **v**
List of table ..... **ix**
List of figures ..... **x**
List of symbols and abbreviations ..... **xi**
Introduction **1**
1.1 Background 1
1.2 Statement of the problems 2
1.3 Objectives 4
1.3.1. General Objective 4
1.2.2. Specific Objective 4
1.3. Significance of the work 4
1.4. Limitation of study 5
Literature review **6**
2.1 Scientific classification of *sisnu* 6
2.2 Botanical description of sisnu 6
2.3 Some common species of nettle and their importances 8
2.3.1 Characters of some common species 8
2.3.2 Medicinal importance of *Sisnu* 10
2.3.3 Consumption pattern of *Sisnu* 12
2.3.4 Religious belief of nettle 12
2.4 Sisnu products 12
2.5 Blanching 12
2.5.1 Effect of blanching on foods 13
2.5.2 Methods of blanching 15
2.6.1 Boiling 17
2.6.2 Steaming 17
2.7 Ascorbic acid 18
2.7.1 Stability or retention of vitamin C 18
2.7.2 Losses of vitamin C 20
2.7.3 Physiological and biochemical functions of vitamin C 21
2.7.4 Effect of deficiency of vitamin C in humans 22
2.7.5 Side effect from over dosage of Vitamin C 22

2.7.6 Recommended daily allowances of ascorbic acid 22
2.7.7 Source of ascorbic acid in some fruits and vegetables 23
2.7.8 Occurrence of ascorbic acid in human body 24
2.8 Anti-nutritional factors 24
2.8.1 Oxalates 24
2.8.2 Phytic acid 31
2.9 Reduction of anti-nutrients 35
2.9.1 Reduction of phytate 37
2.9.2 Reduction of Oxalate 39
Materials and methods 40
3.1 Materials 40
3.1.1 Fresh *Sisnu* as raw material 40
3.1.2 Apparatus Required 40
3.1.3 Chemicals Required 41
3. 2 Methods 41
3.3 Methodology 43
3.4 Analytical procedures 43
3.4.1 Determination of moisture 43
3.4.2 Determination of total ash 43
3.4.3 Determination of crude protein 44
3.4.4 Determination of fat 44
3.4.5 Determination of crude fiber 44
3.4.6 Determination of total carbohydrate and energy value 44
3.4.7 Estimation of vitamin C 44
3.4.8 Determination of oxalate 44
3.4.9 Phytate content 45
3.5 Data Analysis 45
RESULTS AND DISCUSSION 46
4.1 Chemical composition of fresh sisnu (*Urtica plaviflora*) 46
4.2 Effect of cooking methods 46
4.2.1 Effect of heat treatments on the proximate composition of sisnu 47
4.2.2 Effect on the vitamin C content 49
4.2.3 Effect of cooking methods on anti-nutrients 51
4.2.4 Percentage loss of vitamin C, phytate and oxalate 54
Summary 59

References **61**
Appendices **79**
Appendix A 79
Appendix B 82
Appendix C 87

List of tables

Table No.	Title	Page No.
Table 2.1	Nutritional composition of nettle	10
Table 2.2	Time-temperature relationships for blanching of some vegetables	13
Table 2.3	RDA proposed by ICMR and FAO	22
Table 2.4	Ascorbic acid contents of some fruits and vegetable	23
Table 2.5	Oxalate content in some vegetables and beans	26
Table 2.6	Phytate content in plant derived human food	33
Table 4.1	Chemical composition of *Sisnu*	46
Table 4.2	Effect of cooking on proximate composition of *Sisnu*	47
Table 4.3	Percentage loss/reduction of phytate, oxalate and vitamin C	55
Table A.1	Mean value of phytate	79
Table A.2	Mean value of oxalate	79
Table A.3	Mean value of vitamin C	80
Table A.4	Mean value of protein	80
Table A.5	Mean value of fiber	80
Table A.6	Mean value of fat	81
Table A.7	Mean value of ash	81
Table A.8	Mean value of moisture	81

List of figures

Fig No.	Title	Page No.
Fig 2.1	An image of Urtica	7
Fig 2.2	Structure of oxalate	25
Fig 2.3	Structure of phytic acid	32
Fig 2.4	Experimental flow chart	43
Fig 4.1	Effect of cooking on vitamin C content	50
Fig 4.2	Effects of cooking on phytate content	51
Fig 4.3	Effects of cooking on oxalate content	53
Fig 5.1	Collection of fresh *Sisnu* sample	87
Fig 5.2	Steaming of sample	87
Fig 5.3	Lab work on progress	88

List of symbols and abbreviations

Symbols and abbreviation	Full Form
%	Percentage
µg	Microgram
^{o}C	Degree Celsius
ANOVA	Analysis of variance
BPH	Benign prostatic hyperplasia
CCT	Central campus of Technology
DM	Dry matter
et al.	Et alibi, and others
etc	Et cetera
FAO	Food and agricultural organization
Fe	Iron
FeCl3	Ferric chloride
Fig.	Figure
FW	Fresh weight
g	Gram
HW-5	Hot water immersion cooking for 5 min
HW-15	Hot water immersion cooking for 15 min
ICMR	Indian Council of Medical Research
Kcal	Kilo calorie
Kg	kilograms
KMnO4	potassium permanganate
M	Mole
MC	Moisture content
mg	Milligrams
Min.	Minute
ml	Milliliter
ppm	Parts per million

RDA	Recommended dietary allowance
S-5	Steam cooking for 5 min
S-15	Steam cooking for 15 min
Zn	Zinc

Part I

Introduction

1.1 Background

Stinging nettle (*Urtica dioica, L. Urticaceae*) is a ubiquitous herb which is available in large part of the world. *Urtica dioica* is a moderately shade-tolerant species, which occurs on moist or damp, weakly acid or weakly basic, fertile soils. Its stems and leaves are densely covered with stinging hairs, which release potentially pain-inducing toxins, is rarely eaten by castles and rabbits (Taylor, 2009). The plant *sisnu* (*Urtica plaviflora*) is evenly distributed in Himalayas especially in middle and lower zone between 450 to 3500 m from sea level (Watanabe et al., 2013). Stinging nettle (*Urtica plaviflora*) locally called *sisnu* is an important traditional food item along with an important medicinal plant (Panta and Sundriyal, 2016).

Stinging nettles (*U. urticoia and U. plaviflora*) have been also consumed in Iran and in certain parts of Europe and Africa. The tender leaves of young shoots are cooked and eaten as a vegetable or also mixed with cereals especially millet flour to make porridge in the households of the Hills and Himalaya regions in Nepal. It is known as a good source of protein, vitamins and minerals(Mishra, 2007).

Four species of *urtica* viz; *dioica, girardinia, plaviflora and trema* are used for vegetable purpose. All species are armed with stinging hairs on the leaves and stems, which on contact with the skin cause irritation and urtication or nettle-rash. The leaves of *Rumex nepalensis,* a herb usually found growing closely with *Rumex patientia* are rubbed over the affected parts for relief(Chopra, 1958). The genus name *Urtica* comes from the Latin verb *urere,* meaning 'to burn', because of these stinging hairs. The species name *dioica* means 'two houses' because the plant usually contains either male or female flowers. Nettle has a well-known reputation for giving a savage sting when the skin touches the hairs and bristles on the leaves and stems (Baytop, 1989).

In the last few years, *Urtica dioica* L., has been accepted as a healing plant because of its considerable effects on human health in many countries all over the world (Does *et al.,* 1999; Wetherilt, 1989). From ancient times, the fresh stinging nettle is used for flailing arthritic or paralytic limbs with fresh stinging nettle to stimulate circulation and bring warmth to joints and extremities in a treatment known as "urtication". The plant leaves has been reported to have important therapeutic properties such as anti-inflammatory, antioxidant, anti-rheumatic

and acute diuretic and hypotensive effect (Gulchin et al., 2007;Tahri *et al.*, 2000)cardiovascular effect and stimulation of proliferation of human lymphocytes (Wagner et al., 1989). Nettle can be used to foster health and vitality of the people. Due to the nutritional and functional qualities of nettle, it has been utilized to alleviate symptoms associated with allergic rhinitis and improve oxidative stability in brine anchovies. It is also rich in fatty acids, carotenoid, and phenolic compounds, while its extracts have been reported to improve oxidative stability in brined vegetables (Rutto *et al*., 2013). one of the most commonly used herbal remedies for Benign Prostatic Hyperplasia is nettle, which causes anti-inflammatory, anti-tumor, antiviral effects, modulating of immune system, and relieves the symptoms of benign prostatic hyperplasia due to the compounds phytosterols, lignans and polysaccharides (Mills and Bone, 2000).

Nutritionally, *sisnu* leaf and shoot are rich sources of minerals (especially iron, calcium and potassium), antioxidant and vitamins (such as vitamin C, carotenoids and vitamin E) (Bhattarai *et al*., 2017). Besides, it also possesses good quality protein and good source of fibres as compared to other leafy vegetables (Rafajlovska *et al*., 2013). Analysis of nettle powder showed significantly higher level of bioactive compounds: phenolic compounds as 129 mg Gallic acid equivalent/g; carotenoid level 3497μg/g; tannin 0.93 mg/100g; anti-oxidant activity 66.3 DPPH inhibition (%), as compared to wheat and barley. This study further established that nettle plants as very good source of energy, protein, high fiber, and a range of health benefitting bioactive compounds (Adhikari *et al*., 2015).

Effect of drying and fermentation on nutrient and organoleptic quality of sisnu was carried out by Adhikari *et al.* (2015). Likewise, effect of hot water blanching and cabinet drying on retention of vitamin C and some phytochemicals were also carried out by (Mishra, 2007). A significant reduction in anti-nutritional factors like oxalate, phytate and tanins after blanching of chaya leaf (Babalola and Alabi, 2015)and other vegetable (Fabbri and Crosby, 2016)has been reported. But the study related to effect of cooking on anti-nutritional factors of *sisnu* is limited. Hence, the study was aimed at studying the effect of household cooking methods on the nutritional quality of sisnu.

1.2 Statement of the problems

Studies made on sisnu had revealed that the amount of protein and minerals (iron, calcium, phosphorus) were six times higher than those of rape leaves and other leafy vegetables (Manandhar, 1989). It is consumed in Nepal as curry, soup and porridge. The importance of

sisnu relies not only as a traditional food item but also as an important medicinal plant. It is believed that plenty of mineral content might be the fact for its medicinal value. As reported by Manandhar (1989) sisnu is used to lower high blood pressure due to its high potassium content.

Inspite of nutritional and medicinal values, anti-nutritional factors such as oxalate and phytate are naturally present in it. These anti-nutritional compounds interfere with digestion, absorption and proper utilization of nutrients. Oxalate found in green leafy vegetables reduce the absorption of the calcium and other minerals and also contribute to kidney stone(Voss *et al*., 2006). Phytates are cationic salts of phytic acid (myo-inositol1, 2, 3, 4, 5, 6 hexakisphosphates) bound to minerals. These are 2natural chelators with negatively charged sites, bind polyvalent metal cat ions more strongly than the monovalent ones(Lott *et al*., 2000).Phytic acid is the major phosphorous storage compound in vegetables. Phytates reduce the bioavailability of metal ions like iron and zinc as well as affect protein and starch digestion in the body (Hurrell et al., 2003;Khokhar and Apenten, 2003).

In 2016, the number of undernourished people in the world increased to an estimated 815million from 777 million in 2015. Similarly, while the prevalence of undernourishment is projected to have increased to an estimated 11 percent in 2016, this is still well below the level of a decade ago. Nonetheless, the recent increase in malnutrition has caused for greater concern and posed a significant challenge for international commitments to end hunger by 2030 (FAO, 2017). In the last few years, food scientists have laid an emphasis on the effects of mineral nutrient deficiencies and it has become increasingly evident that the lack of minerals may have severe negative consequences on human health (Bouis, 2000). These deficiencies have major negative effects on human health, and development; working ability and quality of life (Shailen *et al.*, 2005; Swindale and Bilinsky, 2006; Welch and Graham, 2004; White and Brown, 2010). Although micronutrient requirement is very small, every one in three humans worldwide is not getting enough quantity, especially the poor, women and children (Gibson, 1994; Ramakrishna *et al*., 2006). A few cases such as iodine, zinc, iron and selenium micronutrient deficiencies can be attributed to particular geological conditions where the soils are low in these minerals (Coelho *et al.*, 2007; Raboy et al., 2001). Fortification and strategies for supplementation of food have proved to be unrealistic in several developing countries for economic reasons (Turner *et al*., 2002).

It has been shown that wet processing like soaking, germination and fermentation reduced phytic acid content and increased the solubility of nutrients (Bilyeu *et al.*, 2008; Cakmak *et al.*, 1999; Selle and Ravindran, 2008). Studies have shown that a long soaking period before fermentation or germination, leads to a reduction in phytate content and an enhancement of mineral bioavailability (Liang et al., 2008;; Nunes *et al.*, 2005; Paulik et al., 2005). Cooking is a common form of processing in plants that are consumed as a food source. Cooking causes changes in the phytochemistry of vegetables affecting its bioaccessability and health benefit properties (Odhav *et al*., 2007). But the study related to effect of cooking on anti-nutritional factors and retention of functional properties of *sisnu* shoots is still limited.

1.3 Objectives

1.3.1. General Objective

The general objective of this work is to study on the effect of various pretreatments and cooking time on the anti-nutritional factor and vitamin C contents of sisnu (*Urtica Plaviflora*).

1.2.2. Specific Objective

The specific objectives are as follows:

- To determine Proximate composition, phytate, oxalic acid and vitamin C content of fresh sisnu.
- To determine changes in proximate composition, phytate, oxalic acid and vitamin C contents in steamed and hot water based cooking.

1.3. Significance of the work

Sisnu is a traditional leafy vegetable of Nepal which grows wild. *Sisnu* is available at a cheap cost in Nepal, as it does not need to be cultivated. It is cheaper cost as well as has high therapeutic and nutrition values. In Nepal and few other countries, it has been used as a medicine for rheumatoid arthritis, back pain, fever, diarrhea, fatigue, hemorrhoids, hypertension, etc. Consumption rate of *sisnu* is high at hilly region of Nepal but it is still underutilized in urban areas. Recently, due to its functional value and good taste, its value and demand has emerged. As its consumption rate has been drastically increased, it is important to find out the possible household means to decrease the anti-nutrient content in it. As this study determines the anti-nutritional contents of raw sisnu and changes in nutritional

composition and antinutrient factor of hot water and steam blanching and cooking method; this study can provide the optimized process of reducing the antinutrient, so the nutrient in sisnu is available to the full potential. The outcome of the study can be helpful in effective optimization of the way of cooking of sisnu in household level and also provides the opportunities for the use of sisnu in various processed foods like incorporation of sisnu in biscuits, sarbottam flour, noodles, instant soup and as nutrient supplement without wasting the potential nutrient content.

1.4. Limitation of study

- Varieties of sisnu could not be included during the study.
- Only the limited cooking methods were adopted due to the time constraint.
- Combined salt and spices cooking treatment could not be done due to the lack of time period.

Part II

Literature review

2.1 Scientific classification of *sisnu*

Sisnu (*nettle bud*), a genus of annual or perennial herbs, commonly known as stinging nettle is a medicinal herb belonging to Urticaceae family (Mishra, 2007). Four common species of *Urtica* are used for vegetable purpose in Indian region viz. *dioica, girardiinia, plaviflora and trema.* Scientific classification of sisnu (*nettle bud*) (Urtica dioica) is shown in table 2.1.

Table 2.1 Systematic position of Urtica dioica

Kingdom: Plantae

Subkingdom: Tracheobionta (Vascular plants)

Superdivision: Spermatophyta (Seed plants)

Division: Magnoliophyta (Flowering plants)

Class: Magnoliopsida (Dicotyledons)

Subclass: Hamamelididae

Order: Urticales

Family: Urticaceae

Genus: *Urtica* L.

Species: *Urtica dioica* L.

Source: (Cronquist, 1981)

2.2 Botanical description of sisnu

Urtica dioica (stinging nettle) is the name given to common nettle, garden nettle and hybrids of these two plants. Originally from the colder regions of northern Europe and Asia, today this herbaceous shrub grows all over the world. Stinging nettle grows well in nitrogen-rich soil, blooms between June and September, and reaches nearly 3 feet high. The branching stems underground multiply by themselves and has multiple shoots. The leaves are heart-shaped, finely toothed, and tapered at the ends. The entire plant is covered with tiny stinging

hairs, mostly on the underside of the leaves and stem(Anonymous, 2015). The figure for sisnu is shown in figure 2.1 below:

Source: Wikipedia (2006)

Fig 2.1 An image of Urtica

Urtica dioica is a native British perennial growing in damp forests or wherever land has been disturbed by man. It has a richly-branched yellow rhizome, which spreads over large areas, and from which grow numerous erect, quadrangular stems. These are up to 120 cm tall and are covered with long stinging hairs and short bristly hairs. The opposite, stalked, cordate or lanceolate leaves are serrated at the margin and covered on both sides with stinging hairs. The flowers are unisexual, the plants dioecious, although monoecious ones do occur. The flowers are arranged in drooping panicles, growing in groups from the upper leaf axils. The male inflorescences are erect and shortly branched, with four perianth segments and four stamens. The female flowers have two perianth segments and a superior ovary with a stalk less stigma. The fruit is an achene. It consumes the phlegmatic superfluities in the body of man that the coldness and moisture of winter has left behind. It has a creeping root, sharp-pointed leaves

and greenish flowers. The irritant substance which causes the sting when the prickly haris are touched is a mixture of histamine and formic acid (Anon, 2015).

A detailed description of this familiar plant is hardly necessary, it's heart-shaped, finely toothed leaves tapering to a point, and its green flowers in long, branched clusters springing from the axils of the leaves are known to everyone. The flowers are incomplete: the male or barren flowers have stamens only, and the female or fertile flowers have only pistil or seed-producing organs. Sometimes, there different kinds of flowers are to be found on one plant; but usually a plant will bear either male or female flowers throughout, hence the specific name of the plant, dioica, which means 'two houses'. The male flower consists of a perianth of four greenish segments enclosing an equal number of stamens, which bend inwards in the bud stage, but when the flower unfolds spring backwards and outwards, the anthers with the sudden uncoiling, exploding and scattering the pollen. The flowers are thus adapted for wind-fertilization. The perianth of the female flower is similar, but contains a single, one-seeded carpel, bearing in style with a brush like stigma. The male flowers are in loose sprays or racemes, the female flowers are more densely clustered together (Anon,2015).

2.3 Some common species of nettle and their importance's

2.3.1 Characters of some common species

The species *dioica* (Hindi - *Bichhu booti*) is a dioeciously herb, up to 2 m high, with grooved stem abundantly armed with stinging hair, found in the Himalayas from Kashmir to Kumari at altitudes of 2100-3200 m. Leaves are ovate or calceolate, usually chordate, serrate; flowers are greenish, in axillary's cymes (Mishra, 2007). Stinging nettle, *U. dioica* is widespread and probably native throughout Europe and Asia from the arctic regions to the Mediterranean. Of the other members of the *U. dioica* group, *U. sondenii* occurs in northern Finland, Norway, Russia and Sweden; the non-stinging *U. galeopsifolia* is found in western, central and eastern Europe, and *U. pubescens*, confined to the Volga delta in Russia and lower Dnepr in the Ukraine. Worldwide *U. dioica* is alien in other temperate regions in North and south Africa, China, India, Australia, New Zealand and North and South America, but not found in tropics (Greig-Smith, 1948).

The species *Plaviflora* (Nepal- *sisnu,* Bengali- *Pharah-bichuti*) is a slender, sparingly branched perennial herbs up to 3 m tall and copiously armed with stiff stinging hairs, abundantly found in the temperate region of Himalayas from Kashmir to Sikkim, in Darjeeling in western Bengal, Mishmi hill in Arunanchal Pradesh, and in the Nilgiri hills in

the south of India. Stems obtusely angles; leaves ovate or ovate chordate or lanceolate; flowers small, monoecious, green clustered on lax axillaries cymes; achenes small with persistent sepals (Manandhar, 1989). The leaves of *plaviflora* with their hairs intact contain acetylcholine (318.4µg/g), histamine (38.8µg/g) and 5-hydroxy tryptamine (0.25µg/g). In addition, the presence and histamine liberating substance is strongly indicated in the extract and leaves (Saxena, 1965). A chromatographic study of the healthy and diseased stems of the host revealed the presence of malic acid and an undefined non- volatile carboxylic acid, glucose, fructose, sucrose, asparagines aspartic acid, serine, tyrosine and tryptophan. The concentration of amino acid and sugar was considered to be double or more in the infected parts than the healthy ones (Gupta, *et al.*,1967). The seeds of *plaviflora* contain about 10-12% of oil, which can be used for making soap and other oil based industrial products (Manandhar, 1989). The byproducts of the plant can also be used for paper making, blue and green shades can be obtained from thus plant, which can be used as dyeing materials (Bredemann, 1959).

Urtica urens are annual and not perennial. Stems are branched, 10.60 cm tall, sparsely puberulent and are somewhat densely armed with stinging hairs. Stipules are free, narrowly triangular, 1.2.5 mm, ciliate; petiole 1.2.5 cm, puberulent, sparsely armed with stinging haris; leaf blade broadly elliptic, sometimes ovate or obovate, 1.2.6 × 0.6.3 cm, 5-veined, often subglabrous except for sparse stinging hairs on both surfaces, base broadly cuneate or rounded, margin 6.11- dentate, apex obtuse-rounded; cystoliths punctiform, distinct adaxially. Inflorescences contain proximal female flowers and distal male flowers, spicate, 0.5-2.5 cm. Male flowers are short and pedicellate. Female flowers have; perianth lobes connate at base and have dorsal-ventral lobes ovate. Achene brownish gray, ovoid, compressed, 0.8 mm, verrucose, invested by persistent perianth lobes (Chen, 2003).

Urtica taiwaniana are perennial and monoecious with rhizomes present. Stems are simple or shortly branched and their length varies up to 30-80 cm tall. The stem is armed with stinging hairs. Stipules are free, oblong and linear of 4.7 mm. leaf blade are ovate to ovate-lanceolate of size 3.6 × 1.5.4 cm and are 3 to 5 veined, with stinging and setulose hairs on both surfaces. Inflorescences contain proximal female flowers and distal male flowers. Male flowers are short whereas female flowers are as long as achene. In female flowers, the lateral lobes are shorter reaching to apical 1/3 of dorsal ventral lobes. Achene is ovoid, compressed (Chen, 2003).

Urtica artichocaulis are perennial and monoecious herbs with woody rhizomes. Stems are gracile, simple or branched, and may be up to 30 to 150 cm tall and are armed with stinging hairs, particularly on nodes. Stipules are free, oblong and linear of 4.7 mm. Leaf blade are ovate or narrowly ovate and are rarely lanceolate with size 2.5 × 1.3 cm, and are 3-veined. Inflorescences contain proximal female flowers and distal male flowers. Male flowers are short and perianth lobes connate ½ of length. Female flowers: perianth lobes connate ½ of length, dorsal ventral lobes elliptic-ovate, as long as achene. The lateral lobes are shorter reaching to 1/3 of distal part of larger lobes (Chen, 2003).

Table 2.1 Nutritional composition of Nettle-bud

Constituents	Amount(100g)
Moisture	81.7g
Protein	6.9g
Fat	0.2g
Carbohydrate	5g
Minerals	4.2g
Fiber	1.8g
Energy	53kcal
Calcium	981.3mg
Vitamin C	5.5mg

Source: Food composition table (DFTQC, 2012)

2.3.2 Medicinal importance of *Sisnu*

The irritant property of species *dioica* has long been used externally to excite activity in paralyzed limbs, and internally for the treatment of hemorrhages and their uses still survive in some parts of the western Himalayas. In addition to these long-time traditional uses, nettles are currently in use for the treatment of prostate problems. Germany has approved a mixture of saw palmetto and nettle root for the treatment of benign prostatic hyperplasia, BPH, which can develop into prostate cancer if left unattended. This mixture is also for sale in the united States (Wagner *et al.*, 2007).

One retrospective interview study of 18 self-selected patients suggested improvement in joint or muscular pain by use of nettle, mostly by rubbing, beating or touching (apparently) fresh nettle leaves to the affected area (i.e. counterirritation) (Randall *et al*., 1999). The only

significant side effect was transient urticarial rash. Two underpowered randomized trails of thumb pain,(Randall *et al*., 2000) and knee pain, (Randall *et al.*, 2008) respectively, with only 1 week follow-up were reported. In vitro studies have demonstrated a number of effects that would explain a potential role for stinging nettle extracts in the treatment of allergic rhinitis or asthma. Nettle were found to be more effective than allergy medications(Mittman, 1990). Experts have expressed concern for the use of stinging nettle extracts for allergic rhinitis given their potential for side effects and the availability of generally safe antihistamines. However, except for obvious potential adverse dermatologic effects of topical application, reported side effects of *Urtica dioica* products have been generally mild in most types of trials (Bielory, 2004).

The roots of species *Plaviflora* are employed for the treatment of fractures and in florescene are prescribed as tonic. A decoction of the herb is given in fevers (Rhonda, 2004). The leaves of some species are applied for headaches and swollen joints, and decoction of them are given in fevers. According to Duke (1983), the most widespread and consistent folk use for nettles is the treatment of arthritis or rheumatism. Time-tested therapeutic application ranges from flagellating oneself externally with nettle leaves on the skin, causing welts, to taking freeze-dried nettle leaf capsules internally. Nettles in the form of freeze-dried capsules are often recommended for asthma. Due its relatively high iron content, some cultures use stinging nettles as a remedy for anemia (Kavalali, 2003).

The most animal studies are in favor of the use of *Urtica* dioica in diabetes. The blood sugar lowering effect of *Urtica* dioica has been mentioned in old script such as those written by Avicenna. There has been some reports indicating the benefit of plant in diabetes such as antidiabetic effect of hydroalcholic *Urtica dioica* leaf extracts in rats with fructose-induced insulin resistance, streptozocin-induced Type 1 Diabetes Mellitus and in patients with type 2 diabetes by inhibition of alpha-glucosidase and induction of insulin secretion in perifused Islets of Langerhans (Domola *et al*., 2010; Simoes-Pires *et al*., 2009). Two recent randomized controlled trails have examined the effects of stinging nettle on subjects with type 2 diabetes mellitus. In One, 100 mg/kg daily of nettle extract did not improve insulin sensitivity but did favorably reduce inflammatory biomarkers (Namazi *et al*., 2011). In the second study, insulin-requiring subjects took a 500 mg capsule of nettle leaf extract every 8 hours for 3 months. This treatment significantly lowered fasting blood glucose, hemoglobin A 1 c values and 2-hour postprandial glucose (Kianbakht *et al*., 2013).

2.3.3 Consumption pattern of *Sisnu*

The leaves of *girardinia* are said to be used as vegetable in western Himalayas. The leaf of *U. hyperborea* jacquem, a low, tufted herb, occurring in the alpine regions of the central and eastern Himalayas, is eaten as a pot-herb (a traditional leafy vegetable) (Chopra, 1958). Nettles in the form of freeze-dried capsules are served usually in the form of a soup or as a vegetable dish that incorporates the tops of young nettles. For example, in Scotland, yound nettle tops are combined with leeks or onions, broccoli or cabbage, and rice, boiled in a muslin bag and served with butter or gravy. In turkey, they are an ingredient in many spring recipes, such as spinach and nettle pie in which a mixture of spinach and nettles is layered between thin layers of *phyllo* (a traditional baked product of turkey) (Duke, 1983).

The tender leaves of species *plaviflora* are cooked and eaten as a green vegetable. Tender shots and leaves are collected with the help of bamboo or iron pincers, and cooked as soup. The plant is boiled with maize, millet or wheat flour by adding salt and chili to make a sort of porridge, which is a favorite food item of the villagers (Manandhar, 1989). The infected parts of the plants are deformed and very much hyper trophies; they become soft and yellowish green. These hypertrophied portions of the plants are sweet and delicious; they are called *Sishun Kakri* and are relished by the hills – tribes. The plant is sometimes feed to cattle, through cases and determinants have been reported. The seeds of species *Plaviflora* are nutritious and yield edible oil (Chopra, 1958).

2.3.4 Religious belief of nettle

It is believed that sprinkled nettle around the room protects from evil spirit and it is burned during ceremonies for exorcism. It is stuffed in a puppet and sent back to the sender of a curse or bad spell as it is believed to end the negativity (Hartl and Vogl, 2002).

2.4 Sisnu products

Sisnu are brought fresh from wild and cooked. Generally, sisnu is cooked as a soup and consumed along with dhido or rice. Nowadays, in some eastern hilly region gundruk of sisnu is also prepared. Some locals also prepare sisnu powder for off season (Rai, 2017).

2.5 Blanching

Heating of food for a short period prior to canning, freezing and dehydration followed by cooling is called blanching. It is generally applied to fruit and vegetables, and primarily carried out to inactivate enzymes. Unblanched frozen or dried foods undergo relatively rapid

changes during storage in food quality such as color, flavor, texture and nutritive value due to continuous enzymatic activity (Kharel, 2004). In plant tissues, enzymes such as lipoxygenase, polyphenoloxidase, polygalacturanase and chlorophyllase, cause loss of nutrition, flavor and texture. In addition, peroxidase and catalase are the two most heat resistant enzymes although they are not implicated as a cause of deterioration during storage, their activity is used to evaluate the effectiveness of blanching. If both of these enzymes are inactivated, then it can be safely assumed that other significant enzymes are also inactivated. Peroxidase is the more heat resistant of the two and the absence of residual peroxidase activity indicates that other less heat resistant enzymes are also destroyed (Kharel, 2004). An illustration of time temperature relationships for blanching of some vegetable is given in table 2.1.

Table 2.2 Time-Temperature relationships for blanching of some vegetables

Vegetables	Temperatures (º C)	Time (minutes)
peas	85-90	2-7
Green beans	90-95	2-5
Cauliflower	Boiling	2
Carrots	90	3-5
Peppers	90	3

(Source: Siddappa, 1986)

2.5.1 Effect of blanching on foods

The heat received by the food during blanching inevitably causes some changes to sensory and nutritional qualities. In, general, the time- temperature combination used for blanching is a compromise which ensures adequate inactivation but prevents excessive softening and loss of flavor in the food (Fellows, 2000). Small vegetables may be adequately blanched in boiling water in a minute or two but large vegetables may require several minutes (Kordylas, 1990). Some of the effects on foods are discussed below.

2.5.1.1 Nutrients

Some minerals, water-soluble vitamins and other water-soluble components are lost during blanching. Losses of vitamins are mostly due to leaching, thermal destruction and, to a lesser extent, oxidation (Fellows, 2000). The extent of vitamin loss depends upon on a number of factors including:

- The maturity of food and variety
- Methods used in preparation of the food, particularly the extent of cutting, slicing or dicing
- The surface-area-to volume ratio of the pieces of food
- Method of blanching
- Time and temperature of blanching (lower vitamin losses at higher temperature for shorter times)
- Method of cooling

Blanching as a unit operation is a short time heating in water at temperatures of 100^{o}C or below. In order to reduce losses of hydro soluble substances (mineral salts, vitamins, sugars, etc.) during water blanching, several methods have been developed (Jones *et al.*, 1996):

- Setting temperature at 85-95^{o}C instead of 100^{o} C
- Adjusting blanching time, just sufficient to inactivate enzymes catalase and peroxidase.

2.5.1.2 Color and flavor

Blanching brightens the color of some foods by removing air and dust on the surface and thus altering the wave length of reflected light. The green color of chlorophyll is protected by using alkaline blanching, although the increase in pH may increase losses of ascorbic acid. Blanching water is often added with sodium carbonate to neutralize the natural acidity of the products. When, correctly blanched, most foods have no significant changes to flavor or aroma, but under blanching can lead to the development of off-flavors during storage of dried or frozen foods (Fellows, 2000). Green tender peas when blanched with the use of blanching aids, 0.125% MgO and 0.1% $NaHCO_3$retained maximum percentage of Chlorophyll (D. Kumar, 1991).

2.5.1.3 Texture

One of the purposes of blanching is to soften the texture of vegetables to facilitate filling into containers prior to canning. Calcium chloride (1-2%) is added to the blanched water to form insoluble calcium pectate complexes and thus to maintain firmness in the tissue (Fellows, 2000).

Blanching is essential where fruits and vegetables are to be frozen or dried because drying or freezing operations only slow down enzymatic action but do not completely stop it. If blanching is not done prior freezing or drying then the frozen or dried product, which is often held in frozen or dried state for many months, will slowly develop off flavors and off colors and also other kinds of enzymatic spoilage might result. Under blanching may cause more damage to food than the absence of blanching does. Heat, which is sufficient to disrupt tissue but not to inactivate enzymes, causes the mixing of enzymes and substrates. In addition, only some enzymes may be destroyed which causes increased activity of other enzymes and this accelerates deterioration (Kharel, 2004).

2.5.1.4 Antinutrient

Boiling is one of the effective methods in reducing water soluble anti-nutrients. For example, boiling of root crops such as taro and cassava will lead to the significant reduction of oxalates and cyanide respectively. Boiling also found to decrease some amount of soluble phytate. Boiling may cause considerable rupturing of plant cell due to high temperatures and facilitate leakage of soluble anti-nutrients into cooking water (Albihn and Savage, 2001; Inchuen *et al.*, 2011) under the influence of concentration gradient (Uzogara, et al., 1990;Vijayakumari *et al.*, 1997). Boiling also facilitates the formation of water soluble complexes (Bakr and Gawish, 1991; Uzogara *et al.*, 1990).

2.5.2 Methods of blanching

During blanching a raw food material is immersed in hot water or exposed to live steam. Water temperature must be well controlled at desired level. The blanching operations varies according to the maturity and type of vegetable used. In practice, immersion blanching and steam blanching are two general methods of blanching. Less frequently, microwave blanching can be used (Kharel, 2004).

2.5.2.1 Immersion blanching

It involves passing the food at a controlled rate through a perforated drum rotating in a tank of water which is thermostatically controlled to the blanching temperature (70-100^{o}C). In a small plant, food to be blanched is passed on the wire of a perforated basket, which is first dipped in hot water for a short period of time (2-5 minute) and then in cold water. Hard water toughens tissues and destroys the natural texture of foods. A disadvantage of immersion blanching is that water soluble nutrient will pass into the blanching water, but an important

advantage is that undesirable oxidation can be easily controlled by appropriate additions to the blanching bath (Kharel, 2004).

2.5.2.2 Steam blanching

It uses saturated steam at atmospheric or at low pressure (150 kN/m^2). The food is conveyed through the steam chamber on a mesh belt or by the means of helical screw, the residence time being controlled by the conveyer speed. Typical equipment is 15 m long, 1 to 1.5 m wide and up to 2 m high. In conventional blanching there is often poor uniformity of heating in the multiple layers of food. The time temperature combination required, ensuring enzyme inactivation at the center of the bed results in the overheating of food at the edges and this results in losses in texture and other sensory characteristics of the food , individual quick blanching which involves blanching in two stages, overcomes this problem (Kharel, 2004).

In the first stage, food is heated in a single layer at a required temperature, and in the second stage, a deep bed of food is held for sufficient time to allow complete enzymes inactivation. This reduces the steaming time from a conventional 3 minute to about 75 seconds (25 seconds for heating and 50 seconds for holding). The blanched product is discharged through an outlet into a cooler (Kharel, 2004).

2.5.2.3 Microwave blanching

Microwave blanching has been applied to fruits and vegetables packaged in film bags and would appear to offer some advantages such microbiological cleanliness and low losses of nutrients. Blanching with microwave energy in order to apply heat at the center of large item before the surface are overcooked, is receiving interest in application but is not yet used commercially on a large scale due to its high cost (Kharel, 2004).

2.6 Cooking

Vegetables and fruits are mainly carbohydrates, and carbohydrates are robust molecules; even boiling temperatures simply disperse them more evenly in the tissue moisture, so the texture becomes soft and succulent. However, the cooking of vegetables and fruits does have its fine points. Plant pigments, flavor compounds, and nutrients are sensitive to heat and to the chemical environment. The challenge of cooking vegetables and fruits is to create an appealing texture without compromising color, flavor, and nutrition. Many plants, contain chemicals meant to discourage animals from eating them. The fruits and vegetables that we eat are no exception. Some animals have developed specific detoxifying enzymes that enable

them to exploit an otherwise toxic plant. Human invented their own ingenious detoxifying methods, including plant selection and breeding and cooking. Cultivated varieties of such vegetables as cabbage, lima beans, potatoes, and lettuce are less toxic than their wild ancestors. And many toxins can be destroyed by heat or leached away in boiling water. Hot water and steam are excellent carries of heat, these are efficient methods as well, ideal for the rapid cooking of green vegetables that minimizes the loss of color (McGee, 2004).

2.6.1 Boiling

Boiling means to cook in a liquid that is bubbling rapidly and greatly agitated. Water boils at 100^{o}C (near sea level, with predictably lower temperatures at higher elevations). No matter how high the burner is turned, the temperature of the liquid will go no higher. Boiling is generally reserved for vegetables and starches. In the case of boiling green vegetables, pH and dissolved mineral content of cooking water should be at optimum condition. Ideally it should be neutral or just slightly alkaline (pH 7-8), and not too hard, because acidity dulls chlorophyll, and acidity and calcium both slow softening and so prolong the cooking. A large volume of rapidly boiling water will maintain a boil even after the cold vegetables are added, cut into pieces small enough to cook through in about five minutes. Salt in the cooking water at about the concentration of 3% will speed softening and also minimize the loss of cell contents to the water. When just tender enough, the vegetables should be removed and either served immediately (McGee, 2004).

Starchy vegetables, especially potatoes cooked whole or in large pieces, benefit from a different treatment. Their vulnerability is a tendency for the outer portions to soften excessively and fall apart while the interiors cook through. Hard and slightly acid water can help them maintain their surface firmness, as will starting them in cold water and raising the temperature only gradually to reinforce their cell walls. Salt is best omitted from the water, since it encourages early softening of the vulnerable exterior (McGee, 2004).

2.6.2 Steaming

Steaming is a good method for cooking vegetables at the boiling point, but without the necessity of heating a whole pot of water, exposing the food directly to turbulent water, and leaching out flavor or color or nutrients. It doesn’t allow the cook to control saltiness, calcium cross-linking, or acidity (steam itself is a slightly acid pH 6, and plant cells and vacuoles are also more acid than is ideal for chlorophyll); and evenness of cooking requires that the pieces be arranged in a single layer, or that the pile be very loose to allow the steam

access to all food surfaces. Steaming leaves the food tasting exclusively of its cooked self, though the steam can be also be aromatized by the inclusion of herbs and spices (McGee, 2004).

2.7 Ascorbic acid

L-ascorbic acid, which is also known as vitamin C, is an important naturally occurring nutrient essential for human nutrition (Chauha et al., 1998). Vitamin C is a white crystalline compound with sour taste but no smell. Identification of its formula ($C_6H_8O_6$) was presented by group headed Professor Haworth in 1933 and it was the same group that proposed the first synthetic method for its molecular weight of 176,and melting point of 190ºC (Mottram, 1974).It is a derivative of glucose called hexose, Chemically it is 7-threo-2, 4, 5, 6 pentoxy hexen 2carboxyllic acid lactone (Jain, 1996).

Ascorbic acid has four isomers, L-ascorbic acid, D-ascorbic acid, L-arabo ascorbic acid and Disoascorbic acid. Among the four isomers, the D-forms have no biological activity and used asfood additives. The presence of enediol group on ascorbic acid imparts acidic and reducing properties. It behaves as mono basic and can give salt when reacted with alkalies. Only Lascorbicacid has important vitamin activity (Deman, 1976; Lee, 1975). The unusual properties of L-ascorbic acid are derived from the fact that it shows acidic properties in the absence of carboxylic group, it has strong reducing properties and has one unusually stable 1, 4-lactonering (Herbert *et al*., 1993).

Ascorbic acid is highly soluble in water (30g/100 ml), slightly soluble in alcohol and insoluble in chloroform, ether and benzene (Jain, 1996; Mottram, 1974). It is very stable when dry, moderately stable in acid solution and unstable in alkali. It is rapidly lost due to oxidation by exposure to the air; the oxidation is speeded up by the heat, light, alkali oxidative enzymes and traces of copper and iron (Mottram, 1974; Rajalakshmi, 1990).

2.7.1 Stability or retention of vitamin C

Stability or retention of vitamin C depends upon the following factors:

2.7.1.1 Optimum harvest condition

For the maintenance of a maximum level of vitamin C, it is essential to ensure that the fruit is picked at optimum maturity (Gresswell, 1974). Fresh fruit, even of some variety, may differ enormously in their nutritive value. The condition of growth, whether the season has been

wet or dry, sunny or dull, the maturity of the fruit and the time that has been elapsed after picking , which all influence the vitamin content of the fruit which enter the processing line (Tucker, 1990).

2.7.1.2 Shortest possible processed time

Once the fruit has entered the process chain, it is important to complete the operations in the shortest possible time thus reducing to minimum exposure of the fruit or juice to atmospheric air especially at elevated temperature (Gresswell, 1974).

2.7.1.3 Blanching

Even though blanching can result in severe loss of ascorbic acid that which remains must be stabilized to some extent because air has been driven out of the tissues and any oxidative enzymes have been at least practically inactivated (Henshall, 1974). Less oxidation of ascorbic acid occur at higher temperatures because of the elimination of oxygen from the blanched water and or the inactivation of enzymes, thus HTST blanching gives the best retention of ascorbic acid (Gresswell, 1974).

2.7.1.4 Construction and maintenance of equipment's

Choice of plant construction materials and the maintenance of such equipment in the best state of repair are also important. As ascorbic acid oxidation is significantly increased by trace contamination with metals ions especially Copper and to a lesser extent Iron (Gresswell, 1974).

2.7.1.5 Removing oxygen

The use of vacuum deareation can play a valuable role in vitamin C retention by reducing oxygen content and aside benefit is reduction in frothing. According to Gresswell (1974) if vacuum deareation is not possible, the use of blanket of CO2 or N2 will help to displace air.

2.7.1.6 Acidic condition

Ascorbic acid is stable in acidic condition but destroyed by alkali (Gresswell, 1974).

2.7.1.7 Uses of sulphites

The activity of sodium metabisulphate or sulphite is scavenging oxygen in solution tends to increase the stability of ascorbic acid (Ritter, 1982). According to Gresswell (1974) the oxidative enzyme (peroxidase) can be inhibited by the addition of a low level of SO2.

2.7.1.8 Best storage condition

Stability of ascorbic acid increase as the temperature decreases (Henshall, 1974). Fruits and vegetables conserve ascorbic acid best by storage at low temperature (Wilson *et al.*, 1971).

2.7.1.9 Quick freezing

A slow freezing form ice crystal which damages the tissue and the vitamin C is lost through coming into contact with the enzyme ascorbic acid oxidase. Quick freezing at temperature of -10 to -20ºC prevents the formation of large ice crystals and the plant tissues are undamaged, so vitamin C loss is minimized. Vegetables show no loss of vitamin when storage at-27ºC for one year but the time decreases to 4 month at -18ºC (Fisher and bander, 1975).

2.7.1.10 Powder form of sisnu

In powder form of a product, vitamin stability is generally good because of the low available Water content. According to Gresswell (1974) such products can even be packed in sachets provided that a laminate is chosen which reduces moisture permeability to minimum.

2.7.2 Losses of vitamin C

Some factors responsible for the losses of vitamin C are discussed below

2.7.2.1 Pre-harvest factor

Variations in the vitamin content of raw material can affect the content of vitamins in the final food products to a considerable extent. Raw food may vary widely in the vitamin content because of climatic and soil condition, genetic variation and maturity at the time of harvest (Ritter, 1982).

2.7.2.2 Oxidation of ascorbic acid

Vitamin C may be oxidized both by air and by enzymes (Fisher and bander, 1975). Enzymes containing copper or iron in their prosthetic groups are most efficient catalysts of ascorbic acid decomposition. The most important enzymes of this group are ascorbic acid oxidase, phenolase, cytochrome oxidase and peroxidase. Among them only ascorbic acid oxidase involves a direct reaction between enzymes, substrate and molecular oxygen. The other enzymes oxidize the vitamin indirectly. Phenolases catalyses the oxidation of mono and dihydroxy phenols to quinines and this reacts directly with ascorbic acid. Cytochrome oxidase oxidizes cytochromes to the oxidized form and this react with L-ascorbic acid. Peroxidase in combination with phenolic compounds utilizes H_2O_2, to bring out oxidation

(Deman, 1976). The enzymes do not act in intact fruits because of the physical separation of enzymes from the substrates. When the fruit is damaged or cellular fragments occurred, reductase is more liable and therefore oxidases are free to react with ascorbic acid. The route and rate of oxidation is influenced by several factors, including pH, trace metals, enzymes, oxidation reduction potential, presence of oxygen as well as time and temperature (Deman, 1976; Paulik *et al.*, 2005).

2.7.2.3 Anaerobic destruction

Anaerobic destruction of ascorbic acid following oxidative changes is also significance. The rate of this reaction is virtually independent of pH except in the range 3-4 where it is slightly increased. Accelerators of this reaction are fructose, Fructose 6-phosphate, fructose 1, 6 diphosphate, sucrose and caramelized fructose. Furfural and carbon dioxide are appeared to be the major end products of decomposition (Henshall, 1974).

2.7.2.4 Drying or dehydration

Vitamin C is the most difficult of the vitamins to preserve during the dehydration of the food. It is generally considered that the preserve of vitamin C is highly correlated with overall quality of food products (Birch *et al.*, 1974; F. A. Lee, 1975). A better retention of vitamin C is observed in rapid drying at higher temperatures than in slow drying at lower temperature (Desrosier and Desrosier, 1987).

2.7.2.5 Non-enzymatic change

Non-enzymatic changes which are of the catalytic effects of the copper which are enhanced by iron, resulting the formation of dehydro ascorbic acid and H_2O_2. The H_2O_2 produced in this reaction further reacts with ascorbic acid and copper catalyst to give directly or indirectly oxygen and water (Henshall, 1974).

2.7.2.6 Loss during storage

The destruction rate of ascorbic acid during storage is affected by moisture content, enzyme, temperature and time. Significant losses begin to occur during storage and rate of loss is time and temperature dependent (Henshall, 1974).

2.7.3 Physiological and biochemical functions of vitamin C

The principle function of ascorbic acid is the formation of collagenous intercellular substances. Ascorbic acid helps to reduce the ferric ion to ferrous state in the intestine and

thus helps in the absorption of iron. It is involved in the synthesis of cortical hormones and metabolism of tyrosine (Swaminathan, 1991). A high dose of ascorbic acid depresses alimentary hypercholesterol anemia and protects from the disease and resulting from atherosclerosis. However, the effect on lipid oxidation as a function of ascorbic acid is a controversial one (Ginter, 1974). Ascorbic acid is concerned with the formation of red blood corpuscles. In the old age deficiency of ascorbic acid is frequently observed, hence it is also regarded as anti-ageing agent (Jain, 1996).

2.7.4 Effect of deficiency of vitamin C in humans

The deficiency of vitamin C in humans results in the defective formation of the intercellular cement substances. Fleeting joint pains, irritability, retardation of the growth in the infants or child, anemia, poor wound healing and increased susceptibility to infection are among the signs of deficiency (Swaminathan, 1991).

2.7.5 Side effect from over dosage of Vitamin C

The reported side effects based on biochemical theory are gastro-intestinal disturbance, increase peristalsis, abdominal colic, gastro-enteritis and anal irritation, looseness of bowels, occasional diarrhea, abnormal uric acid metabolism, and production of gout, stone formation, bone demineralization and increased collagen catabolism, calcium desorption, allergic symptoms, haemolytic crisis, and human infertility. These effects were observed taking daily dose in the range between 200 mg to 300 mg (Wilson *et al.*, 1971).

2.7.6 Recommended daily allowances of ascorbic acid

The recommended daily allowances of ascorbic acid for different group of people are shown in Table 2.3.

Table 2.3 RDA proposed by ICMR (2010) and FAO (2017) are as given below. (The requirements are expressed in mg per day)

subject	ICMR	FAO
Man	40	45
Women	40	45
Women (Pregnancy)	60	55
Women (lactation)	80	70
Infant (0-6 Month)	25	25

Children (7-12 Months)	25	30
Children (1-3 years)	40	30
Children (4-6 years)	40	30
Children (7-9 years)	40	35
Adolescents (10-12 Boys)	40	40
Adolescents (10-12 Girls)	40	40
Adolescents (13-15 Boys)	40	40
Adolescent (13-15 Girls)	40	40
Adolescent (16-17 Boys)	40	40
Adolescent (16-17 Girl)	40	40

1. ICMR (2010) - Nutritional expert group of ICMR India
2. FAO (2001) - Food and Agricultural Organization

2.7.7 Source of ascorbic acid in some fruits and vegetables

Ascorbic acid contents in some fruits and vegetable are given in Table 2.4.

Table 2.4 Ascorbic acid contents mg per 100g fresh weight of some fruits and vegetable.

Fruits	mg per 100 gram	Vegetable	mg per 100 gram
Rich source		Amaranth leaves	173
Amla	700	Cabbage	124
Guava	300	Coriander leaf	135
Good source		Spinach	48
Orange	68	Radish leaves	65
Pine apple	63		
Mango	24		
Papaya	46		
Tomato ripe	32		
Fair source			
Apple	2-8		
Banana	2-6		
Grape	2-6		

Source :(Swaminathan, 1991)

2.7.8 Occurrence of ascorbic acid in human body

The normal human body when fully saturated contains about 5 gm of vitamin of which perhaps 30 mg are in the adrenal glands, 200 mg in the extra cellular fluids and rest distributed in varying concentration throughout the cells of the body. Blood contains about 1 mg/100ml ascorbic acid (Lehninger, 1982).

2.8 Anti-nutritional factors

Foods are complex substances that contain many chemical compounds, more than 50 of which are required to nourish the body. These nutrients include water, proteins, lipids, carbohydrates, minerals and vitamins. Additionally, most plant foods also consist of thousands of natural compounds, depending on the situation may have beneficial or deleterious effect on consuming them. These compounds, with the exception of nutrients, are referred to as allelochemicals. Anti-nutritional factors may be regarded as the class of these compounds that are generally not lethal. They diminish animal productivity but may also cause toxicity during the periods of scarcity or confinement when the food rich in these substances is consumed by animals in large quantities (Rosenthal and Janzen, 1979).

Anti-nutrients are potentially harmful and give rise to a genuine concern for human health in that they prevent digestion and absorption of vitamins, minerals and other nutrients. They can reduce the nutritional value of a plant by causing a deficiency in an essential nutrient or preventing through digestion when consumed by humans or animals (Prathibha *et al*., 1995). Several anti-nutritional factors are present in root and tuber crops and are partially neutralized during ordinary cooking (Bhandari and Kawabata, 2004). The remaining anti-nutrients can, however, be responsible for the development of serious gastric distress and may interfere with digestion of nutrients, which inevitably results in chronic deficits in absorption of nutrients (Brune et al., 1989; Jood et al., 1986; Kelsay, 1985). Anti-nutritional factors include cyanogens, glycosides, saponins, phytate, enzyme inhibitors (trypsin and amylase inhibitors), lectins (haemagglutinins), oxalate and total polyphenols.

Some of the anti-nutrients are described below: -

2.8.1 Oxalates

The plant *oxalis,* commonly known as wood sorrel, gave rise to oxalic acid (chemical formula HOOC-COOH), a strong, organic acid which has been found to be widely distributed in plants (Liebman, 2002), occurs ubiquitously in nature, sometimes as a free acid, but more

commonly as soluble potassium, sodium or ammonium oxalate or as insoluble calcium oxalate. Biosynthesis of oxalate occurs in members of all five kingdoms. Oxalate is associated with metabolic disorders and infectious disease (Holmes and Assimos, 1998; Nakagawa *et al.,* 1999).See fig 2.2 for structure.

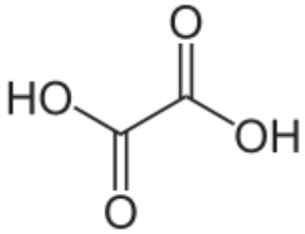

Fig 2.2 Structure of oxalic acid

Oxalate is of primary concern among the anti-nutritional factors due to its strong oxidizing and corrosive nature with good chelating activity, synthesized by a broad range of animals, plants and microorganisms (Stewart *et al.,* 2004). Oxalate-producing plants, which include many crop plants, accumulate oxalate in the range of 3%-80% (w/w) of their dry weight (Libert and Franceshi, 1987). The diversity of calcium oxalate crystal shapes and sizes, as well as their prevalence and spatial distribution, have led to a number of hypotheses regarding crystal function in plants. The proposed functions include roles in ion balance, in plant defense, in tissue support, in detoxification, and in light gathering and reflection (Franceshi and Horner, 1980). Recently, Nakata and McConn (2000) hypothesized the roles of calcium oxalate formed in plants in supporting tissue structure and in regulating excess tissue calcium. Oxalic acid is a common and wide spread constituent of plants, being found in almost all plant families usually at low levels. It occurs as the free acid, as soluble salts of potassium and sodium and as insoluble salts of calcium, magnesium and iron (Noonan and Savage, 1999). High oxalate concentrations in the leaves and corms of plants consumed daily are of concern because of the harmful health effects associated with the intake of high amounts of oxalates (Savage and Catherwood, 2007).

Table 2.5 Oxalate content in some vegetables and beans (mg/100g fresh weight)

Food types	Oxalate (mg/100g)
Spinach	978 ± 5
Carrot	49 ± 7
Beet root	67 ± 12
White bean	158 ± 16
Red bean	113 ± 15
Soybean	497 ± 22

Source: (Akhtar *et al.*, 2010)

2.8.1.1 Occurrence in plants

Calcium oxalate crystals occur in more than 215 higher plant families, as well as the algae, lichen and fungi, in the form of whewellite (CaC2O4.H2O) or weddelite (CaC2O42H2O). They can form in any organ or tissue within plants, including in stems, leaves, roots, tubers, and seeds, and have a variety of functions including calcium storage, defense and providing structural strength (Crowther, 2005). The highest levels of oxalates are found in the following families: amaranth family for example *Amaranthus* (amaranth); aroid/arum family, for example *Colocasia* (Taro) and *Xanthosoma* (caladium), *goothfoot* family, for example, *Atriplex* (orach), *Beta* (beet, beetroot) and *Spinachia* (Spinach); ice-plant family for example, *Tetragonia* (NZ spinach);Wood sorrel family for example *Oxalis* (sorrel yam);buckwheat family, for example, Rheum (rhubarb) and *Rumex* (sorrel); and the purslane family, for example *portulaca* (purslane) (Noonan and Savage, 1999).

The oxalic acid content is variable within some species; some cultivars of spinach contain 400- 600mg/100g, while others range from 700-900mg/100g. Oxalic acid accumulates in plants especially during dry season. The distribution of oxalic acid within plants is also uneven. In general, oxalic acid is highest in the leaves followed by seeds; it is lowest in the stems. High oxalate levels in tropical plants are of concern. Taro (*Colocasia esculenta*) and sweet potato (*Ipomoea batatas*) were reported to contain 278-574mg/100g and 470mg/100g, respectively (Noonan and Savage, 1999).

2.8.1.2 Biosynthesis of oxalates in plants

Oxalate is considered as an end-product of ascorbic and tartaric acid metabolism. Several pathways have been described for oxalate biosynthesis, but there are two main pathways (Debolt *et al.*, 2007). In major pathway oxalate is formed from L-ascorbic acid and this lead to calcium oxalate crystal formation in plants (Franceshi and Nakata, 2005). In the minor pathway oxalate is formed through the oxidation of oxaloacetate, glycolate and glyoxylate. The activation of isocitrate lyase and glycolate are responsible for the formation of oxalate in the minor pathway (Giachetti *et al*., 1987). Although glycolate, glyoxylate and their oxidizing enzymes are abundant in green plants, this is still considered a minor pathway (Franceshi and Nakata, 2005). (Guo *et al*., 2005)reported that increased feeding of ascorbic acid to plants increased oxalate levels, especially soluble oxalate, whereas studies have shown that glycolate or glyoxylate were relatively poor precursors of oxalate biosynthesis (Kostman *et al*., 2001). L-ascorbic acid is cleaved between carbons two and three to give oxalic acid and L- threonic acid, which can be oxidized to give tartaric acid and the biosynthesis in plants occurs in crystal idioblasts (Li *et al*., 2003). Calcium oxalate crystal formation increases with increasing calcium in the growth medium of plants. It has been hypothesized that this phenomenon can be affected by growing plants in high-calcium soil (Keates *et al*., 2000).

2.8.1.3 Role of oxalates in plants

Oxalates are inactive and cannot be used for energy production and oxalates could be viewed as a metabolic waste. However, in plants, there are a number of professed functions carried out by oxalates. Oxalates offer protection and defense to plants and may contribute to maintaining cell homeostasis and photosynthesis (Noonan and Savage, 1999). These functions are outlined below.

2.8.1.3.1 Photosynthesis

Kuo-Huang *et al.* (2007) described a possible correlation between calcium oxalate crystals and photosynthesis. In six different plant species, druse crystals were found in the photosynthetic palisade cells rather than in cells specialized for crystal formation, suggesting their role in photosynthesis. The position of druse crystals within the palisade cells alters in response to different light intensities. Kuo-Huang *et al.* (2007) concluded that the crystals migrated to the top of the palisade cells, perhaps to reflect some high-intensity light, as a form of light regulation and protection for shade-adapted plants.

2.8.1.3.2 Calcium regulation

The formation of calcium oxalate crystals has been considered to be a way of regulating calcium levels in plants as it converts the calcium into an inactive form. In plants, calcium uptake is dependent on the availability of calcium in the root zone and is not regulated by metabolic requirements (Franceshi and Nakata, 2005). The number and size of calcium oxalate crystals in plants changes with the changing calcium levels in the growing medium (Mazen *et al.*, 2004). In plants, calcium has important physiological roles in signal transduction pathways and other biochemical and cellular processes (Franceshi and Nakata, 2005).

2.8.1.3.3 Protection

In plants, both oxalic acid and calcium oxalate provide possible defense mechanisms. Soluble oxalates can have toxic poisoning effects on grazing animals and are associated with calcium oxalate accumulation in rumen walls, arteries and kidneys (Franceshi and Nakata, 2005). Calcium oxalate crystals provide protection from omnivores due to the formation of needle-shaped raphide crystals, styloid crystals and small angled crystals, etc (Franceshi and Nakata, 2005). These crystals can pierce the skin of grazing animals and make eating unpleasant so animals avoid high oxalate containing plants. Styloid crystals may span the entire cross-section of the leaf and potentially pierce and injure the mouth of grazing animals (Franceshi and Nakata, 2005).

2.8.1.3.4 pH regulation and osmoregulation

Many cellular processes are pH dependent and thus the concentration of H^+ ions within a cell is important. Oxalate (the anion) has been found to be important in maintaining homeostasis by counteracting inorganic cations such as potassium and sodium. Free oxalic acid acts as an H^+ source (Ruiz and Mansfield, 1994). Soluble salts of oxalates may also play an important role in osmoregulation, as they can reversibly bind inorganic cations such as sodium and potassium that are important in osmoregulation (Libert and Franceshi, 1987).

2.8.1.3.5 Detoxification of heavy metals

Plants produce oxalates to protect themselves from being poisoned with heavy metals (e.g., lead and cadmium) through exclusion and internal mechanisms. The exclusion mechanism involves the release of oxalate by the roots into the environment and this is induced by

aluminum stress. The internal mechanism is the chelation of aluminum by oxalate to form non-toxic aluminum oxalate (Ma *et al.*, 1998). Further studies have demonstrated oxalate binding detoxifies other heavy metals, such as lead (Yang *et al.*, 2000), strontium (Franceshi and Schueren, 1986) and cadmium (Choi *et al.*, 2001).

2.8.1.4 Absorption and fate of oxalate in the human body

There are some contradictions about the major sites of free oxalate absorption in the human body. However, most studies have suggested the small intestine as the major site for oxalate absorption (Savage & Martensson, 2010). The amount and rate of oxalate absorption varies depending on the diet of the individuals (Noonan and Savage, 1999). In general, the oxalate absorbed from the diet is relatively low. In normal people absorbed oxalate is only a fraction of the total oxalate ingested. This fraction is estimated in the range of 5-15% depending on the co-ingestion of calcium, magnesium and dietary fiber (Noonan and Savage, 1999; Savage & Martensson, 2010). During fasting the rate of oxalate absorption is higher (~12%) compared to in the non-fasting state (~7%) (Noonan and Savage, 1999). The absorption of oxalates also varies markedly depending on the food source, for example, 1% from rhubarb and spinach to 22% from tea. A fraction of the oxalate is absorbed from foods and once absorbed free/soluble oxalates bind to calcium ions to form insoluble calcium oxalate (Noonan and Savage, 1999).

While passing through the gastrointestinal tract, oxalate can be broken down by oxalate degrading bacteria or enzymes in the colon (Savage & Martensson, 2010). Anaerobic *Oxalobacter formigenes* influences the gastrointestinal absorption of oxalate (Kwak *et al.*, 2003). This bacterium uses oxalate as an energy-yielding substance for growth. In combination with enzymes *O. formigenes* can convert oxalates to formate and carbon dioxide (CO_2). The absence or decreased activity of oxalate-degrading bacteria is associated with the increase risk of hyperoxaluria (Savage, 2002). A study carried out by Kwak *et al.* (2003)suggested that patients with calcium oxalate kidney stones who tested negative for oxalobacteria, showed higher than average level of urinary oxalate (0.36 mmol/day) compared to patients positive for oxalobacteria (0.29 mmol/day).

Animals models demonstrate that diseases including chronic renal failure, hyperoxaluria and oxalate-associated diseases can alter oxalate absorption in the intestine. These diseases may change cellular oxalate transport, transcellular and paracellular pathways and promote oxalate absorption or secretion. Intestinal oxalate absorption is increased in patients with ileal

dysfunction and kidney stones. A number of studies have shown that hyperoxaluric stone formers absorb more oxalate than non-stone formers. On average, stone formers may absorb up to 50% more oxalate than normal individuals (Chai and Liebman, 2004; Voss *et al.*, 2006).

2.8.1.5 Health Implications of oxalate

Oxalic acid and its salts are extensively spread in numerous plant tissues as the end products of metabolism. Oxalic acid and its salts are extensively spread in numerous plant tissues as the end products of metabolism. Oxalic acid content in foodstuffs has long been a concern in human diets, due to the negative health effects connected to a high intake of oxalic acid. Incidences of kidney stones, hypocalcemia and hyposideremi (low plasma levels of calcium and iron) correspond strongly with the intake of oxalic acid that perform as an absorption inhibitor are common (Palaniswamy *et al.*, 2002). High oxalate content in urine and blood causes several diseases such as hyperoxaluria and vitamin deficiencies (Jiang *et al.*, 1996). Small doses of oxalate in the body may result in pain, headaches, and twitching in muscles and cramps. Larger doses can result in a drop-in blood pressure, weak, irregular heartbeat, and signs of heart failure. Large doses of oxalate may rapidly put a person in a shock-like state, causing convulsions (because of low plasma calcium), coma, and even death. The mean fatal dose for an adult is about 15 to 30g, but lowest reported fatal dose is merely 5 g (or about 70mg/kg) (Noonan and Savage, 1999; Tsai *et al*., 2005).

Consumption of foods high in oxalic acid in the long term can be troublesome. Healthy persons can securely consume such foods moderately, but those with gout, rheumatoid arthritis, kidney disorders, or certain forms of chronic vulvar pain (vulvodynia) are normally advised to stay away from foods high in oxalated or oxalic acid (Roy *et al*., 2010).Oxalate is an anti-nutrient which under normal conditions is confined to separate compartments. However, when it is processed and/or digested, it comes into contact with the nutrients in the gastrointestinal tract (Kaushaiya *et al.*, 1988).

Ingestion of foods containing oxalates has also been reported to cause caustic effects, irritation to the intestinal tract and absorptive poisoning. It could therefore be recommended that the intake of calcium oxalate in one meal does not exceed two-third of this lethal dose. Oxalic acid forms water soluble salts with Na, K+ and (NH4)2 + ions and it also binds withCa2+, Fe2+ and Mg2+ and rendering these minerals unavailable to animals. However Zn appears to be relatively unaffected (Noonan and Savage, 1999). High oxalate foods have been

known to exert a negative effect on the absorption of calcium and iron. The adverse effect of oxalate is greater if the oxalate: calcium ratio exceeds 9:4. The adverse effect of oxalates must be considered in terms of oxalate: calcium ratio in the food. This ratio varies widely and can be classified into three groups: (i) plants with oxalate to calcium ratio of greater than 2, (ii) plants with ratio of approximately one and (iii) plants with a ratio of less than one (Noonan and Savage, 1999).

Foods that have a ratio greater than two and that contain no utilizable calcium have exceeds oxalates which can bind calcium in other food eaten at the same time. Food stuffs having a ratio of approximately one do not encroach on the utilization of calcium provided by other products and, therefore, do not exert any dematerializing effects. However, these foods are not good source of calcium. Foods with a ratio of one do not reduce the availability of calcium as far as other calcium sources are concerned (Noonan and Savage, 1999).

Oxalates are poorly absorbed under non-fasting conditions. It has been demonstrated that only 2- 12% of the oxalate is absorbed from foods but that once absorbed, free oxalates bind to calcium form insoluble calcium oxalate. This may result in a functional hypocalcemia with tetany in acute cases. Free oxalate and calcium precipitate in the urine and may form kidney stones. These stones are comprised mainly of calcium oxalate (80%), which is relatively insoluble in urine, and calcium phosphate (5%). Oxalates crystallizes with calcium in the renal vasculature and infiltrates vessel walls causing renal tubular obstruction, vascular necrosis and hemorrhage, which leads to anuria, uraemia, electrolyte disturbances or even rupture. Oxalic acid may cause greater decreases in mineral availability if consumed with a high fiber diet, although the decrease may only be temporary (Noonan and Savage, 1999).

Prevention of calcium oxalate stone formation can be achieved by avoiding large amounts of oxalate containing foods and consuming calcium rich foods such as dairy products together with oxalate containing foods (Martensson and Savage, 2008).

2.8.2 Phytic acid

Phytic acid is a hexaphosphoric ester of the hexahydric cyclic alcohol meso-inositol, see fig 2.2. phytic acid (known as inositol hexakiphosphate (IP6), or phytate when in salt form) is the principal storage form of phosphorus in many plant tissues. Inositol penta- (IP5), tetra- (IP4) and tripohospate (IP3) are also called phytates. The chemical description for phytic acid is myoinositol (1,2,3,4,5,6) hexakiphosphoric acid.(Kumar *et al*., 2010). Phytate is formed

during maturation of the plant seed and in dormant seeds it represents 60-90% of the total phosphate (Loewus, 2002).

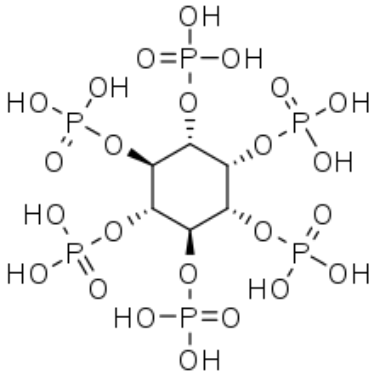

Fig 2.3 Structure of Phytic Acid

The unique structure of phytic acid offers it the ability to strongly chelate with cations such as calcium, magnesium, zinc, copper, iron and potassium to form insoluble salts (Kumar *et al*., 2010). Phytic acid is a major anti nutritional factor that binds with cationic nutrients like zinc and iron, and makes them unavailable for human intestinal absorption. Zinc and iron are among the important nutrients required for human growth and development. The presence of certain forms of a particular nutrient can hinder the uptake of other nutrients especially micronutrients. PA forms insoluble complexes with polycations like zinc and iron due to reactive phosphorus groups attached to its inositol ring (Pederson et al., 2007Sandberg and Svanberg, 1991), which in turn renders these essential nutrients unavailable for human intestinal absorption.

Monogastric animals, whose diets are largely cereal, legume and oilseed based do not produce sufficient amounts of intrinsic phytases necessary to hydrolyze the phosphorus binding phytic acid molecule (Smith et al., 2004). The six phosphate groups in the phytate molecule make it highly charged (Lehninger, 1982). Phytic acid binds calcium, magnesium and zinc very tightly at the multiple phosphate groups preventing their absorption (Lehninger, 1982).

Phytic acid especially reduces the bioavailability of divalent cations. Zinc and iron are the essential micronutrients and are key co-factors for many enzymes, involved in growth and developmental processes (Duhan *et al.,* 2002). Zn deficiency is affecting 3 to 4 billion people which accounts for 49% of the world population (Ramakrishna *et al.,* 2006). The importance of Fe in vital metabolic functions is evidenced by Fe being an intrinsic component of

hemoglobin, myoglobin and cytochromes (Hurrell *et al.*, 2003). As humans and animals are dependent on plant based foods for their nutrient requirement except Vit B12, the deficiency of any nutrient can lead to malnutrition or under nutrition (P.J. White and Broadley, 2009). Phytate is a common constituent of plant derived foods like cereals or legumes, which are the main staple food of people in developing countries. The daily intake of phytate for humans or vegetarian diets, on an average, is 2000-2600 mg whilst, for inhabitants of rural areas in developing countries, on mixed diets, it is 150-1400 mg (N. R. Reddy, 2002). Phytate content in plant-derived human foods is shown in table 2.4.

Table 2.6 Phytate content (mg/g DM) in plant-derived human food

Food types	phytate (mg/g)
Rice (polished, cooked)	1.2-3.7
Rice (unpolished, cooked)	12.7-21.6
Wheat bread	3.2-7.3
green peas (cooked)	1.8-11.5
soybeans	9.2-16.7
lentils (cooked)	2.1-10.1
chickpea (cooked)	2.9-11.7
buckwheat	9.2-16.2
amaranth grain	10.6-15.1
cowpea (cooked)	3.9-13.2

Source: (Greiner and Konietzny, 2006)

2.8.2.1 Negative aspects of phytate

2.8.2.1.1 Effect on mineral uptake

The presence of phytate in the human diet has a negative effect on mineral uptake. Minerals of concern in this regard include zinc, iron, calcium, magnesium and copper (Konietzny and Greiner, 2003; Lopez et al., 2002). Among them, bioavailability of Zn^{2+} was reported to be the most adverse effect in humans (Lopez *et al.*, 2002). First reports of Zn^{2+} -deficiency in humans were reported in 1963 among Egyptians, feeding mainly on bread and beans (Prasad *et al.*, 1963). The order of the ability of the mineral cations to form complexes with phytate *in vitro* has been found to be: $Cu^{2+}>Zn^{2+}>Cd^{2+}$ at pH 3-7 (Persson *et al*., 1998).

Uptake of non-haem iron from plant foods is lower than that of haem iron from meat products. Phytic acid acts a main inhibitor for the absorption of non-harm iron from plant foods. The phosphate groups of phytic acid are negatively charged under physiologically relevant conditions, resulting in phytate chelation of cations such as iron and zinc, making these minerals less available for absorption (Bohn *et al*., 2008; Schlemmer *et al*., 2009). For vegetarians, elimination of meat coupled with high intakes of phytate-rich whole grains is known to lower iron absorption, increasing the risk of iron deficiency (Hunt, 2003).

The stability and solubility of the complexes depend on the pH value, the individual cation, the phytate to cation molar ratio and the presence of other compounds in the solutions (Oberleas, 1983). The pH is an important factor influencing the solubility of phytate (Cheryan, 1980), it is being more soluble at lower than at higher pH values (Torre *et al*., 1991). Ca^{2+}, Cd^{2+}, Zn^{2+} and Cu^{2+} salts tend to be soluble at pH lower than 4-5, whereas Mg-phytate is soluble at acid pH up to pH 7.5 (Brown *et al*., 1961). In contrast, ferric phytate is soluble at pH values in the range 1.0-3.5 at equimolar Fe3+ to phytate ratios and solubility increases above pH 4 (Askar *et al*., 1983). However, solubility studies of bran phytate prove that , at gastric pH (approximately pH 2), Ca actually does not bind and this component does not contribute to the solubility of the Ca ion (Siener *et al*., 2001).

Phytate also interacts directly and/or indirectly with various dietary minerals to reduce their bioavailability. In this context the synergistic effect of secondary cations (Ca2+) has been most prominently exhibited (Wise, 1983). Two cations may, when present simultaneously, act jointly to increase the quantity of phytate precipitation. In the presence of phytate and calcium, absorption of other mineral is depressed due to formation of insoluble complexes (Sandber et al., 1983). For example, calcium-bound phytate shows more affinity for Zn and forms co-precipitates, thereby reducing the reabsorption of endogenous Zn as well as affecting availability of dietary Zn (Hardy, 1998).

2.8.2.1.2 Effect on protein digestibility

Phytate forms a strong complex with some proteins and resists their proteolysis. In general, the interaction of phytate with protein is dependent on pH (Cheryan, 1980). At a pH value lower than the isoelectric point of proteins (Cosgrove, 1966), phosphoric acid groups of phytate bind with the cationic group of basic amino acid, e.g., arginine, histidine, lysine, and form binary protein-phytate complexes. They are insoluble complexes that dissolve only below pH 3.5. Such complex formations may affect the protein structures that can hamper

enzymatic activity, protein solubility and protein digestibility (Kemme *et al*., 1999). *In vitro* studies have shown that phytate-protein complexes are less likely to be digested by proteolytic enzymes (Ravindran *et al*., 1995) and even digestive enzymes, such as pepsin, trypsin, chymotrypsis (Deshpande and Damodaran, 1989; Inagawa et al., 1987; Singh and Krikorian, 1982), lipase (Knuckles, 1988) and amylase (Deshpande and Cheryan, 1984; Knuckles and Betschart, 1987) are inhibited by phytate.

2.8.3.1.3 Effect on carbohydrate utilization

Phytate intake reduces the blood glucose response (glycemic index) (Lee *et al*., 2006). This may be because phytate forms complexes with carbohydrates of feedstuffs thereby reducing their solubility and adversely affecting the digestibility and absorption of glucose. Phytate may bind with starch either directly, via hydrogen bonds, or indirectly via proteins associated with starch (Rickard and Thompson, 1997). Moreover, the reduction in glucose response, i.e., low glycemic index, as a result of cereal and legume foods consumption may aid diabetics to control glucose (Thompson *et al*., 1987; Yoon *et al*, 1983).

2.8.3.2 Health benefits of Phytic acid

Its consumption provides protection against a variety of cancers mediated through antioxidation properties, interruption of cellular signal transduction, cell cycle inhibition and enhancement of natural killer (NK) cells activity. It has therapeutic use against diabetes mellitus, atherosclerosis and coronary heart disease and reduces kidney stone formation, HIV-1 and heavy metal toxicity; however, information on the dosage for humans for eliciting beneficial effects is limited (Kumar *et al*., 2010). L. U. Thompson (1998) suggested that the interaction among phytate, dietary starch and protein could be beneficially utilized in the treatment of diabetes and hyperglycemia. Indeed, it is possible to use phytic acid as an uncommon, versatile food preservative because of its anti-oxidant or iron-chelating properties (Hix *et al*., 1997). This apparent discrepancy between unhealthy and healthy properties of phytate clearly calls for a re-evaluation of this storage compound (Grases *et al*., 2001).

2.9 Reduction of anti-nutrients

Different processing techniques are often utilized in order to reduce anti-nutritional factors. Some techniques are performed on household level or domestically and others are performed on a larger scale in industry (Raes *et al.*, 2014). According to many studies in literature, soaking, cooking and boiling have generally achieved significant reduction of anti-nutrients.

Therefore foods high in anti-nutrients should be processed adequately in order to make them wholesome for consumption (ILeke, 2014). The term "Food Processing" covers an enormous field, from simple boiling to the use of irradiation. The types of cooking methods differ in countries around the world and also vary with the ethnic background of the family. Processing (cooking) can be both beneficial and detrimental to nutrient composition of foods. It is known that processing techniques may decrease the food value of some nutrients (Nestares *et al*., 1996): for example, there is some inevitable leaching of nutrients into the cooking water during processing. The cooking water may or may not be discarded, depending upon cultural and personal preference.

Processing method is one of the most common and widely used methods in the reduction of anti-nutrients from foods. Foods processing is aimed at reducing the toxic substances in food, increasing the palatability of foods, developing aroma, increasing the shelf life of foods, and minimizing the post-harvest loses. There are different kinds of processing method that are effective in reducing anti-nutritional factors in plant foods. These may include; extruder cooking, germination, roasting, soaking, boiling, fermentation, radiation (Tilahun, 2009).

On the other hand, cooking may enhance the nutritional quality of food by reducing or destroying the anti-nutrients present in it, as well as increasing the digestibility of proteins and starches. Elimination of inactivation of anti-nutritional compounds is absolutely necessary to improve the nutritional quality and effectively utilize human foods to their full potential. A typical example is the protein in legumes, which is made more digestible by heating because of inactivation of anti-nutrients such as trypsin inhibitors (Siddhuraju and Becker, 2001). The use of some processing methods, such as boiling, baking, microwave and pressure cooking are known to achieve reduction or elimination of anti-nutritional factors (Bhandari and Kawabata, 2006; Habiba, 2002; Khokhar and Chauhan, 1986; Udensi et al., 2007).

Extrusion cooking was found to be a versatile, quick and efficient method to reduce anti-nutrients when compared with other traditional processing methods (Alonso *et al.*, 2000). Soaking, sprouting, fermentation and cooking methods have also been investigated. Combination of cooking and fermentation improved nutrients quality and drastically reduced the antinutrient factors to safe levels much greater than any of the other processing methods tested (Obizoba and Atii, 1991). Excessive heat processing, however, should be avoid, since it adversely affects the protein quality of foods. It is therefore important that processing is

done within the recommended guidelines e.g. for heat, pH, as over processing will further destroy not only nutrient content but also taste and appearance (Morris *et al*., 2004).

2.9.1 Reduction of phytate

2.9.1.1 Mechanical treatment

There are numerous types of mechanical treatments, however, dehulling stands out. The technique, often used in cereal grains involves the removal of the bran (outer layer) from the grain and seeds. However, it upsets the location of the anti-nutrients and minerals due to cell degradation and in turn affects mineral bioacessability (Raes *et al*., 2014).

2.9.1.2 Soaking

This process is often used for legumes, grains and seeds. It is done by submerging biological material in a water at a specific temperature of 4-80^{o}C. Upon soaking, water is absorbed by cells and the pH changes, which results in the activation of endogenous enzymes. Soaking has had great results in phytate reduction, it also allows for minerals such as zinc and iron to be lost as an unwanted side effect (Raes *et al*., 2014).

2.9.1.3 Germination

Dry seeds are usually soaked in water which begins the process of a series of biochemical reactions. Other types of germination are malting and sprouting. Malting involves drying after germination in order to stop enzymatic reaction. Sprouting is a process in which soaked and drained seeds are left to germinate and sprout. Studies have shown that a long soaking period before fermentation or germination, leads to a reduction in phytate content and an enhancement of mineral biovailability (Liang *et al.*, 2008; Nunes *et al.*, 2005; Paulik *et al.*, 2005).

2.9.1.4 Use of Phytase

Various food processing and preparation techniques, along with the addition of exogenous enzyme, are the major efforts made to reduce the amount of phytate in foods. Hydrolysis of phytate during food processing (and then preparation, for example by germination, soaking, cooking and fermentation) is a result of the phytate-degrading activity of phytase, which is naturally present in plants and microorganisms. Thus, phytases have an important application in human nutrition both for degradation of phytate during food processing and in the gastrointestinal tract. However, the capability to dephosphorylate phytate differs greatly

among different plant and microbial species due to differences in their intrinsic phytate-degrading activities (Egli *et al*., 2002).

2.9.1.5 Fermentation

Fermentation is a process whereby microbial enzymes are synthesized and the pH altered. These are the two processes that allow the increase of shelf-life for the altering of sensory properties of the material to be fermented. The pH is either increased or decreased. Both situations affect endogenous and microbial enzyme activities which in turn affect the complexation of anti-nutrients with minerals. The most commonly used micro-organisms for fermentation are lactic acid bacteria as well as yeasts and fungi. Phytate degradation during fermentation has been recorded between 0 and 90%. This is due to the endogenous phytase activity of the plant matrix (Raes *et al*., 2014).

2.9.1.6 Heat processing

Thermal processing is a domestic technique that is used to reduce anti-nutritional factors in green leafy vegetables, however, it is temperature, pH and species dependent. Heat processing, specifically moist heat, such as boiling, autoclaving, extrusion, cooking and microwaving have been known to decrease vast levels of hydrogen cyanide as well as tannins and phytate by up to 40% (Inyang *et al*., 2013). Blanching has been recorded to reduce phytate and tannin levels in green leafy vegetables by 5-15%(Inyang *et al*., 2013). Cooking is a common form of processing in plants that are consumed as a food source. Cooking causes changes in the phytochemistry of the leafy vegetable affecting its bioaccessability and health benefit properties. The degree of these changes depends largely on the cooking methods as well as the type of vegetable (Odhav *et al.*, 2007).

Though the complete removal of phytic acid has not been shown, wet processing techniques can help to reduce phytic acid which in turn increases the availability of minerals in food (Mahesh *et al*., 2015). However, phytate being a heat stable component in plant food stuff, is not easily degraded whilst cooking; prolonged exposure to high temperature may lead to the inactivation of endogenous phytase enzyme (Kumar *et al*., 2010). Heat treatment causes cell wall to rupture and the leaching of soluble anti-nutrient into the cooking medium (i.e. water) (Essack *et al.*, 2018).

2.9.2 Reduction of Oxalate

Reduction of oxalate(soluble) was observed (i.e., 30% to 87% reduction) in boiled vegetables (Chai and Liebman, 2005). In carrots and spinach, the reduction in total oxalate corresponded to the amount of oxalate found in the cooking water. Similarly, (Jaworska, 2005; Judprasong et al., 2006; Savage, et al., 2000) discovered reduction of oxalate in a boiled vegetable from Thailand and New Zealand. Losses of soluble oxalates in Pakistan vegetables ranged from 16%-77% (mean 64.7%), the highest loss of soluble oxalates was observed when vegetables were boiled (Akhtar et al., 2010). Al-Wahsh *et al.* (2005) indicated that, different cooking methods have different effects on food oxalate. Bhandari and Kawabata (2006) pointed out; cooking treatments were found to be an effective measure to reduce oxalate content in wild yam tubers. Decreased in oxalate content was highest in boiling compared to pressure cooking and baking. Additionally, Linda and Massey (2007) reported that boiling vegetables may be a choice to decrease soluble oxalate, if the cooking water is not consumed, but baking potatoes or roasting peanuts or sesame seeds does not affect oxalate content. It was Noonan and Savage (1999) who reported that cooking has proved to be effective in terms of the reduction of total oxalate and thus high oxalate foods should be cooked to reduce the oxalate content.

Part III

Materials and methods

All chemicals used were reagent grade and distilled water was used throughout the work. All operations were performed at room temperature, unless otherwise stated.

3.1 Materials

3.1.1 Fresh *Sisnu* as raw material

Fresh *sisnu* (*U.plaviflora*) sample were collected from Sunsari district of Nepal. Its proximate content, vitamin C and antinutrient content were determined before and after cooking.

3.1.2 Apparatus Required:

i. Pans (stainless steel) "dekchi''
ii. Heating arrangement
iii. Thermometer
iv. Muslin cloth
v. Fire tong
vi. Gloves
vii. Glass wares
viii. Weighing balance.
ix. Grinder, mortar and pestle
x. Buchner filter assembly
xi. Silica crucible
xii. Linen cloth
xiii. Suction pump
xiv. Whatman filter paper (rapid)
xv. Hot air oven
xvi. Muffle furnace
xvii. Soxhlet Extraction apparatus
xviii. Kjeldhal protein analysis set
xix. Steam Distillation apparatus
xx. Magnetic stirrer
xxi. Desiccator

3.1.3 Chemicals Required:

i. Sodium bicarbonate ($NaHCO_3$).
ii. Magnesium oxide (MgO).
iii. Potassium metabisulphite
iv. Ascorbic acid standard.
v. Dye solution (2,6 dichlorophenol Indophenol)
vi. Metaphosphoric acid (3%)
vii. Sodium Hydroxide
viii. Sodium Carbonate
ix. Ammonium Hydroxide
x. Silver Nitrate
xi. Conc. Sulphuric Acid
xii. Catalyst mixture
xiii. Diethyl ether or petroleum ether
xiv. Hydrochloric acid
xv. Potassium Permanganate
xvi. Potassium ferric-cyanide
xvii. Potassium Iodide
xviii. Oxalic acid
xix. Phenolphthalein indicator
xx. Methyl orange indicator
xxi. Boric acid
xxii. Mixed indicator

3. 2 Methods

The young shoots of *Sisnu* were harvested manually with the help of fire tong and gloves which were put in black colored polythene bags to prevent the degradation of vitamin C from sunlight as vitamin C is liable to both heat and light. The leaves were sorted and graded according to maturity and uniformity. After completion of preliminary operations, fresh shoots were analyzed for proximate components (moisture, fat, protein and fiber) and also for vitamin C and antinutrient content. The samples were subjected to cooking by different methods.

The samples were divided into lots of 200 g weight. The samples were given heat treatments by:

(i) submerging in boiling water for 5 and 15 minutes, draining followed by cooling (HW-5 and HW-15) and

(ii) exposing under live steam for 5 and 15 minutes, draining followed by immediate cooling (S-5 and S-15).

For the steam cooking, the sample was introduced into the steam cooker when the vigorously boiling water was generating adequate steam. The best cooking method was choose based on retention of vitamin C and reduction of anti-nutritional factor (oxalate and phytate content) over the fresh samples as a control.

3.3 Methodology

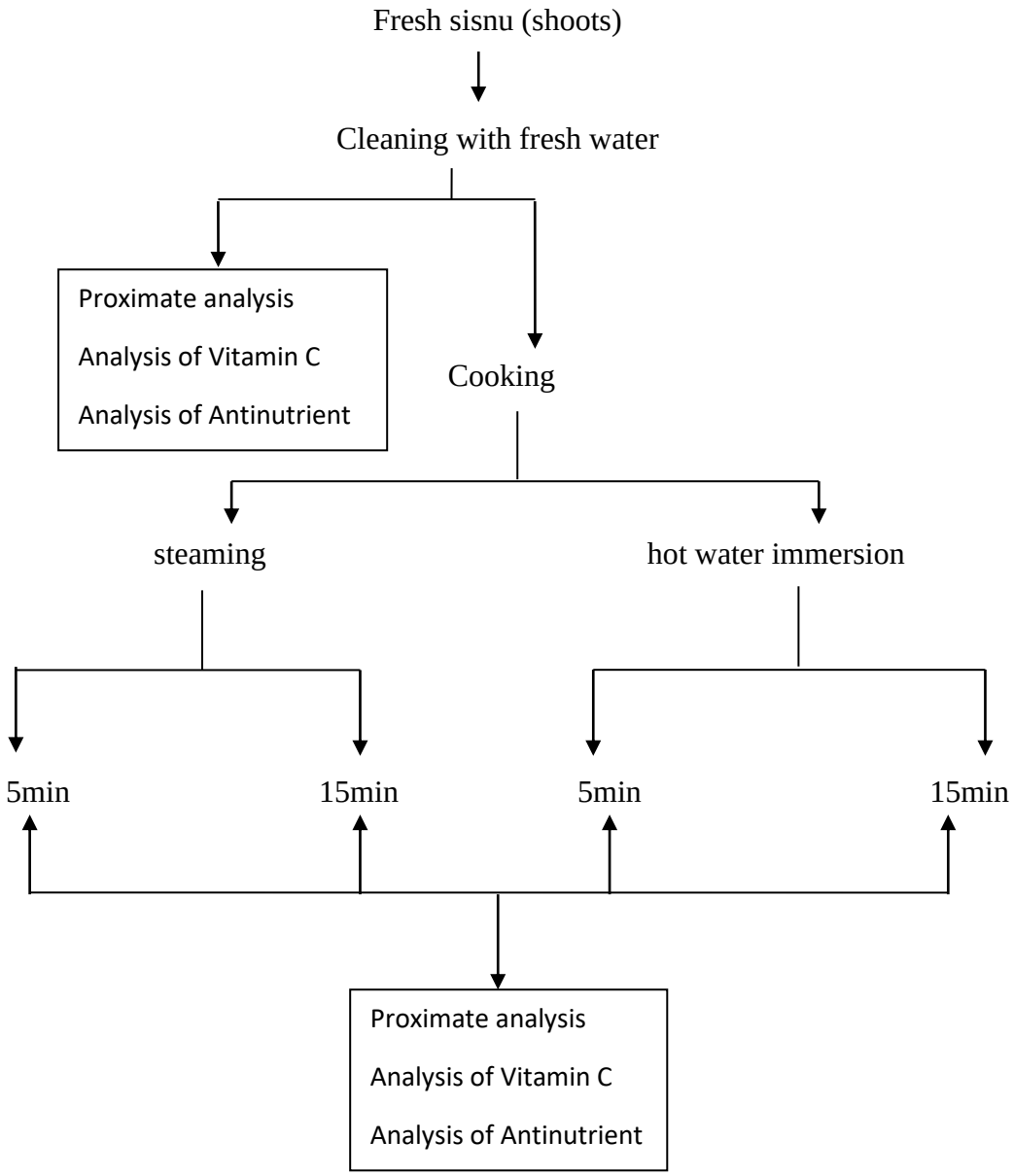

Fig 2.4 Flow diagram showing experimental methodology

3.4 Analytical procedures

3.4.1 Determination of moisture

Moisture content was determined by drying in electric hot air oven as described in (Ranganna, 2010).

3.4.2 Determination of total ash

The ash content was determined by incinerating the dried Shoot samples (2 g) in a muffle furnace at 600 ^{o}C for 12 h (Ranganna, 2010).

3.4.3 Determination of crude protein

Crude protein was determined by the Kjeldahl method, total protein was calculated by multiplying the nitrogen content by a factor of 6.25 (Ranganna, 2010).

3.4.4 Determination of fat

The total fat content was obtained by exhaustive extraction of shoot samples (10 g) with n-hexane using a Soxhlet extractor (Ranganna, 2010).

3.4.5 Determination of crude fiber

For crude fiber, fat-free samples were digested with 0.128 M H_2SO_4 followed by 0.313 M NaOH. The insoluble residue was then washed with hot water and dried at 130 ºC and weighed to constant mass. The dried residue was incinerated at 600 ºC for 3 h and the ash was weighed to determine the crude fiber content (Ranganna, 2010).

3.4.6 Determination of total carbohydrate and energy value

Total carbohydrate was obtained by difference, and the energy value was determined using equation.

Energy value per 100g = [Carbohydrate x 4 + Protein x 4 + Fat x 9] Kcal

3.4.7 Estimation of vitamin C

Vitamin C was determined by titration method as per (Ranganna, 2010).

The vitamin C content in the sample was calculated as follow

$$\text{Vitamin C } \left(\frac{\text{mg}}{100\text{g}}\right) = \frac{\text{Titer} \times \text{Dye factor} \times \text{Volume made up} \times 100}{\text{Aliquot of extract taken (ml)} \times \text{wt. of sample (g)}}$$

3.4.8 Determination of oxalate

The oxalate content was determined by the method of Day and Underwood (1986). Ground sisnu shoot (1g) was mixed with 50 ml of 3 M sulphuric acid in a conical flask and stirred for 1 hour using a magnetic stirrer. The mixture was filtered and a 25 mL of aliquot of the filtrate was titrated against 0.05M $KMNO_4$ solution until violet color persisted at least for 30 seconds. The oxalate content of the sample was calculated using the following equation.

1 ML 0.05 $KMNO_4$= 2.2 mg oxalate

Calculation,

$$\text{Oxalic acid(mg oxalate/gram)} = \frac{Volume\ of\ KMNO4\ consumed x 2.2\ x 50}{sample\ Weight\ x 25}$$

3.4.9 Phytate content

The phytate content was determined by the method of M. B. Reddy and Love (1999) with some modifications. *Sisnu* leaves (4g) was soaked in 100 ml of 2 % hydrochloric acid for 3 hours and then filtered. A 25 ml of aliquot of filtrated was taken and 0.3% ammonium thiocyanate solution was added as an indicator and titrated against a standard $Fecl_3$ solution till a brownish yellow color persisted for 5 min. The phytate content of the sample was calculated using the following equation.

Phytate (mg/kg) = volume of $Fecl_3$ consumed × 5.64

3.5 Data Analysis

The experimental data were analyzed using Two-way ANOVA- with no blocking and with replication using GenStat Discovery Edition. The difference between the means was compared using Least Significant Difference (LSD) method at 5% level of confidence.

PART IV

RESULTS AND DISCUSSION

The objective of this research was to study the effects of various processing methods on proximate composition, oxalate, phytates and vitamin C contents of *sisnu* to reduce the synergistic hazardous effect of these anti-nutrients on health. The processing treatments applied were simple household boiling and steaming method. A common variety of the sisnu *Urtica plaviflora* collected from Bishnupaduka was used in this study.

4.1 Chemical composition of fresh sisnu (*Urtica plaviflora*)

Various parameters of fresh *sisnu* were analyzed and the results are shown in Table 4.1.

Table 4.1 Chemical composition of fresh *sisnu* shoots

Parameters	**Values[a]**
Mositure (%)	82.4 ± 0.7
Fat (%)	3.11 ± 0.18
Crude fiber (%)	8.22 ± 0.08
Ash (%)	17.86 ± 0.5
Protein (%)	31.31 ± 1.87
Ascorbic acid (mg/100g)	33.14 ± 1.58
Carbohydrates (%)	39.47±2.25
Oxalic acid (mg/100g)	1471.32 ± 109.56
Phytate (mg/100g)	2.99± 0.28

*[a]Values are the mean ± sd of three determinations. Except moisture, all values are expressed on dry basis

4.2 Effect of cooking methods

For studying the effects of cooking methods on sisnu, samples were subjected to cooking by different method such as steaming for 5 and 15 min and boiling water cooking at 100 ^{o}C for 5 and 15 mins. After cooking, samples were analyzed to study the reduction pattern of anti-nutrients.

4.2.1 Effect of heat treatments on the proximate composition of sisnu

The effect of different cooking methods on the proximate composition of the sample is shown in the table 4.2. The moisture content of the fresh sample was determined to be 82.402% which was closer to the finding of Mishra and Kharel (2010) and Aryal (2011). Steam cooking for 5 and 15 min resulted in the increase of moisture content to 84.3% and 85.42% respectively, Whereas, moisture content was 89.4 and 91.7% when cooked in hot water for 5 and 15 min respectively. The increase in moisture content could be as a result of water absorption by the fibers and other natural chemical component of the vegetables.

Table 4.2 Effect of cooking methods on the proximate composition of sisnu

Treatment	moisture	Protein(db)	Fat(db)	Fiber(db)	Ash(db)
Fresh	82.402 ± 0.7[a]	31.314 ± 1.877[a]	3.113 ± 0.179[a]	8.228 ± 0.079[a]	17.868 ± 0.5[a]
S-5	84.3 ± 0.953[b]	30.586 ± 0.914[a]	3.063 ± 0.087[a]	8.227 ±0.211[a]	17.862 ± 0.04[a]
S-15	85.42 ± 0.584[b]	30.92 ± 0.953a	3.056 ± 0.163[a]	8.216 ± 0.138[a]	17.877 ± 0.08[a]
HW-5	89.4 ± 0.8[c]	29.781 ± 0.835[a]	3.044 ± 0.156[a]	8.217 ± 0.086[a]	17.834 ± 0.133[a]
HW-15	91.7 ± 0.964[d]	29.58 ± 0.751[a]	3.049 ± 0.07[a]	8.2 ± 0.07[a]	17.767 ± 0.078[a]

*Values are means of triplicate

*means having similar super script in a column are not significantly different by LSD at 5% significance

In the fresh sample, protein content was found to be 31.314% (db). Similar results of protein content were also reported by Aryal (2011) (30.25 %) and Mishra and Kharel (2010) (34.43%) which was higher than the result of this study. From Table 4.2 protein content did not reduced significantly ($p>0.05$) in both steam and water cooking (at 100 ^{o}C) for 5 and 15 min. Steaming for 5 min and 15 min, protein was reduced to 30.586% and 30.92%, respectively. Statistical analysis showed that, there was no significant effect ($p<0.05$) of steaming on the protein content of the sample. The reduction of protein while steaming for 5 and 15 min was 0.91% and 0.95%, respectively. Similar result was also reported by Zhang *et*

al. (2011). Steaming of bamboo shoots for 10 min has shown no effect on the level of the protein content, while hot water cooking for 5 min and 15 min, protein was reduced to 29.781% and 29.58%, respectively. Statistical analysis shows that, there was no significant effect ($p<0.05$) of hot water cooking on the protein content of the sample. Acho *et al.* (2015) has reported the retention of protein in various vegetables (*Basella alba, Solanum melongena, Corchorus olitorius*) during steam blanching for 15 min. Likewise, hot water boiling of vegetables (Amaranth, black nightshade) for 30 min showed retention of protein content (Traore *et al.*, 2017). Kala and Prakash (2004) reported no significant changes in the protein content of green leafy vegetables (Amaranth, Kilkeerai, shepu, spinach) during conventional cooking.

In the fresh sample, fiber content was found to be 8.228% (db). Similar results of fiber content were also reported by Aryal (2011) (7.71%). After steam cooking for 5 min and 15 min, fiber content was 8.227 and 8.216%, respectively. Statistical analysis showed that, there is no significant effect ($p<0.05$) of steam cooking on the fiber content of the sample. Acho *et al.* (2015) had reported insignificant changes in the fiber content of leafy vegetables (*Corchorus esculenta, Basella alba, Solanum melongena, Talinum triangulare*), on steam blanching for 15min. . After water cooking for 5 min and 15 min, fiber content was 8.21% and 8.2%, respectively. Statistical analysis shows that, there was no significant effect ($p<0.05$) of hot water cooking on the fiber content of the sample. Kala and Prakash (2004) had also reported insignificant changes in the fiber content of green leafy vegetables (Amaranth, Kilkeerai, shepu, spinach) on cooking.

In the fresh sample, crude fat content was found to be 3.11% (db). Similar results of crude fat content was also reported by Mishra and Kharel (2010) (3.3%). After steam cooking for 5 min and 15 min, crude fat content was 3.063% and 3.056%, respectively. Statistical analysis showed that, there was no significant effect ($p<0.05$) of steam cooking on the crude fat content of the sample. Water cooking for 5 min and 15 min, also did not showed the significant ($p<0.05$) change in the crude fat content, (3.044% and 3.049%, respectively). Similar results were reported by Ilelaboye *et al.* (2013), were insignificant changes in the crude fat content on the various green leafy vegetables (*Amarranthus hybridus,Telifera occidentalis, Solanum nigrum, Cnidoscolus acontifolus*) were reported.

Ash content of the fresh sample was found to be 17.868% (db). Similar results of ash content was reported by Mishra and Kharel (2010). After steam cooking for 5 min and 15 min, crude

fat contents were 17.862% and 17.877%, respectively. Water cooking for 5 min and 15 min, also did not showed the significant($p<0.05$) change in the ash content (17.834% and 17.767%, respectively). There was no cooking-related significant difference($p<0.05$) in the ash content of the sample. Similar result was found in *Amaranthus cruentus* when cooked for 15 min (Kamela *et al.*, 2016). Traore *et al.* (2017) reported that there were no significant changes in the ash content of Amaranth and Black night shade when blanched and cooked.

4.2.2 Effect on the vitamin C content

The vitamin C contents in control, 5 min boiling water (HW-5), 15 min boiling water (HW-15), 5 min live steam (S-5) and 15 min live steam (S-15) treated sisnu samples were 34.142, 15.689, 11.4, 28.883 and 25.01 mg/100g db respectively. From the fig 4.3, vitamin C content seems to have reduced significantly ($p>0.05$) in both steam and water cooking (at 100^oC) for 5 and 15 mins. Statistical analysis showed that methods of cooking had a significant effect on the vitamin C reduction. LSD indicated that the vitamin C contents among control, 5 min steam, 15 min steam, 5 min water cooked and 15 min water cooked were significantly different ($p>0.05$), while boiling water treated samples had significantly lower vitamin C contents compared to control and steam treated ones. In both the boiling water and steam treatments, treatment time had significant ($p>0.05$) effect on vitamin C retention.

Larger variation were found in the different studies in regards to the content of vitamin C in fresh Sisnu. Rutto *et al.* (2013) had reported the amount of vitamin C to be 1.1 mg/100g (wet basis) which was comparatively lower than the observation of our study. Mahlangeni *et al.* (2015) reported that *Urtica dioica* contained 26.2 mg/100g (DM) of vitamin C, which was comparatively less than the current observation. However, Thapaliya (2010) reported that fresh sisnu contained 33.12 mg/100g (DM) of vitamin C, which was similar to the current observation.

While steaming for 5 min and 15 min, ascorbic acid was reduced to 28.88 mg/100g and 25 mg/100g db, respectively. Statistical analysis showed that, there was a significant effect ($p>0.05$) of steaming on the vitamin C content of the sample. The reduction of vitamin C while steaming for 5 and 15 min was 12.84% and 24.53%, respectively. The reduction percentage obtained from the study was similar to that of observation by Zeng (2013) in spinach (11.1% while steaming for 5 min), whereas, steaming of *Amaranthus hybridus* by Adefegha and Oboh (2011) for 10 min showed reduction of ascorbic acid by 29.2% which was higher than the present observation (28.53%). LSD indicated that steaming time (5 min

and 15 min) had a significant effect on the reduction of vitamin C content. It is well established that vitamin C content destroyed during cooking due to the fact that it is not stable at high temperature (Adefegha and Oboh, 2011).

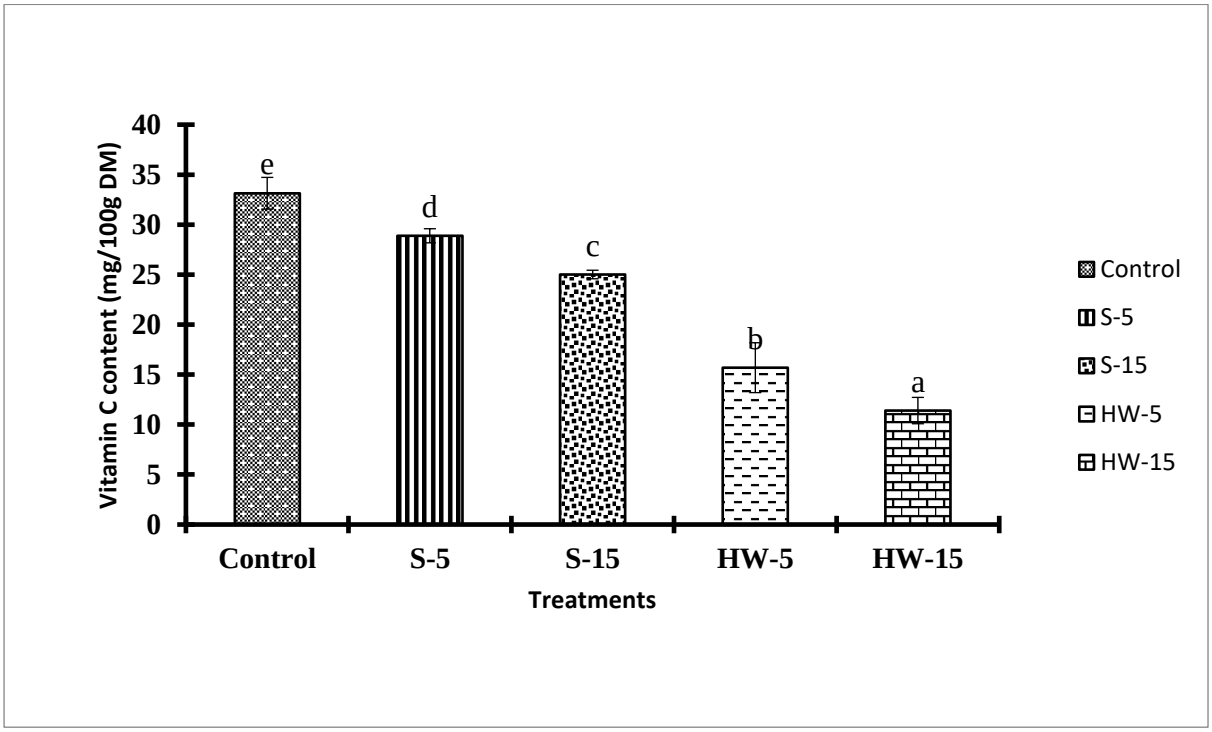

Fig 4.1 Effect of cooking on Vitamin C content

Note: Control – fresh sisnu, S-5 – steamed for 5 min, S-15 – steamed for 15 min, HW-5 – boiled for 5 min and HW-15 – boiled for 15 min

While hot water cooking for 5 min and 15 min, ascorbic acid was reduced to 15.68 mg/100g and 11.39 mg/100g db, respectively. Statistical analysis showed that, there was a significant effect ($p>0.05$) of hot water cooking on the vitamin C content of the sample. The reduction of vitamin C during hot water cooking for 5 and 15 min were 52.66% and 65.6%, respectively. The reduction obtained from the study was similar to the observation made by Zeng (2013) in spinach (50.5% on cooking for 5 min). Observation by Kala and Prakash (2004) reported that conventional cooking (boiling for 22 min) resulted in the reduction of vitamin C by 66.21%. Vitamin C being water soluble leaches into cooking water and gets degraded (Igwemmar *et al.,* 2013). LSD indicated that boiling water cooking time (5 and 15 min) had significant effect on the reduction of vitamin C content.

Vitamin C is easily oxidized, especially in aqueous solutions and losses are enhanced by higher temperatures, physical damage and relative humidity (Lee and kader, 2000). A

prolonging of the residence time resulted in additional losses by thermal destruction. The loss of vitamin C at cooking time was mainly due to the enzymatic destruction (Burg and Fraile, 1995).

4.2.3 Effect of cooking methods on anti-nutrients

4.2.3.1 Effect on the phytate content

Effects of different cooking methods on phytate content of sisnu are shown in Fig. 4.1. The phytate contents in control, 5 min boiling water (HW-5), 15 min boiling water (HW-15), 5 min live steam (S-5) and 15 min live steam (S-15) treated sisnu samples were 2.991, 2.246, 2.189, 2.913 and 2.789 mg/100g DM respectively. Akubugwo *et al.* (2007) reported that phytate content in *Amaranthus hybridus* leaves was 1.32 mg/100g DM which was comparatively lower than found in this study. Statistical analysis showed that methods of cooking had a significant effect on the phytate reduction. LSD indicated that the phytate contents among control, 5 min steam and 15 min steam treated samples were not significantly different ($p>0.05$), while boiling water treated samples had significantly lower phytate contents compared to control and steam treated ones. In both the boiling water and steam treatments, treatment time had no effect on phytate reduction.

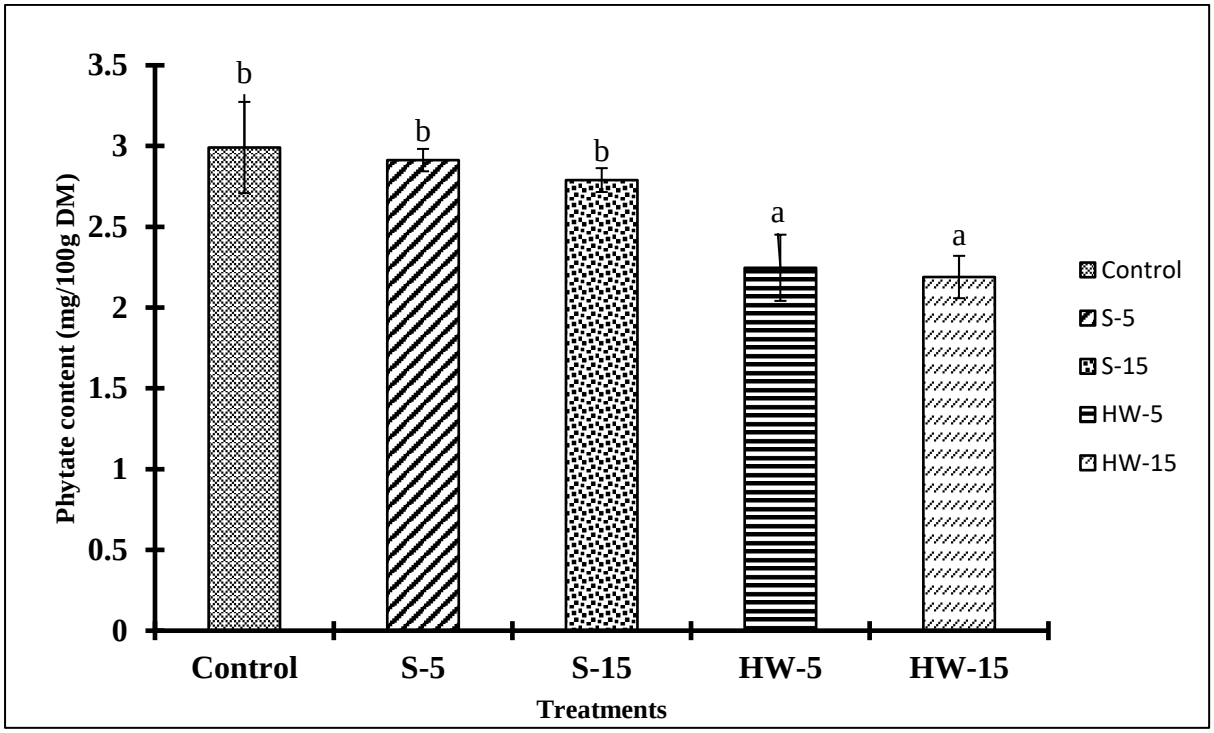

Fig 4.2 Effects of cooking on phytate content

Note: Control – fresh sisnu, S-5 – steamed for 5 min, S-15 – steamed for 15 min, HW-5 – boiled for 5 min and HW-15 – boiled for 15 min

Steam cooking (1 kg/cm^2) of cow pea (*Vigna unguiculata*) has shown significant reduction in the phytate content (Deol and Bains, 2010). But Phytate, being a heat stable component in plant, is not easily degraded whilst heat treatment (Kumar *et al*., 2010). However, Daneluti M and Matos (2013) has observed that thermal decomposition of phytic acid occurred when heated to 150 ^oC for around one hour.

While hot water immersion cooking for 5 min and 15 min, phytate was reduced to 2.246 mg/100g and 2.189 mg/100g(DM), respectively. Statistical analysis shows that there was a significant effect ($p<0.05$) of hot water cooking on the phytate content of the sample. Similar reduction of phytate was also observed when Chaya leaf (*Cnidoscolus aconitifolius*) was boiled at 100^oC for 15min (Babalola and Alabi, 2015). LSD indicated that there exists no significant difference between the time variation of hot water immersion cooking. Similar results were obtained for screening of anti-nutrient on traditional south African leafy vegetables when cooked for 5 and 15 min (Essack *et al.*, 2018). The reason could be that during cooking, endogenous phytases are inactivated by the heat and are broken down with high temperatures (Amalraj and Pius, 2015).

LSD indicated that there is significant loss of anti-nutrient on hot water immersion cooking compared to that of steaming. This might be due to the fact that, phytate is a heat stable component in plant food stuffs. The observed degradation of phytate might be attributed to its water-soluble nature, so a considerable amount of phytate is removed to the water (Kumar et al., 2010). The interaction of the leaves with the hot water causes the cell wall to be ruptured and soluble phytic acid may leach into the medium which can account for phytic acid losses (Yadav and Sehgal, 2003).

The phytic acid concentration was minimal in all the sample. This was consistent with work done by Akubugwo *et al.*, (2007). Phytate was reported much lower than work done by Akwaowa *et al.* (2000) on traditional leafy vegetables (*Telfairia occidentalis*). Similarly, blanching reduced the phytic acid content of leaves (Yadav and Sehgal, 2003).

4.2.3.2 Effect on the oxalate content

Effects of different cooking methods on oxalate content of sisnu are shown in Fig. 4.2. The oxalate contents in control, 5 min boiling water (HW-5), 15 min boiling water (HW-15), 5

min live steam (S-5) and 15 min live steam (S-15) treated sisnu samples were 1471.328, 763.491, 704.949, 1162.713 and 1051.514 mg/100g db respectively. Statistical analysis showed that methods of cooking had a significant effect on the oxalate reduction. LSD indicated that the oxalate contents among control, 5 min steam and 15 min steam treated samples were significantly different ($p>0.05$), boiling water treated samples had significantly lower oxalate contents compared to control and steam treated ones. In both the boiling water and steam treatments, treatment time had no effect on oxalate reduction. But cooking method showed significant difference on the reduction of the oxalate.

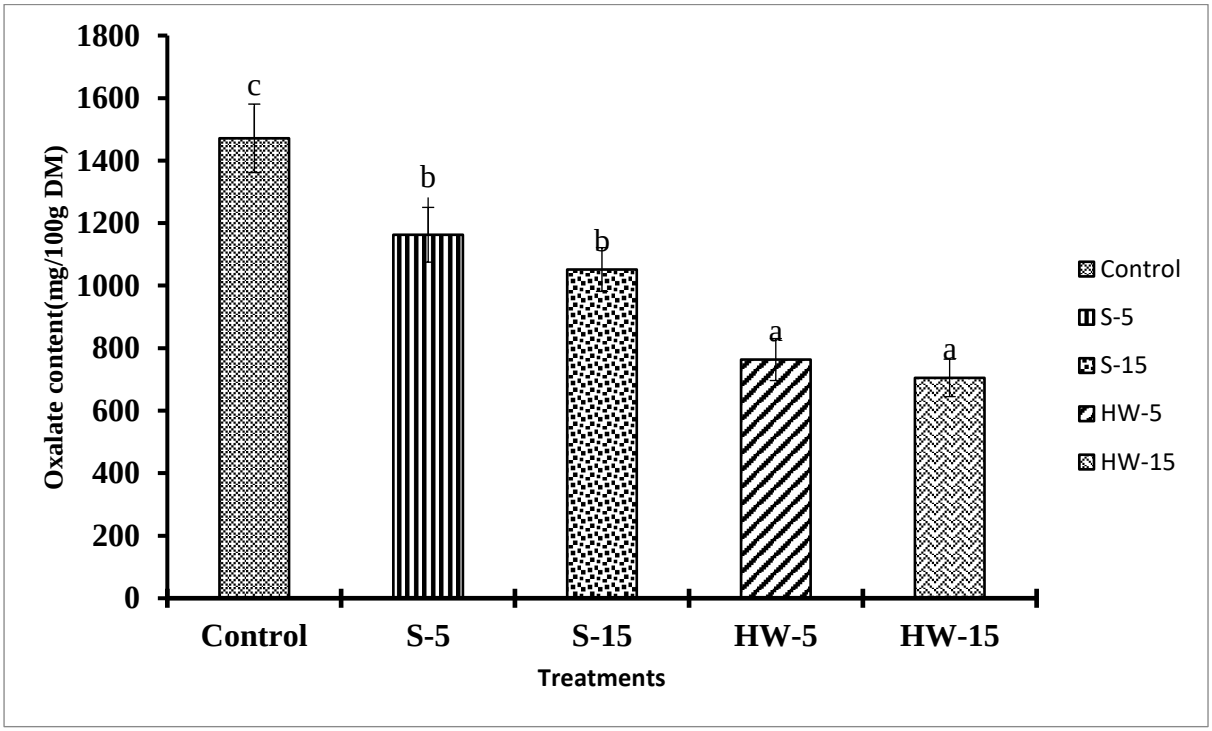

Fig 4.3 Effect of cooking on oxalic acid content

Note: Control – fresh sisnu, S-5 – steamed for 5 min, S-15 – steamed for 15 min, HW-5 – boiled for 5 min and HW-15 – boiled for 15 min

In the fresh sample (control), oxalate content was found to be 1471.328 mg/100g db. Observation by Prakash and Gupta (2011) suggests that *Amaranthus paniculatus* contains 928.1 mg/100g(DM) of oxalate, which is less than the content of control of this study. However, Beiquan (2008) reported to contain oxalate in spinach ranging from 53.4 to 116.2 mg/g (DM) which is much higher than that of fresh sisnu sample. Total oxalate contents of leaves, shoots and roots of the fresh Thai vegetables ranged from 249.5 ± 12.1 to 7597.9 ± 77.6 mg oxalate/100g DM (Juajun *et al.*, 2012). According to Juajun *et al.* (2012), among various Thai vegetables, *Sesbania grandiflora* and *Lobelia begonifolia* had similar oxalate

contents (1254.2 and 1549.8 mg/100g as of fresh sample) which is similar to the control (1471.328 mg/100g) of this study. Observation of various Underutilized green leady vegetables of North India by Gupta and Yadav (2016) shows the range of oxalate content from 101.82 to 825 mg/100g(dry basis), which is less than that of the fresh sisnu sample.

While steaming for 5 min and 15 min, oxalate was reduced to 1162.713 mg/100g and 1051.514 mg/100g, respectively. Statistical analysis showed that, there was a significant effect ($p<0.05$) of steaming on the oxalate content of the sample. Steaming time (5 min and 15 min) had no significant difference on the reduction of oxalate content. Reduction of oxalate was similar to that of observation on *Basella alba* (steam blanched for 15 min) by Acho *et al.* (2015), whereas, loss of oxalate during steaming was also found to be similar to the range of investigation done by Chai and Liebman (2005).

While hot water immersion cooking for 5 min and 15 min, oxalate was reduced to 763.4914 and 704.9493 mg/100g(DM), respectively. Statistical analysis showed that there was a significant effect ($p<0.05$) of hot water cooking on the oxalate content of the sample. LSD indicated that there was no significant difference between the time variation of hot water immersion cooking. Hot water immersion cooking was observed to be effective even only at 5 minutes of boiling. Similar result was observed by Essack *et al.* (2018), most of the vegetables, specifically; *Physalis viscosa, Amaranthus hybridus, Chenopodium album* and *Guilleminea densa* required only 5 minute boiling to reduce their oxalic acid content significantly and did not showed significant reduction of oxalic acid between 5 and 15 mins of boiling.

4.2.4 Percentage loss of vitamin C, phytate and oxalate

In this study, reduction of phytate ranges from 2.59 - 26.79 % on steam and boiling cooking. Similar result was obtained on boiling of cowpea (21 % reduction) (Akinyele, 1989). The report of this study is lesser than that reported by (Aye, 2012). These percentage losses were within the range to those (7 – 56%) of phytates obtained for blanched leafy vegetables from Thailand (Weenanan *et al.,* 2008). Even 5 min of hot water immersion cooking showed significant reduction of phytate in this study. Likewise, five-minute boiling was adequate to eliminate the phytic acid content in *Solanum nigrum, Momordica balsamina, guileminea densa, Galinsoga parviflora, Emex australis* and *Amaranthus dubius* whereas *Physalis viscosa* and *Asystasia gangetica* required a total of fifteen minutes boiling to completely eliminate the phytic acid content (Essack *et al.*, 2018). On this study, there was no significant

difference on the reduction of phytate on the different cooking time. Similarly, there was no significant difference in the phytic acid between 5 and 15 minute boiling in all leafy vegetables except for *A. hybridus*. *A. hybridus* attained a significant effect in the decrease of phytic acid content after 15 minute boiling (Essack *et al.*, 2018).

Table 4.3 Percentage loss/reduction of phytate, oxalate and vitamin C

Treatment	phytate loss %	oxalate loss %	vitamin C loss %
S-5	2.59 ± 2.31	20.97 ± 5.95	12.84 ± 2.13
S-15	6.73 ± 2.47	28.53 ± 4.75	24.53 ± 1.29
HW-5	24.88 ± 6.85	48.1 ± 4.55	52.66 ± 7.52
HW-15	26.79 ± 4.38	52.087 ± 4.07	65.6 ± 3.97

Note: Control – fresh sisnu, S-5 – steamed for 5 min, S-15 – steamed for 15 min, HW-5 – boiled for 5 min and HW-15 – boiled for 15 min

In this study, reduction of oxalate ranges from 20.97 – 52.08%. From Table 4.3 reduction percentage of oxalate during steam and hot water cooking is similar to the result obtained by Acho *et al.* (2015), where loss of oxalate during steam blanching for 15, 25 and 45 min ranges from 2.16 – 42.62% in different leafy vegetables (*Basella alba, Colocasia esculenta, Corchorus olitorius, Solanum melongena* and *Talinum triangulare*) consumed in Ivory coast. During steaming reduction of oxalate ranged from 5 - 53% (Chai and Liebman, 2005). Five-minute steam and hot water cooking was effective on the reduction of the oxalate. Similar result was observed by Essack *et al.* (2018), most of the vegetables, specifically; *Physalis viscosa, Amaranthus hybridus, Chenopodium album* and *Guilleminea densa* required only 5 minute boiling to reduce their oxalic acid content significantly. There was no significant difference on the reduction of the oxalate at different cooking time in this study. Similarly, Essack *et al.* (2018) did not showed significant reduction of oxalic acid between 5 and 15 mins of boiling.

From table 4.3 The reduction of vitamin C while steaming for 5 and 15 min was 12.84% and 24.53%, respectively. The reduction percentage obtained from the study was similar to that of observation by Zeng (2013) in spinach (11.1% while steaming for 5 min), whereas, steaming of *Amaranthus hybridus* by Adefegha and Oboh (2011) for 10 min showed reduction of ascorbic acid by 29.2% which was higher than the present observation (28.53%). Steaming

time (5 min and 15 min) had a significant effect on the reduction of vitamin C content. It is well established that vitamin C content destroyed during cooking due to the fact that it is not stable at high temperature (Adefegha and Oboh, 2011). The reduction of vitamin C during hot water cooking for 5 and 15 min were 52.66% and 65.6%, respectively. The reduction obtained from the study was similar to the observation made by Zeng (2013) in spinach (50.5% on cooking for 5 min). Observation by Kala and Prakash (2004) reported that conventional cooking (boiling for 22 min) resulted in the reduction of vitamin C by 66.21%. Vitamin C being water soluble leaches into cooking water and gets degraded (Igwemmar *et al.*, 2013).

PART V

Conclusions and Recommendation

5.1 Conclusions

From this research work following conclusions were drawn.

1. The mean value of moisture, fat, crude fiber, ash and protein were found to be 82.4, 3.11, 8.22, 17.86 and 31.31% respectively on the dry basis.
2. The mean values of phytate, oxalate and vitamin C contents in the *Sisnu* were found to be 2.99, 1471.328 and 33.142 mg/100g respectively on the dry basis.
3. There were insignificant changes in the proximate composition in steam and hot water cooking.
4. Maximum reduction of Phytate (26.79%) and oxalate (52.08%) were found when boiled for 15 min in plain water.
5. Retention of vitamin C was found maximum while steaming for 5 min (12.8% loss) compared to hot water cooking for 5 min (52.6% loss).
6. Hot water cooking was efficient on reducing the anti-nutrient factor but there was higher loss of vitamin C. whereas, steam cooking was more preferable for the retention of vitamin C.

5.2 Recommendations

The following recommendations are made for further study:

1. Steam blanching for drying and further processing of sisnu is better than hot water blanching.
2. Study on the effect of salt and spices on anti-nutrient content of sisnu during cooking
3. Study on the effect of fermentation in the anti-nutrient content of *Sisnu.*

PART VI

Summary

Stinging nettle (*urtica dioica, L. Urticaceae*) is a ubiquitous herb which is available in large part of the world. *Urtica dioica* is a moderately shade-tolerant species, which occurs on moist or damp, weakly acid or weakly basic, rich fertile soils. Its stems and leaves are densely covered with stinging hairs, which release potentially pain-inducing toxins, is rarely eaten by castles and rabbits (Taylor, 2009). The plant *Sisnu* (*Urtica plaviflora*) is evenly distributed in Himalayas especially in middle and lower zone between 450 to 3500 m from sea level (Watanabe et al., 2013). Stinging nettle locally called *sisnu* is an important traditional food item along with an important medicinal plant (Panta and Sundriyal, 2016). The young shoots of *Sisnu* were harvested manually from Bishnupaduka, Dharan-20, Sunsari district, Nepal with the help of a fire tong and gloves which were put in black colored polythene bags to prevent the degradation of vitamin C from sunlight as vitamin c is liable to both heat and light. The leaves were sorted and graded according to maturity and uniformity. After completion of preliminary operations, fresh shoots were analyzed for proximate components (moisture, fat, protein and fiber), vitamin C and antinutrient content. And the Changes in Nutritional Quality of *Sisnu* in Common Household Cooking Methods were studied on the basis of loss of anti-nutrient factors and retention of vitamin C in different cooking practices at different time.

The samples were divided into sets of 200 g weight. Sample lot were Submerged in boiling water for 5 and 15 minutes, drained and cooled (HW-5 and HW-15) and other sample lot were Steamed over boiling water in a water bath for 5 and 15 minutes, drained and cooled immediately (S-5 and S-15).

Phytate, oxalate and vitamin C content in the raw *Sisnu* were found to be 2.99, 1471.32 and 33.14 mg/100g respectively on dry basis. Among the common household cooking method, boiling at 15 min showed higher reduction of phytates (2.18 mg/100g) and oxalates (704.94 mg/100g) but vitamin C (11.4 mg/100g) is found to be lost. Therefore, on the basis of reduction of phytate (26.8%) and oxalate (52.08%), boiling at 15 min was preferred but loss of vitamin C (65.6%) was prominent. From the statistical analysis, it was found that, different cooking condition had significant effect on reduction of phytate and oxalate. But Steaming for 5 (2.91 mg.100g db) and 15 min (2.78 mg/100g db) didn’t showed the significant reduction of phytates. Deterioration of vitamin C while boiling was higher than steaming.

Boiling for 5 and 15 min reduced vitamin C to 15.68 and 11.39 mg/100g respectively on dry basis. But retention of vitamin C was found to be higher while steaming for 5 min (28.88 mg.100g db) and 15 min (25.01 mg/100g db). Whereas, there was significant reduction in oxalate content on both steaming for 5 (1162.713 mg/100g db) and 15 min (1051.514 mg/100g db). But boiling for 5 (763.49 mg/100g db) and 15 min (704.49 mg.100g db) showed higher reduction of oxalate content.

Boiling for 15 min shows higher reduction of antinutrient but destruction of vitamin C is higher. Among, all the cooking methods steaming for 15 min shows less reduction of vitamin C and significant loss of oxalate. Despite there is no significant loss in phytate level. It is within the safe level. So, steaming for 15 min is considered better cooking method on the basis of loss of antinutrient and retention of vitamin C.

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Appendices

Appendix A

Mean values for anti-nutritional factors during cooking of sisnu.

Table A.1 Mean value of phytate content (mg/100g on dry basis)

Treatment	Mean value
Fresh	2.991^{b}±0.282
S-5	2.913^{b}±0.069
S-15	2.789^{b}±0.074
HW-5	2.246^{a}±0.205
HW-15	2.189^{a}±0.131

*The values are mean values of triplicate determination ± standard deviation.

Mean values within a column and a row with different superscripts are significantly different at $p<0.05$. LSD between samples = 0.3143

Table A.2 Mean value of oxalate content (mg/100g on dry basis)

Treatment	Mean value
Fresh	1471.328^{c}±109.563
S-5	1162.713^{b}±87.566
S-15	1051.514^{b}±69.943
HW-5	763.491^{a}±67.086
HW-15	704.949^{a}±59.98

*The values are mean values of triplicate determination ± standard deviation.

Mean values within a column and a row with different superscripts are significantly different at $p<0.05$. LSD between samples = 147

Table A.3 Mean value of Vitamin C content (mg/100g on dry basis)

Treatment	Mean value
Fresh	33.142^{e}±1.583
S-5	28.883^{d}±0.706
S-15	25.01^{c}±0.428
HW-5	15.689^{b}±2.493
HW-15	11.4^{a}±1.318

*The values are mean values of triplicate determination ± standard deviation.
Mean values within a column and a row with different superscripts are significantly different at $p<0.05$. LSD between samples = 2.716

Table A.4 Mean value of protein content (% on dry basis)

Treatment	Mean value
Fresh	31.314^{a}±1.877
S-5	30.586^{a}±0.914
S-15	30.92^{a}±0.953
HW-5	29.781^{a}±0.835
HW-15	29.58^{a}±0.751

*The values are mean values of triplicate determination ± standard deviation.
Mean values within a column and a row with different superscripts are significantly different at $p<0.05$. LSD between samples = 2.08

Table A.5 Mean value of fiber content (% on dry basis)

Treatment	Mean value
Fresh	8.2^{a}±0.079
S-5	8.216^{a}±0.211
S-15	8.218^{a}±0.138
HW-5	8.227^{a}±0.086
HW-15	8.229^{a}±0.069

*The values are mean values of triplicate determination ± standard deviation.
Mean values within a column and a row with different superscripts are significantly different at $p<0.05$. LSD between samples = 0.2338

Table A.6 Mean value of crude fat content (% on dry basis)

Treatment	Mean value
Fresh	3.045^{a}±0.179
S-5	3.05^{a}±0.087
S-15	3.056^{a}±0.163
HW-5	3.063^{a}±0.156
HW-15	3.114^{a}±0.07

*The values are mean values of triplicate determination ± standard deviation.
Mean values within a column and a row with different superscripts are significantly different at $p<0.05$. LSD between samples = 0.2524

Table A.7 Mean value of ash content (% on dry basis)

Treatment	Mean value
Fresh	17.77^{a}±0.5
S-5	17.83^{a}±0.05
S-15	17.86^{a}±0.086
HW-5	17.87^{a}±0.133
HW-15	17.88^{a}±0.078

*The values are mean values of triplicate determination ± standard deviation.
Mean values within a column and a row with different superscripts are significantly different at $p<0.05$. LSD between samples = 0.4297

Table A.8 Mean value of moisture content (%)

Treatment	Mean value
Fresh	82.4^{a}±0.7
S-5	84.3^{b}±0.95
S-15	85.42^{b}±0.58
HW-5	89.4^{c}±0.8
HW-15	91.7^{d}±0.96

*The values are mean values of triplicate determination ± standard deviation.
Mean values within a column and a row with different superscripts are significantly different at $p<0.05$. LSD between samples = 1.483

Appendix B

ANOVA Results

Table 1.1 ANOVA for Phytate

Source of variation	Degree of freedom	Sum of squares	Mean squares	Variance ratio	F probability ratio
Sample	4	1.73056	0.43264	14.49	<0.001
Residual	10	0.29854	0.02985		
Total	14	2.02909			

Since F pr<0.05, there is significant difference between the samples so LSD testing is necessary. LSD between samples = 0.3143

Table 1.2 LSD for phytate

Sample	Mean score	Mean difference	LSD @ 0.05= 0.3143
A	2.991	A-B	<LSD*
		A-C	<LSD*
		A-D	>LSD
		A-E	<LSD
B	2.913	B-C	<LSD*
		B-D	>LSD
		B-E	>LSD
C	2.789	C-D	<LSD*
		C-E	<LSD*
D	2.246	D-E	<LSD*
E	2.189		

***=** not significantly different

Table 1.3ANOVA for oxalic acid

Source of variation	Degree of freedom	Sum of squares	Mean squares	Variance ratio	F probability ratio
Sample	4	1168582.	292145.	44.72	<0.001
Residual	10	65325.	6532.		
Total	14	1233907.			

Since F pr<0.05, there is significant difference between the samples so LSD testing is necessary. LSD between samples = 147

Table 1.4 LSD for oxalic acid

Sample	Mean score	Mean difference	LSD @ 0.05= 147
A	1471.328	A-B	>LSD
		A-C	>LSD
		A-D	>LSD
		A-E	<LSD
B	1162.713	B-C	<LSD*
		B-D	>LSD
		B-E	>LSD
C	1051.514	C-D	>LSD
		C-E	>LSD
D	763.491	D-E	<LSD*
E	704.949		

*= not significantly different

Table 1.5 ANOVA for Vitamin C

Source of variation	Degree of freedom	Sum of squares	Mean squares	Variance ratio	F probability ratio
Sample	4	988.136	247.034	110.82	<0.001
Residual	10	22.291	2.229		
Total	14	1010.428			

Since F pr<0.05, there is significant difference between the samples so LSD testing is necessary. LSD between samples = 2.716

Table 1.4 LSD for ascorbic acid

Sample	Mean score	Mean difference	LSD @ 0.05= 2.716
A	33.142	A-B	>LSD
		A-C	>LSD
		A-D	>LSD

		A-E	<LSD
B	28.883	B-C	>LSD
		B-D	>LSD
		B-E	>LSD
C	25.01	C-D	>LSD
		C-E	>LSD
D	15.689	D-E	>LSD
E	11.4		

***=** not significantly different

Table 1.5 ANOVA for protein

Source of variation	Degree of freedom	Sum of squares	Mean squares	Variance ratio	F probability ratio
Sample	4	6.574	1.644	1.26	0.349
Residual	10	13.069	1.307		
Total	14	19.643			

Since F pr>0.05, there is no significant difference between the samples. So LSD testing is no necessary

Table 1.6 ANOVA for fibre

Source of variation	Degree of freedom	Sum of squares	Mean squares	Variance ratio	F probability ratio	
sample	4	0.00153		0.00038	0.02	0.999
Residual	10	0.16516		0.01652		
Total	14	0.1667				

Since F pr>0.05, there is no significant difference between the samples. so LSD testing is not necessary.

Table 1.7 ANOVA for Crude fat

Source ofvariation	Degree of freedom	Sum of squares	Mean squares	Variance ratio	F probability ratio
sample	4	0.00929	0.00232	0.12	0.972
Residual	10	0.19245	0.01925		
Total	14	0.20174			

Since F pr>0.05, there is no significant difference between the samples so LSD testing is not necessary.

Table 1.9 ANOVA for ash

Source of variation	Degree of freedom	Sum of squares	Mean squares	Variance ratio	F probability ratio
sample	4	0.02417	0.00604	0.11	0.977
Residual	10	0.55785	0.05578		
Total	14	0.58202			

Since F pr>0.05, there is no significant difference between the samples so LSD testing is not necessary.

Table 1.11 ANOVA for moisture

Source of variation	Degree of freedom	Sum of squares	Mean squares	Variance ratio	F probability ratio
sample	4	174.4372	43.6093	65.66	<.001
Residual	10	6.6417	0.6642		
Total	14	181.079			

Since F pr<0.05, there is significant difference between the samples so LSD testing is necessary. LSD between samples = 1.483

Table 1.12 LSD for moisture

Sample	Mean score	Mean difference	LSD @ 0.05= 2.716
A	82.4	A-B	>LSD
		A-C	>LSD
		A-D	>LSD

		A-E	<LSD
B	84.2	B-C	<LSD*
		B-D	>LSD
		B-E	>LSD
C	85.42	C-D	>LSD
		C-E	>LSD
D	89.4	D-E	>LSD
E	91.7		

*= not significantly different

Appendix C

Fig 5.1 Collection of fresh *Sisnu* sample

Fig 5.2 Steaming of *Sisnu*

Fig 5.3 Lab work on progress