NUTRITIONAL EVALUATION AND GLYCEMIC INDEX OF SELECTED VARIETIES OF MULBERRY LEAVES

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Affectionately Dedicated
To My Beloved Grandfather
Late Shri Vitthal Islampure
And my beloved parents
Ishwar, Mahananda,
and Dearest brother Veeresh and
sister Ashwini

CERTIFICATE

This is to certify that the thesis entitled **"Nutritional evaluation and glycemic index of selected varieties of mulberry leaves"** submitted by **Ms. SHWETA ISLAMPURE, PAK 7174** for the award of degree of **MASTER OF SCIENCE (Agriculture)** in **Food Science and Nutrition** to the University of Agricultural Sciences, Bangalore, is a record of research work carried out by her during the period of her study in this University, under my guidance and supervision and no part of the thesis has been submitted for the award of any other degree, diploma, associateship, fellowship or other similar titles.

Bangalore
July, 2009

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Professor and Major Advisor

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(Mrs. K.V. JAMUNA)

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THESIS ABSTRACT

Mulberry leaves are available abundantly but they are not used in the local diets. Nutritional evaluation of selected varieties of mulberry leaves, development of value added products and sensory evaluation of developed products and glycemic index of a selected product was assessed. The macro nutrients, micro nutrients, and others like total sugars, oxalates and tannins were estimated. The mean protein, total lipids, crude fibre, carbohydrate and energy values were 25.66g/100g (20.50 to 30.87g/100g), 2.92g/100g (2.03 to 3.43g/100g), 11.91g/100g (9.41 to 14.94g/100g), 41.01g/100g (31.81 to 46.95g/100g) and 288.25Kcal/100g (277 to 294.88Kcal/100g) respectively in dried mulberry leaf powder. The mean values for ash, calcium, phosphorous, ascorbic acid, β-carotene, total sugars, oxalate and tannin content of mulberry varieties were 13.00g/100g (11.19 to 15.18g/100g), 353.91mg/100g (168.33 to 561.66mg/100g), 8.13mg/100g (4.6 to 12.90mg/100g), 182.47mg/100g (133.33 to 216.66mg/100g), 5324µg/100g, 11.24g/100g (5.0 to 15.66g/100g), 32.04mg/100g (27.15 to 39.95mg/100g) and 8.87mg/100g (4.99 to 15.18mg/100g) respectively. The mean i*n vitro* protein digestibility of mulberry powder was 88.70 percent (66.13 to 99.68 percent). *in vitro* carbohydrate digestibility of different varieties differed significantly. Four products were developed namely, chapathi mix, papad, khakra and chutney powder. The mulberry and spinach (control) powder was incorporated at 2 and 5 percent level. Sensory scores of the products revealed that products were well accepted and had higher micro nutrient content. Microbial load for chapathi mix was low. Storage up to two months did not show any deterioration in sensory characters. Glycemic index of chapathi mix with mulberry leaf powder incorporation at 5 percent was 93.66. Thus, mulberry leaf powder was found to be an excellent source of nutrients and may be used as a low cost nutritional adjuvant in daily diets**.**

Signature of Student **Signature of Major Advisor**

ಆಯ್ದ ಹಿಪ್ಪು ನೇರಳೆ ತಳಿಗಳ ಸೊಪ್ಪಿನಲ್ಲಿ ಪೋಷಕಾಂಶಗಳ ಮೌಲ್ಯಮಾಪನ ಹಾಗೂ ಗ್ಲೈಸಿಮಿಕ್ ಸೂಚಿಕೆ

ಶ್ವೇತಾ ಇಸ್ಲಾಂಪುರೆ

ಪ್ರಭುದ ಸಾರಾಂಶ

ಹಿಪ್ಪು ನೇರಳೆ ಸೊಪ್ಪು ಹೇರಳವಾಗಿ ದೊರೆಯುತ್ತದೆ ಆದರೆ ದೈನಂದಿನ ಆಹಾರದಲ್ಲಿ ಬಳಸುವುದಿಲ್ಲ. ಆಯ್ದ ತಳಿಗಳಲ್ಲಿ ಪೋಷಕಾಂಶಗಳ ಸಂಯೋಜನೆ, ಮೌಲ್ಯವರ್ಧಿತ ಪದಾರ್ಥಗಳ ತಯಾರಿಕೆ ಮತ್ತು ಆಯ್ದ ಪದಾರ್ಥಗಳ ಗ್ಲೈಸಿಮಿಕ್ ಸೂಚಿಕೆಯನ್ನು ಅಧ್ಯಯನ ಮಾಡಲಾಯಿತು. ತೇವಾಂಶ, ಪ್ರೋಟೀನು, ಶರ್ಕರ ಪಿಷ್ಠ, ಕೊಬ್ಬಿನಾಂಶ, ನಾರಿನಾಂಶ, ಶಕ್ತಿ, ಸೂಕ್ಷ್ಮ, ಬೀಟಾ ಕೆರೊಟೀನ್, ಪತ್ರ ಹರಿತ್ತು ಮತ್ತು ಇತರೆ ಪೋಷಕಾಂಶಗಳಾದ ಒಟ್ಟು ಶರ್ಕರ, ಆಕ್ಸಾಲೇಟ್ಸ ಮತ್ತು ಟ್ಯಾನಿನ್ಸಗಳ ಅಂದಾಜು ಮಾಡಲಾಯಿತು. ಒಣಗಿದ ಹಿಪ್ಪುನೇರಳೆ ಸೊಪ್ಪಿನ ಪುಡಿಯಲ್ಲಿ ಸರಾಸರಿ ಪ್ರೋಟೀನು, ಕೊಬ್ಬಿನಾಂಶ, ನಾರಿನಾಂಶ, ಪಿಷ್ಠ ಮತ್ತು ಶಕ್ತಿಯ ಮೌಲ್ಯಗಳು ಕ್ರಮವಾಗಿ ಈ ಕೆಳಗಿನಂತಿವೆ. 25.66ಗ್ರಾಂ/100ಗ್ರಾಂ (20.50 ಇಂದ 30.82ಗ್ರಾಂ/100ಗ್ರಾಂ), 2.92ಗ್ರಾಂ/100ಗ್ರಾಂ (2.03 ಇಂದ 3.43ಗ್ರಾಂ/100ಗ್ರಾಂ), 11.91ಗ್ರಾಂ/100ಗ್ರಾಂ (9.41 ಇಂದ 14.91ಗ್ರಾಂ/100ಗ್ರಾಂ), 41.01ಗ್ರಾಂ/100ಗ್ರಾಂ (31.18 ಇಂದ 46.95ಗ್ರಾಂ/100ಗ್ರಾಂ) ಮತ್ತು 288.25ಕಿಕ್ಯಾಲೋರಿ/100ಗ್ರಾಂ (277 ಇಂದ 294.88ಕಿಕ್ಯಾಲೋರಿ/100ಗ್ರಾಂ). ಒಣಗಿದ ಹಿಪ್ಪುನೇರಳೆ ಸೊಪ್ಪಿನ ಪುಡಿಯಲ್ಲಿ ಸರಾಸರಿ ಬೂದಿ, ಸುಣ್ಣದಾಂಶ, ರಂಜಕ, ಸಿ ಅನ್ನಾಂಗ, ಬೀಟಾ ಕೆರೋಟೀನ್, ಒಟ್ಟು ಶರ್ಕರ, ಆಕ್ಸಾಲೇಟ್ಸ, ಟ್ಯಾನಿನ್ಸಗಳು ಕ್ರಮವಾಗಿ ಈ ಕೆಳಗಿನಂತಿವೆ. 13.0ಗ್ರಾಂ/100ಗ್ರಾಂ (11.19 ಇಂದ 15.18ಗ್ರಾಂ/100ಗ್ರಾಂ), 353.91ಮಿಗ್ರಾಂ/100ಗ್ರಾಂ (198.22 ಇಂದ 561.66ಮಿ.ಗ್ರಾಂ/100ಗ್ರಾಂ), 8.13ಮಿ.ಗ್ರಾಂ/100ಗ್ರಾಂ (4.6 ಇಂದ 12.90ಮಿ.ಗ್ರಾಂ/100ಗ್ರಾಂ), 182.47ಮಿ.ಗ್ರಾಂ/100ಗ್ರಾಂ (133.33 ಇಂದ 216.66ಮಿ.ಗ್ರಾಂ/100ಗ್ರಾಂ), 5324ಮೈಕ್ರೋ ಗ್ರಾಂ/100ಗ್ರಾಂ, 11.24ಗ್ರಾಂ/100ಗ್ರಾಂ (5.0 ಇಂದ 15.66ಗ್ರಾಂ/100ಗ್ರಾಂ), 32.04ಮಿ.ಗ್ರಾಂ/100ಗ್ರಾಂ (27.15 ಇಂದ 36.95ಮಿ.ಗ್ರಾಂ/100ಗ್ರಾಂ) ಮತ್ತು 8.82ಮಿ.ಗ್ರಾಂ/100ಗ್ರಾಂ (4.99 ಇಂದ 15.18ಮಿ.ಗ್ರಾಂ/100ಗ್ರಾಂ). ಹಿಪ್ಪು ನೇರಳೆ ಸೊಪ್ಪಿನ ಪುಡಿಯಲ್ಲಿ ಶೇಕಡಾ ಸರಾಸರಿ ಅಜೀವ ಪ್ರಯೋಗಾಲಯದ ಪ್ರೋಟಿನು ಜೀರ್ಣವಾಗುವಿಕೆಯು 88.70 (66.13 ಇಂದ 99.68) ಮತ್ತು ಅಜೀವ ಪ್ರಯೋಗಾಲಯದ ಶರ್ಕರ ಪಿಷ್ಠ ಜೀರ್ಣವಾಗುವಿಕೆಯು ಬೇರೆ ಬೇರೆ ತಳಿಗಳಲ್ಲಿ ಬೇರೆ ಬೇರೆಯಾಗಿದೆ. ಪ್ರಮುಖವಾಗಿ ನಾಲ್ಕು ಮೌಲ್ಯವರ್ಧಿತ ಪದಾರ್ಥಗಳನ್ನು ತಯಾರಿಸಲಾಯಿತು ಅವುಗಳೆಂದರೆ, ಪೌಷ್ಠಿಕ ಚಪಾತಿ ಹಿಟ್ಟು, ಹಪ್ಪಳ, ಖಾಖ್ರ ಮತ್ತು ಚಟ್ನಿಪುಡಿ. ಹಿಪ್ಪು ನೇರಳೆ ಹಾಗೂ ಪಾಲಕ ಸೊಪ್ಪಿನ (ನಿಯಂತ್ರಕ) ಪುಡಿಯನ್ನು ಶೇಕಡಾ 2 ಮತ್ತು 5 ರ ಅಳತೆಯಲ್ಲಿ ಸೇರಿಸಲಾಯಿತು. ಇಂದ್ರಿಯ ಪರಿಶೀಲನೆ ಆಧಾರದ ಮೇಲೆ ಎಲ್ಲ ಮೌಲ್ಯವರ್ಧಿತ ಪದಾರ್ಥಗಳು ಹೆಚ್ಚಿನ ಪ್ರಮಾಣದಲ್ಲಿ ಸ್ವೀಕಾರಗೊಂಡವು ಮತ್ತು ಹೆಚ್ಚಿನ ಪ್ರಮಾಣದ ಸೂಕ್ಷ್ಮ ಪೋಷಕಾಂಶಗಳು ಕಂಡುಬಂದವು. ಪೌಷ್ಠಿಕ ಚಪಾತಿ ಹಿಟ್ಟಿನಲ್ಲಿ ಸೂಕ್ಷ್ಮಾಣು ಜೀವಿಗಳ ಅಂಕೆ ಕಡಿಮೆ ಪ್ರಮಾಣದಲ್ಲಿ ಕಂಡುಬಂತು. ಎರಡು ತಿಂಗಳ ಶೇಖರಣೆ ನಂತರ ಇಂದ್ರೀಯ ಪರಿಶೀಲನೆ ಗುಣಗಳಲ್ಲಿ ಪರಿಣಾಮಕಾರಿ ಬದಲಾವಣೆಯಾಗಲಿಲ್ಲ. ಹಿಪ್ಪು ನೇರಳೆಯ ಪೌಷ್ಠಿಕ ಚಪಾತಿ ಹಿಟ್ಟಿನ (ಶೇಕಡಾ 5ರ ಅಳತೆಯ) ಗ್ಲೈಸಿಮಿಕ್ ಸೂಚಿಕೆಯು 93.66 ರಷ್ಟು ಕಂಡುಬಂತು. ಈ ಅಧ್ಯಯನದಿಂದ ತಿಳಿದು ಬರುವುದೇನೆಂದರೆ, ಹಿಪ್ಪು ನೇರಳೆಯು ಅತ್ಯಮೂಲ್ಯ ಕಡಿಮೆ ವೆಚ್ಚದ ಪೋಷಕಾಂಶಗಳ ಮೂಲವಾಗಿದ್ದು ಹಾಗೂ ಇದನ್ನು ದೈನಂದಿನ ಆಹಾರಗಳಲ್ಲಿ ಉಪಯೋಗಿಸಬಹುದಾಗಿದೆ.

ವಿದ್ಯಾರ್ಥಿಯ ಸಹಿ

ಮುಖ್ಯ ಮಾರ್ಗದರ್ಶಕರ ಸಹಿ

CONTENTS

LIST OF TABLES

LIST OF FIGURES

LIST OF PLATES

INTRODUCTION

I. INTRODUCTION

Mulberry (*Morus Spp*) (Moraceae) is a small medium sized diocious tree distributed in temperate and sub tropical regions. It is the sole food plant of silkworm *Bombyx mori* L. The cultivated variety of mulberry belongs to the species *M. alba, M. nigra, M. indica, M. laevegata, M. Rubra* etc. This plant is widely distributed in India, China, Japan, North Africa, Arabia, South Europe etc (Kumar *et al,* 2008). Mulberry is known by different names viz. *Morera* or *mora* in Spanish, *moreira* in Portuguese, *murier* in French, *tut* in Urdu, Persian and Hindi and in Kannada it is known as *hippunerale.* In Karnataka mulberry is being cultivated in about 97,000 ha/year. The widely cultivated variety for silkworm raring is V1 with the yield potential of 60,000 to 65,000 Kg/ha/year.

Chemical composition of the mulberry leaves varies according to variety, degree of maturity, leaf position within the branch and fertilization level. Since the mulberry leaves contain all the essential and non essential nutrients required by the mankind, they are considered as rich, nutritious and palatable comparable to the leafy vegetables namely amaranth and spinach etc. Mulberry leaves are found to contain 22.13 per cent protein, 5.9 per cent of crude fibre, 13.53 per cent ash, 3.3 per cent of calcium and 1.43 per cent of phosphorous. (Srivatsava *et al.,* 2009).

Green leafy vegetables occupy most important place among the food crops as they provide adequate amounts of many vitamins and minerals for human nutrition. They are rich sources of carotene, a precursor of vitamin A, vitamin C, riboflavin, folic acid and minerals like calcium, iron and phosphorous. They are also fair sources of proteins Narasing Rao *et al.* (1989). Nutritionally caused blindness, iodine deficiency disorders and iron deficiency anaemia are some of the major

problems of micro nutrient deficiency seen especially in vulnerable group of population Shantala *et al.* (2005). One of the measures to prevent malnutrition among the population is through food based approaches, which concentrate on change or improvement in food habits by creating awareness among the population (FAO 1996, 1997).

Besides being the chief feed for silkworm mulberry leaves other parts of the plant are widely used in traditional medicines. The roots of the plants have been used as diuretic and expectorant. Stem bark is antihelmentic and a purifying agent. Leaves are used in controlling the hyperglycemia and retardation of cataract which is attributed to the presence of DNJ (1-deoxynojirimycin), glycol protein moran A, trigonelline, moranaline and morin (Ansari *et al.,* 2004). The hypoglycaemic effect of this compound has been demonstrated in animal models (Jamshid and Prakash 2008, Nakagawa 2007). The leaf is used as herbal medicine by Chinese herbalists. Being the only plant leaf discovered so far containing 1-deoxynojirimycin (1-DNJ), a potent inhibitor of α-glucosidase, and hence mulberry leaf is effective in lowering blood glucose, and thus can be used in the prevention and treatment of diabetes (Nakagawa 2007, Kimura 2007). The Morus plant is also a rich source of natural isoprenoid substituted phenolic compounds including flavanoids. These compounds have been studied by many investigators with structural, biological and pharmacological interests (Suryanarayana *et al.,* 1997). There are few studies which have demonstrated a hypoglycaemic effect of mulberry leaf powder (Andallu *et al.* 2001, Miyahara *et al.* 2004, Kimura 2007).

Variety of good quality value added products can be produced with a good acceptability score (Anon. 2007). Several products from mulberry are already available in the international market (www.lifeenhancement.com). In the country this trend has already

started. A local brand of mulberry tea known as “Spoorthi” is available at Central Sericulture research and training Institute Govt of India (Anon., 2007).

Mulberry leaves have also been used in leaf stewing, which is considered as a healthy drink for weight reduction. Moreover, the aqueous extract of mulberry leaves rich in flavonoids, acts as a scavenger of lipid radicals and thus acts as antioxidants. *(*Chen and Li *2007).* Different tea product showed significant difference in inhibitory activity against both sucrose and maltase (Hansawasdi and Kawabata 2006). Zong *et al.* (2006) have demonstrated the hypoglycaemic effect of mulberry tea in healthy volunteers.

Mulberry leaves which have been reported to be protein rich and micro nutrient dense can be used like other green leafy vegetables. This can be used to enrich the poor Indian dietaries. Further there are no reports on the hypoglycaemic effect of incorporation of mulberry leaf powder in traditional food matrices. Acceptable products, to suit local community, need to be developed. Similarly, there is a need to assess the nutrient contents of different varieties grown in Karnataka. The present study is planned to evaluate the effect of popular varieties of mulberry leaves for the above characteristics. Following were the objectives framed for the study:

- To evaluate nutritional composition of selected varieties of mulberry leaves
- To study the *in vitro* digestibility of protein and carbohydrate
- To develop value added products from selected varieties of mulberry leaves
- To study the acceptability and shelf life of value added products
- To evaluate glycemic index of selected products developed from mulberry leaves

REVIEW OF LITERATURE

II. REVIEW OF LITERATURE

Mulberry leaf is a good source of protein, macro mineral, dietary fibre and complex carbohydrate. Some reports have suggested that they are an unconventional source of nourishment for humans. The literature pertaining to the chemical composition of leaves, digestibility, glycemic index, health benefits, value added products from leaves, shelf life study and acceptability is reviewed in this chapter. Work carried out by various researchers has been reviewed under the following headings.

2.1 Nutrient composition and digestibility of selected varieties of mulberry and other leaves.

2.2 Glycemic index and other health benefits of mulberry leaves.

2.3 Value added products from mulberry leaf powder.

2.4 Shelf life of value added products using leaf powder.

2.1 Nutrient composition and digestibility of selected varieties of mulberry leaves and other leaves

Nutritional composition, digestibility of various mulberry varieties was studied by Sanchez (2008). He found that crude protein content in leaves varied from 15 to 28 per cent. The variation in the crude protein content was due to the variety, age of the leaves and growing conditions. The high protein content in mulberry leaves was similar to most legume forages. The digestibility of mulberry leaves was found to be more than 80 per cent. Many micro nutrients such as calcium, phosphorous and carotene were found in substantial amounts.

An evaluation of nutritive value of mulberry leaves (*Morus Spp)* was performed on an animal model by Bamikole *et al.* (2005). Thirty weaned (growing) rabbits were sorted by weight and randomly allotted to five experimental diets. The percentage of concentrate in i.e. 100:0, 75:25,

50:50, 25:75 and 0:100. Study lasted for12 weeks. Feed intake, weight gain and nutrient digestibility were assessed. The nutrient digestibilities of diets were high. There were no significant differences among the means for dry matter (75.67 to 82.33 per cent), organic matter (77.83 to 88.67 per cent) and ash (52 to 62.67 per cent). Digestibility of EE (ethanol extract 55.65 to 86.00 per cent) and NFE (nitrogen free extract 76.00 to 87.33 per cent) significantly declined with increasing level of mulberry leaves in the rations. Weight gain in the rabbits on diets containing 25 and 50 per cent mulberry leaves was not significantly different from that of all concentrate rations, but these were significantly higher than those of 25:75 and 0:100 concentrate mulberry diets.

Bongale *et al*. (1991) studied the leaf quality evaluation of mulberry gardens in Karnataka. The mulberry leaf and soil samples were collected from three major sericultural areas of Karnataka and were analyzed for protein, sugar, chlorophyll content of leaf and soil fertility parameters including micro nutrients. The sugar (per cent), protein (per cent) and total chlorophyll (mg/g) of samples from Anekal, kolar and western ghat regions varied significantly. The sugar content was 12.64, 10.0 and 7.97 per cent respectively. Protein (per cent) was 19.88, 15.52 and 19.68 respectively. The total chlorophyll (mg/g) was 3.56, 2.47 and 2.36 respectively.

Bongale and Chaluvachari (1993) evaluated four mulberry varieties by leaf biochemical analysis and bio assay with *Bombyx mori* L.. the varieties namely, kanva 2, DD, S34 and TG were studied with respect to growth, yield, leaf moisture content, total protein, soluble protein, sugars and chlorophyll along with bio assay evaluation of leaf quality. Observations were made for three crops representing three seasons of a year 1991-92. Significant differences were recorded with respect to the varieties and season as also their interaction with respect to bio chemical

and bio assay evaluation. Lower values of total and soluble protein and chlorophyll content in DD variety was observed compared to other varieties. The TG variety had highest total protein and sugar content of leaf 24.43 per cent and 8.99 per cent respectively.

Chaluvachari and Bongale (1992) studied the leaf quality evaluation of selected mulberry genotypes by biochemical and bioassay studies. Four mulberry genotypes namely Mysore local, kanva 2, S36 and S41 with known cytological variations were studied for leaf biochemical components in relation to the bio assay with moulting test. Among the four varieties S41 recorded maximum leaf protein content up to 60 days of growth period. Maximum protein content was recorded on 45th day with increased maturity in all four varieties. The S36 variety recorded consistently higher sugar content over a prolonged period of 45 to 90 days of growth. Similarly M5 variety gave higher sugar content over a longer period (30 to 75 days growth). Mysore local and S41 varieties on other hand did not record uniform pattern of leaf sugar content. In general sugar content showed greater variations both among varieties and growth stages (7.7 to 15.2 per cent of total sugars among all varieties).

Kantwa *et al.* (2006) performed the nutritional evaluation of mulberry leaves (*Morus alba* L), green leaves in sheep and goats. This experiment was conducted in sheep and goats, the animals were fed mulberry green leaves as adlibitum. Mulberry green leaves contained 88per cent dry matter (DM), 15.20 per cent crude protein (CP), 9.85 per cent crude fibre (CF), 7.10 per cent ethanol extract (EE), 55.85 per cent nitrogen free extract (NFE) and 12 per cent ash with an average dry matter content of 32.38per cent in fresh leaves. The difference for dry matter, crude protein, ethanol extract and nitrogen free extract digestibility in sheep and goats were non significant. It was concluded

that the utilization of mulberry green leaves by sheep and goats was adequate to meet their requirement, but supplement of mulberry green leaves with concentrate may result in optimum growth rate in sheep and goats.

Gautam and Awasthi (2007) studied the nutrient composition and physical composition of aloe vera (*Aloe barbadenis)* powder. The process of making aloe vera powder was standardized and its nutritional and physicochemical characters were assessed. The aloe vera powder contained 18.5per cent crude fibre, 4.8per cent crude protein, 2.2per cent crude fat, 14per cent of total ash, 48per cent of carbohydrate and 231Kcal of energy. It contained substantial amounts of iron at 64.8mg/100g, ascorbic acid at 27mg/100g and β-carotene at 335.8mg/100g. The dietary fibre was 21.3 per cent, reducing and non reducing sugars were 76mg/100 g each. The pH of the powder was 4.8 and colour was olive green.

Lakshmi and Vimala (2000) studied the nutritive value of dehydrated green leafy vegetable powders. The green leafy vegetables powders were prepared using dehydration technology. The green leafy vegetables used were amaranth (*Amaranth gangeticus*), curry leaves (*Murayya koenigii*), gogu (*Hibiscus cannabinus)* and mint (*Mentha spicata).* All the green leafy vegetables were blanched before drying after establishing the conditions for blanching including blanching time, temperature and blanching treatment solution. The samples were either sun dried or cabinet dried. Nutritive value of the powder was determined. They concluded that in spite of considerable losses in vitamins, green leafy vegetable powders retained good amount of protein, fibre and minerals and fair amounts of ascorbic acid and β-carotene.

Shingade *et al.* (1995) studied the proximate composition of unconventional leafy vegetables from the Konkan region of Maharashtra.

Ten vegetables were analysed including: math (*Amaranthus tricolour*), katemath (*Amaranthus spinosus*), bharangi (*Clerodendron serratum*) and kwala (*Smithia sensitiva*). These vegetables contained high levels of crude proteins (3.9 to 5.6per cent), fats (0.2 to 0.8per cent), ash (1.1 to 3.4per cent), fibre (1.7 to 2.4per cent) and total carbohydrates (10.0 to 3.7per cent). Drumstick leaves (*Moringa oleifera*) contained high levels (229.9mg/100g) of ascorbic acid and beta-carotene (5080mg/100g) and relatively low levels of oxalic acid (92.5mg/100g) compared to 586.2mg/100g in spinach (*Spinacea oleracea*).

Ndossi and Sreeramulu (1991) determined the nutritional values of *Launaea Cornuta*, a wild leafy vegetable. The authors reported that *Launaea Cornuta* had moisture content of 86.8g, ash 2.5g, crude protein 3 to 9g, crude fat 0.9g, carbohydrate 4.5g, iron 7.2mg, sodium 57.9mg, potassium 869mg, calcium 214mg, phosphorous 13.2mg and vitamin C 18.7mg on fresh weight basis for every 100 g of leaves.

Bharati and Umamaheshwari (2001) carried out a study to estimate the nutritive value of non-traditional leafy vegetables available in Nellore and Prakasham district of Andhra Pradesh. Authors reported that these non-traditional greens were rich source of various nutrients. The protein content ranged from 2.17 to 6.89g/100g, while fat content was 0.48 to 1.57g per cent. Total carotene was maximum in Chenchulaku (47.08 per cent) and minimum in payilaku (11.73 per cent), while β-carotene as per cent of total carotene ranged between 30 to 49 per cent. Similar to most green leafy vegetables these non-traditional greens had a high ascorbic acid content ranging between 29 to 151 mg per cent. Among minerals, iron content of atikamamidi was highest (35.86mg per cent) where as badaku and payilaku had relatively lower levels (6mg per cent). Calcium levels were fairly good ranging from 198 to 853mg per cent.

2.2 Glycemic index and other health benefits of mulberry leaves

During 1970's evidence began to accumulate that the postprandial of blood glucose is not the same for a standard weight of carbohydrate in different carbohydrate containing food. Jenkin's *et al* (1980) introduced the glycemic index (GI) concept. Carbohydrates containing foods differ greatly in the blood glucose responses they produce, when tested under standard conditions both in healthy and diabetic subjects. The Glycemic index (GI) can be defined as the area under the curve of blood glucose after a test meal of food (X), containing 50g (available) carbohydrate, expressed as a percentage of the area under the blood glucose curve after giving about 50g of glucose on different day in the same individual. Both the tests were made in the morning after an overnight fast. The ability of the food item to raise the blood glucose is measured in terms of glycemic index. It is a concept that ranks foods on the basis of their acute glycemic impact.

Andallu and Varadacharulu (2001) studied the effectiveness of mulberry leaves in comparison with that of standard drug Glibenclamide on blood glucose, glycosylated hemoglobin levels and on the activity of certain enzymes in the blood in diabetic rats. The mulberry leaves were washed, dried and powdered. Male Wistar albino rats with body weights ranging from 130 to 150 g were selected and fed with the standard diet along with the experimental diet containing mulberry powder. Blood samples were analyzed for fasting blood glucose, glycosylated hemoglobin and various enzymes. Administration of mulberry leaves to normal as well as diabetic induced rats showed significant reduction in blood glucose level and glycosylated hemoglobin.

Nagakawa (2007) assed the hypoglycaemic property of mulberry leaves which has attributed to a compound called 1-DNJ. This is aza sugar has high potency act as glucosidase inhibitor. They developed the

method for determining the level of plasma DNJ by hydrophilic interaction chromatography coupled to a mass spectrophotometer detector to investigate the absorption and metabolism of orally administered mulberry DNJ in rats. The results revealed that the orally administered mulberry DNJ- is absorbed as an intact form from the alimentary and then quickly excreted from the body.

Kimura (2007) studied the food grade mulberry powder enriched with 1-Deoxynojirimycin (1-DNJ) suppresses the elevation of postprandial blood glucose. The DNJ contents in commercial mulberry were 0.1 per cent, implying that bioavailability of DNJ might not be as expected. They carried out the production of food grade mulberry powder containing a maximally high DNJ content and determination of mulberry powder for the suppression of postprandial blood glucose level through clinical trials. The mulberry leaves from different cultivars, harvest seasons and the leaf locations were determined using hydrophilic evaporative light scattering detection. Healthy volunteers received 0, 0.4, 0.8 and 1.2g of DNJ-enriched powder (corresponding to 0,6 , 12 and 18mg of DNJ respectively), followed by 50g of sucrose. Before and 30 to 180 minutes after the DNJ/sucrose administration, plasma glucose and insulin were determined. The results obtained were: young mulberry leaves taken from the top part of branches in summer contained the highest amount of DNJ. After optimization of the harvesting and drying processes for young mulberry leaves, DNJ-enriched powder (1.5per cent) was produced. A human study indicated that the single oral administration of 0.8 and 1.2g 0f DNJ- enriched powder significantly suppressed the elevation of postprandial blood glucose and secretion of insulin, revealing the physiological impact of mulberry DNJ (effective dose and efficacy). This study suggests that the newly developed DNJ-enriched powder can be used as a dietary supplement for preventing diabetes mellitus.

Miyahara (2004) studied the inhibitory effects of mulberry leaf extract on postprandial hyperglycaemia in normal rats. They examined the inhibitory effects of aqueous ethanol extract from mulberry leaves (ME) on postprandial hyperglycaemia in normal Wistar rats. ME dose dependently superseded the postprandial rise of blood glucose in rats, when ME (0.02 to 0.5g/kg) was given 0.5 hour before the administration of carbohydrates such as sucrose, maltose and starch. The ME dose showing 50 per cent inhibition of the increment of blood glucose was0.11g/kg for sucrose, 0.44g/kg for maltose and 0.38 g/kg for starch. Mulberry leaves (ME) and its basic fraction (MB) containing 1-DNJ were assayed for their inhibitory effects on disaccharidase derived from the small intestine of rats. The inhibitory effect value of mulberry leaves was 3.2µg/ml of sucrose, 10µg/ml of isomaltase and 51µg/ml for maltase. The inhibitory effect of mulberry basic fraction was 0.36 µg/ml of sucrose, 1.1µg/ml for isomaltase and 6.2µg/ml for maltase. The inhibitory effect value of 1-DNJ as the principle component in mulberry leaves was 0.015µg/ml for sucrase, 0.21µg/ml for maltase and this value was compared with the inhibitory effect value of Voglibose (isotope). The inhibitory activity of mulberry leaves in a-amylase was weak. These results suggest that mulberry leaves strongly suppress the postprandial hyperglycaemia after carbohydrate load by inhibiting the activity of disaccharidases in small intestine of rats.

Kong (2008) studied the antiobesity effects and improvement of insulin sensitivity by 1-DNJ in animal models. DNJ was isolated from the silkworm (*Bombyx mori* L*)* and its anti diabetic effects were evaluated in OLETF (Otsuka long-evans tokoshima fatty) rats and in control LETO (long evans tokoshima otsuka) rats. The treatment showed a significant anti diabetic effects in OLFTF rats, significant improvement in fasting blood glucose levels, glucose tolerance and insulin sensitivity and loss in body weight was noticed in both groups. DNJ showed significant anti

hyperglycaemic effects in streptozotocin rats and high fat diet induced hyperglycaemic rats. Its efficacy and dose profiles were better than those of acarbose a typical α- glucosidase inhibitor used in clinical trials. A substantial fraction of DNJ was observed in blood and urine. The results suggests that the anti obesity effect and increased insulin sensitivity were the possible attributes of DNJ for the anti diabetic effect.

Kang *et al.* (2002) studied the effects and mechanism of silkworm powder as blood glucose-lowering-agent. The experiment showed that the extract of silkworm powder prevents hunger and low blood glucose level during empty stomach. The major component of silkworm powder is 1-Deoxynojirimycin (DNJ).

Jamshed and Prakash. (2008) studied the hypoglycaemic effect of *Morus alba.* an animal model (Wistar rats, body weight ranging from 150 to 200g). They evaluated the therapeutic efficacy of mulberry leaves. The experimental animals were divided into five groups. The groups were control group, control group with mulberry leaf extract treatment, diabetic control group, diabetic group treated with 400mg/kg/day of mulberry leaf extract and diabetic group treated with 600mg/kg/day mulberry leaf extract. The result showed that the *M.alba* extract 400mg/kg/day reduced the hyperglycaemia significantly as compared to the diabetic control group. The treatment with extract at 400 and 600mg/kg/day decreased glycosylated heamoglobin significantly in diabetic group. The improvement in glycemic control followed by fall in VLDL production after mulberry treatment (600mg/kg/day) was attributed to the mulberry therapy in diabetic rats. The hypoglycaemic effect of mulberry leaves was due to high fibre content (13.85 per cent) and presence of trigonelline, moran and moranaline.

Zhong *et al.* (2006) studied the effect of black, green and mulberry tea causes malabsorption of carbohydrate but not triglycerol in healthy volunteers. They measured the breath hydrogen and ^{13} Co2 (isotope) to investigate the ability of an extract of black, green and mulberry leaves to induce malabsorption of carbohydrates and triglycerol in healthy volunteers. They selected 20 healthy volunteers and assigned to drink either the extracts of tea with rice and butter meal. Breath samples were collected at hourly intervals for eight hours, at each test period subjects were asked to rate variety of symptoms. Expired air was collected and subjected for hydrogen and ^{13}Co2 analysis. The result showed that the ingestion of tea extract resulted in increase in hydrogen concentration. This clearly indicates the malabsorption of carbohydrates and caloric availability.

Hansawasdi and Kawabata (2006) studied the effect of brewing time on glucose inhibitory active component released from mulberry tea. Different tea product showed significant difference in inhibitory activity against both sucrase and maltase. The most effective enzyme inhibition was observed when 3 to 5 minutes brewing time was applied in tea preparation.

John and Chellappa (2005) studied the hypoglycaemic effect of *Moringa oliefera* (drumstick) leaf powder on human diabetic subjects and albino rats. In human subjects incorporation of (*Moringa oleifera*) drumstick leaf powder at 8g/day for 14 days has reduced the mean fasting and postprandial blood glucose levels. In rats the incorporation of drumstick leaf powder at 50mg/day for 21 days reduced the fasting and postprandial blood glucose levels. Extending the administration period from 14 to 21 days resulted in significant reduction in fasting and postprandial blood glucose levels.

Kumar *et al* (2008*)* are of the opinion that mulberry is a medicinally important plant. In their report they have mentioned that a large number of active bio molecules are present in *Morus alba* and it possesses many medicinal properties. It has been described as astringent and properties such as anthelmintic, anti HIV, anti inflammatory, exudative, diaphoric, anti diabetic, anti hypertensive, anti atherosclerotic and anti tumorogenic.

Butt *et al.* (2008) reviewed the rich photochemistry of mulberry leaves (*Morus alba* L.), its antioxidant potential, inhibition of LDL oxidation, neurodegenerative disorders and mode of action of boosting skin tone. It was reported that mulberry leaves have unique nutritional profile containing proteins, phenolics, flavanoids and anthocyanins that enhances its significance as promising functional tonic. It was also stated that it contains some antimicrobial agents like kuwanon G and leachianone etc. Some other compounds such as 1-DNJ and Moran 20K which have been reported to be effective against hyperglycemia and lipid peroxidation in diabetics. Mulberry protein was found to have nueroprotective functions used against neurodegenerative disorders such as Alzeimer's disease and Parkinsonism.

Doi *et al.* (1999) studied the effect of various fractions extracted from mulberry leaves on lipid metabolism in rabbits fed a cholesterol diet. Effects of ingesting extracts of mulberry leaves were evaluated on hypercholesterolaemic rabbits in order to investigate the active hypolipaemic fractions. Rabbits were fed for 14 wk on a one per cent cholesterol diet containing five or 2.5 per cent of a 1-butanol extract of mulberry leaves, five per cent acetone extract of mulberry leaves or 5 per cent residue of mulberry leaves. Increases in serum total cholesterol and free cholesterol concentration were markedly suppressed in groups receiving diets containing five per cent 1-butanol extract or mulberry leaf

residue compared with those in the cholesterol only, control group. A tendency towards suppressed lipid deposition in hepatocytes was also observed in both the 1-butanol extract and mulberry leaf residue groups. These findings suggest that the hypolipaemic effects of mulberry leaves are exerted by multiple components, and that the 1-butanol extract (recovery 1.03 per cent) contains more active components than the residue (recovery 83.31 per cent).

Robertson (1988*)* studied the physicochemical characteristics of food and digestion of starch and dietary fibre during gut transit. They found that starch digestion varies depending upon starch source, rate and extent of digestion, fibre content, and method of food preparation. The particle size, composition and viscosity change influenced the starch digestibility.

Mitchell (2008) showed the how glycemic concept was being used by food manufacturing industry, its perception by consumers and rate of importance in terms of regulatory provision and consequent labeling implications in different countries. The use of glycemic index (GI) was the most prominent form of label in global market.

Crapo *et al.* (1980) studied the postprandial hormonal responses to different types of complex carbohydrate in individuals with impaired glucose tolerance. They have studied the effect of dextrose, rice, potato, corn and bread on postprandial plasma glucose, insulin and glucagon responses in 11 subjects with impaired glucose tolerance. The results showed that, the dextrose and potato elicited similar plasma glucose responses whereas, rice and bread elicited lower responses with corn intermediate. Dextrose and potato elicited similar plasma insulin responses whereas, rice gave lower response with white bread and corn as intermediate. All the three carbohydrate loads suppressed the plasma glucagon with dextrose causing greatest suppression. They also found

that there is a range of plasma glucose, insulin and glucagon responses to different complex carbohydrates in subjects with impaired glucose tolerance and the different plasma glucose responses may be therapeutic value in controlling the hyperglycemia.

Jenkins *et al.* (1980) studied diets which had high carbohydrate combined with high fibre. In this study 19 diabetics were subjected for 24 to 45 days study, where the guar supplementation was given with carbohydrates. Intakes of carbohydrates ranged from 22 to 61 per cent of total calories. Where carbohydrate formed more than 40 per cent of the total caloric intake, there was a mean 64 per cent reduction in glucosuria. No significant reduction was seen in patients with lower carbohydrate intake. The dietary fibre supplements in diabetic should be given along with the higher carbohydrate intake. Wolver (1990) is also of the opinion that cellulose provide to be best predictor of glycemic response even better than total dietary fibre content.

Thomas *et al.* (2004) reported that high fibre cereal reduces postprandial insulin responses in hyperinsulinemic but not normoinsulinemic subjects. In this study they compared the plasma glucose and insulin response elicited by two ready to eat breakfast cereals (one contained high fibre and another low fibre) and differences in response depended on subject's fasting plasma insulin. The results revealed that high fibre cereal reduced the glucose responses to the same extent in normal and hyperinsulinemic subjects but reduced response was not found in normoinsulinemic subjects.

2.3 Value added products from mulberry leaf powder

In a review Chawdary *et al.* (2009) stated that mulberry is being used as a source of food, leaf protein and source of medicine, timber and fuel. The leaf is a rich source of nutrients like protein, calcium,

phosphorous, carotene and amino acids. The leaf powder is used in food industries of South Korea and China for the preparation of various products such as noodles, biscuits, bread, baked products, yoghurt, sauce, salads, omelets, pudding, ice creams etc. In India mulberry leaf powder is being used in preparations like paratha, curry. Fresh leaf and tender twigs are being used in pakoda, vada, idly, dosa, chapathi etc. Leaf protein is being used in fortification of dishes.

Srivatsava *et al.* (2003) used mulberry leaf powder along with wheat flour to develop paratha, which is most commonly used in Indian diet. The optimum ratio of the mulberry leaf powder and wheat flour mix for preparation of paratha on the basis of sensory quality was 1:4. The products were found to be acceptable.

Charunuch *et al.* (2008) studied the effects of extrusion condition on the physical and functional properties of instant cereal beverage powders admixed with mulberry (*Morus alba* L.) leaves. To study the feasibility of the production, the response surface methodology (RSM) was employed to investigate the interaction of operating conditions at varying screw speed (300,350 and 400rpm), mulberry content (5,10 and 15 per cent) and feed moisture (15, 17 and 19 per cent)on the physical and antioxidant property of product. The results revealed that the effect of mulberry content and feed moisture had significant on bulk density, colour, viscosity, water absorption index, antioxidant activity, total phenolic compound. Increase of mulberry content resulted in finished product with higher bulk density, less lightness, less viscosity, less water absorption index, higher antioxidant activity and total phenolic compounds as a result of in green foods. Ten per cent mulberry content and 17 per cent feed moisture to obtain finished product of high anti oxidant activity and total phenolic compounds with good characteristics

of moderate green colour, easy to dissolve in hot water, palatability and acceptability.

Srivastava *et al.* (2009) studied the mulberry leaf based soups. The fresh leaves were used for soup preparations and onion soup was used as control. The preparation of mulberry soup recipe was standardized and evaluated for sensory characters by semi trained panel members using nine point hedonic scales. The mulberry leaf based onion soup was almost similar to control with regard to preference.

Joshi *et al.* (2008) developed a few acceptable food products. They evaluated the effect of method of cooking on cooking quality. They performed organoleptic profiling of the varieties. The mulberry varieties viz, Vishala, TR8, S1636, S1328, S36 1394, M5 1338 had deep olive green colour, taste was astringent. Products such as mulberry papad, mulberry soup mix, mulberry chapathi mix, mulberry khakra and mulberry chilli biscuits were found to be highly acceptable.

Kang *et al.* (2002) studied the method for preparation of mulberry leaf powder and ice cream containing thereof. A method was described for the preparation of mulberry leaf powder and its use in the manufacture of ice cream. The powder was obtained by blanching mulberry leaves in an aqueous solution containing 0.05 to 0.5per cent sodium bicarbonate, followed by drying and pulverization. The ice cream is prepared by mixing 0.5 to 5 per cent (w/w) mulberry leaf powder with ice cream mix. The ice cream products obtained were claimed to be both functional and palatable.

Singh *et al.* (2004) performed nutritional evaluation of products developed from dried spinach leaves. Fresh and dried spinach leaves were used for the preparation of cake, biscuit, pakora, vada, namakpara and kurmura. The dried samples of products were analyzed for various

nutrients like protein and moisture ranging from 1.43 to 40.87 per cent and 9.16 to 16.62 per cent respectively. Ascorbic acid content was highest in products prepared from fresh spinach as compared to dried powder. β-carotene content was maximum in namakpara from dried leaves. Total iron content of spinach products ranged from 4.10 to 15.00mg/100g on dry weight basis. Ionisable and in vitro iron (per cent of total iron) was found to be highest in biscuits.

Kowsalya and Mohandas (1999) studied the acceptability and nutrient profile of cauliflower leaves (*Brassica oleracea, var Botryttis).* The objective of the study was to find out the feasibility of using cauliflower leaves in common south Indian recipes and assess their nutritional profile. The leaves were cooked in the traditional south Indian meal forms namely poriyal and kootu. The leaves were also incorporated into common preparations like adai, vadai and chapathi at 10 and 20 per cent levels. The drumstick leaves and amaranth leaves were used for comparison and without incorporation of standard recipe were used as control. The mean scores for acceptability of various preparations in terms of colour, appearance, texture, flavor and taste were judged by a panel of 10 members. They concluded that the cauliflower leaves were accepted in the poriyal form as well as in the incorporated recipes such as adai, vadai chapathi.

Hansawasdi and Kawabata (2006) studied the effect of brewing time on dry weight content of and glucosidase inhibitory active component released from mulberry tea. Different tea product showed significant difference in inhibitory activity against both sucrase and maltase. The most effective enzyme inhibition was observed when 3 to 5 minutes brewing time was applied in tea preparation.

Goyle and Gujral (1992) studied the sensory evaluation and acceptability trials of biscuits from raw and malted wheat (*Triticum*

aestivum) – bengal gram (*Cicer arietinum*) mixes with or without green leafy vegetable. The biscuits were prepared by varying the amounts of basic ingredients to arrive at the most acceptable recipe. The final recipe contained 40g of mix, 40g of jaggery and 20g of ghee. Sensory evaluation of biscuits was containing five and ten per cent colocasia leaf powder by composite scoring test and hedonic scale showed that the former type of biscuit was preffered over the latter. The acceptability trial conducted on three to six year old children showed that the biscuits from either malted mix with or without 7.5per cent colocaisa leaf powder and raw mix were equally acceptable.

Singh *et al.* (2001) studied the nutritional composition of selected green leafy vegetables, herbs and carrots. Six green leafy vegetables and herbs, viz., spinach, amaranth, bengal gram, cauliflower, mint, coriander and carrots were analyzed for moisture, protein, ascorbic acid, *β*-carotene, total iron, ionizable iron (as per cent of total iron) in vitro iron (per cent of total iron), copper, manganese and zinc. Moisture content of the leaves and carrots varied from 75.1 per cent to 95.4 per cent bengal gram and in carrot and protein from 9.83 per cent in carrots to 30.9 per cent in mint. Ascorbic acid, *β*-carotene, total iron and ionizable iron contents were at a maximum in case of bengal gram leaves whereas level of ionizable iron and *in vitro* iron as a per cent of total iron was highest in carrots. Copper, manganese and zinc contents were maximum in spinach.

Bhavani and Kaminidevi (1996) studied the stability of curry leaves and carrot powder in snack preparation. Curry leaves and carrots were dried and powdered to 250 micron size. Maize puff was standardised using maize grits, pepper, salt and groundnut oil. Sensory evaluation was carried out by 12 penelists for the samples prepared either with carrot powder and curry leaf powder at various levels and were evaluated

at 5, 10, 15, 20, 25, 30, 35 and 40g per 100g of the product respectively. The authors observed that the scores for products with 30 per cent incorporation of curry leaf powder and carrot powder were highest in terms of taste (98 and 97) and overall acceptability (100 and 97) respectively. The authors concluded that in addition to providing satiety value to the consumers, it also enhanced the carotene value of daily requirement.

Begum *et al.* (2000) developed the products incorporating cauliflower leaf powder to enhance the β-carotene and iron content. Products were assessed for their sensory attributes and also analyzed for the nutritive components. Cauliflower leaves which are unconventional were obtained from local market, processed and utilized in products viz, masala ban, gingilly chikki, wheat soya halwa and nippattu. Cauliflower leaf powder was incorporated at 10, 15 and 20 per cent level. The products found were acceptable at 10 per cent level of incorporation of cauliflower leaf powder with mean acceptability scores 3, 4, 3.6, 3.4 and 3.9 for masala biscuits, masala ban, gingilly chikki, wheat soya halwa and nippattu respectively as judged by five point scale. The nutritive values of best accepted products were analyzed and were expressed for 100g on fresh weight basis.

Punia *et al.* (2004) studied the nutrient composition of amaranth (*Amaranthus tricolour*) and kondhara (*Digeria arvensis*) leaves and their products. Recipes of amaranth and kondhara leaves were standardized in the laboratory and analyzed for their nutrient content. The protein, fat, mineral, crude fibre, carbohydrates and energy content of raw leaves varied from 27.89 to 28.44, 1.74 to 4.55, 20.26 to 22.61, 5.50 to 8.00 , 38.90 to 42.11 and 294.64 to 310-31 K cal/100g on dry weight basis respectively. Calcium, iron, and β-carotene content of raw leaves were 3135.0 to 3289.58, 3.35 to 8.98, 104.34 to 170.39mg/100g and 13464

to 14057µg/100g on dry weight basis respectively. Paratha and poori were prepared by incorporating amaranth leaves. Bengal gram dhal, green gram dhal, raita and sag were prepared by incorporating kondhara leaves. Protein, fat, minerals, crude fibre, carbohydrates and energy content of their products varied from 11.48 to 30.44, 7.25 to 28.77, 2.64 to 21.33, 0.25 to 5.75, 38.56 to 70.72g/100g and 367.33 to 533.29Kcal/100g on dry weight basis respectively. Calcium, iron, ascorbic acid and β-carotene content of their products were 127.30 to 3350.0, 1.50 to 4.10, 5.41 to 60.83mg/100g and 1710 to 10557µg/100g on dry weight basis respectively. It was concluded that these leaves and their products were good sources of protein, calcium, iron and β-carotene.

Nandini and Salimath (2001) studied the carbohydrate composition of wheat, wheat bran, sorghum and bajra with good chapathi/roti (Indian flat bread) making quality. Carbohydrate composition of various cereal var. which exhibit good chapati and roti making properties was evaluated. Samples of wheat (Triticum aestivum cv. Sonalika), the corresponding wheat bran fraction, sorghum (Sorghum bicolour cv. M354) and bajra (Pennisetum typhoideum cv. S203) were analysed for total carbohydrates, uronic acids, starch content, proteins, sugars, and soluble and insoluble dietary fibre. Results showed arabinoxylans as the main polysaccharide with varying ratios of arabinose to xylose according to fraction and cereal species. Wheat bran was richest in dietary fibre (insoluble dietary fibre comprised of 35.8 and soluble dietary fibre 4.0 per cent of defatted wheat bran).

2.4 Shelf life of value added products using leaf powder.

Srivatsava *et al.* (2003) developed mulberry leaf powder and incorporated it with wheat flour to develop paratha, which is most commonly used in Indian diet. The storage stability of mix was estimated

for a period of two months in polyethylene bags at room temperature. They found that there was a non significant difference between paratha prepared from fresh and stored mix. This indicated that the mix can be stored for a period of two months without loss of quality.

Goyle and Gujral (1993) developed mixes and biscuits from malted and raw wheat, bengal gram grains with or without green leafy vegetable. Mixes were prepared from malted and raw wheat (4per cent) and bengal gram grains (1per cent). The same were used in preparation of biscuits. The incorporation of colocasia leaf powder in biscuit was acceptable at 7.5 per cent level. The authors also studied the keeping quality of mixes and the biscuits. It was observed that malted and raw mixes could be kept for 28 days under both accelerated (37° C and 90 per cent and RH) and room (25° C to 34° C and 46 to 99per cent RH) conditions based on moisture gain, alcoholic acidity and peroxide value. While biscuits had a keeping quality of seven days at accelerated condition and 28 days for room temperature.

Udal and Sagar (2008*)* studied the influence of packaging and storage temperature on the quality of dehydrated selected leafy vegetables. Three leafy vegetables viz, amaranth (*Amaranthus Spp*), fenugreek (*trigonella foenum-graecum*), and palak(*Beta vulgaris var.bengalensis*) were dried in cabinet at 58±2° C and packed in Low Density Polyethylene (200 gauge and 400 gauge), High Density Polyethylene (HDPE 200 gauge)and Polypropylene (150 gauge). Products were stored at ambient (25 to 35°C) and low temperature (7±1°C) for 3 months to evaluate the best package and storage temperature for maximum retention of nutrients. HDPE (200 gauge) and storage 7±1° C was best for dehydrated leafy vegetables for storage up to three months. The dehydrated leafy vegetables retained higher β-carotene, ascorbic

acid, total chlorophyll content, rehydration ratio and sensory score and had less moisture and non-enzymatic browning in the dried product.

Devraju *et al.* (2006) prepared pasta products made from finger millet composite flour which was extruded using 50 per cent finger millet flour, 40 per cent refined wheat flour and 10 per cent defatted soy flour (DFS). Packed in double sealed polyetylene cover was kept at room temperature. The organoleptic characteristics and microbial load were studied during the storage by analysis at regular intervals over a period of 3 months. Products had good acceptability even after 3 months storage.

Marero *et al.* (1988) formulated weaning food from germinated cereals and legumes and stored for a period of 6 months at room temperature packed in polypropylene bags and plastic container. Results showed that formulations were microbially safe for consumption by the infants, being negative for coli forms and staphylococci as well having a much lower count for total bacteria count, yeast and mold count compared to control.

As per Rizk and Ebid (1989), the total aerobic bacteria in seven different flours were 3.0×10^6 to $9.1 \times 10^{7.}$ Graves and Hesseltine (1967) found that 27.3 per cent of wheat samples examined contained *Bacillus cereus.*

Chandrashekar *et al.* (1988) studied the evaluation of a malted food based on low cost locally available food and reported that polythene bags were suitable and easy for the storage of mixes at house hold level than in plastic container, tins or bottles. This study was conducted over a period of 45 days; total bacterial count was 10 to 150 per gram in the beginning and 38 to 550 in polythene bags at the end of the study.

Solinka (1986) reported on bacterial count of malted mix powder stored for 42 days. The observations showed that the mixes had low bacterial count even on 42nd day which indicate that mixes could remain acceptable after 42 days under.

Masayasu *et al* (2001) studied the toxicology of mulberry leaf powder in rats. The leaves powder was administered orally to male and female rats at dosage levels of l% (low dose) and 5% (high dose) in food for 4 weeks. Any disturbance of the growth was not found in the whole experimental period and dose related changes, or disorders did not appear in the organ weight, and in the hematologic, biochemical and pathological examination.

METHODOLOGY

III. MATERIAL AND METHODS

The present study was carried out in the Food Science and Nutrition Department, University of Agricultural Sciences, Bengaluru-65, Karnataka. The study was aimed at finding out the nutrient content and digestibility of leaves of different cultivars of mulberry; preparation and evaluation of value added products and assessing glycemic index of selected product. The details of material and methods used are presented in this chapter.

Experimental details

3.1 Procurement of samples and raw materials

3.2 Processing of mulberry leaves

3.3 Chemical analysis of leaf samples

3.4 *In-vitro* protein and carbohydrate digestibility

3.5 Product development

3.6 Sensory evaluation of products developed

3.7 Shelf life study of the products

3.8 Microbial load of products.

3.9 Glycemic index of selected product

3.10 Statistical analysis

3.1 Procurement of samples and raw materials

Leaves from different varieties of mulberry namely RFS-175, S-13, S-41, S-30, S-34, DD-1, Mysore local, M5 were procured from the Department of Sericulture, UAS, Bengaluru. Spinach leaves served as control and these were procured from local market in Bengaluru. Spinach leaves were employed in this study as a reference since mulberry leaves were an entirely new food, thus, a yard stick had to be devised. Spinach is very common green leafy vegetable which is locally,

nationally and globally used. For product development raw material was sourced from the local market in Bengaluru. The ITC brand of wheat flour was used. Other ingredients such as bengal gram flour, rice flour, sago flour, puffed bengal gram, soya dal and other condiments were obtained from the local shops.

3.2 Processing of mulberry leaves

The freshly collected leaves were washed in running water, wiped and stalks were removed. Leaves were dried in vacuum oven at 65 °C. The dried leaves were made into powder and were stored in polyethylene bags in refrigerator at 4°C.

3.3 Chemical analysis of leaf samples

Dried leaf powders were analyzed for the nutrients namely moisture, protein, fat, ash, crude fibre, β-carotene, chlorophyll, ascorbic acid, calcium, phosphorus, iron, oxalates, tannins and in-vitro digestibility of protein and carbohydrates. Samples were worked in triplicates.

3.3.1 Estimation of moisture (AOAC, 1980)

Moisture was determined by taking about 10g of sample in Petri dish and dried in an oven at 105º C till the weight of the Petri dish with its content was constant. Each time before weighing, the Petri dish was cooled in a desiccator. Moisture content of the sample was expressed in g/100g of sample

$$\text{Moisture content (g/100g)} = \frac{\text{Initial weight (g) - Final weight (g)}}{\text{Weight of the sample}} \times 100$$

3.3.2 Estimation of Protein (AOAC, 1980)

The protein content of the dried samples was estimated as per cent total nitrogen by the Micro-kjeldahal method (AOAC, 1980) and then computed by multiplying the per cent nitrogen using conversion factor 6.25 and for the digestion of samples the Gerhardt Turbotherm digestion unit was used. The distillation was carried out in Gerhardt Vapodest automatically (Annexure-VI).

3.3.3 Estimation of total lipids

Total lipids were estimated using the Bligh and Dyer (1959) method. The lipids extracted in a mixture of chloroform and methanol (2:1 v/v) (Annexure-VII).

3.3.4 Estimation of crude fibre (AOAC, 1980)

Crude fibre of the sample was estimated by using moisture and fat free samples. Fibre was estimated by boiling in acid (sulphuric acid 0.255 N) and subsequent alkali (0.31 N NaOH) using the Gerhardt fibre bag system. Then it was filtered and washed with distilled water and dried at 80°C to 100°C. Samples were ashed in muffle furnace. The ash content was cooled in desicators and weighed. The difference represents the crude fibre content of the sample and was expressed as gram per 100g or per cent (Annexure-VIII).

$$\text{Crude fibre (g/100g sample)} = \frac{[100-(\text{moisture}^{*}+\text{fat})] \times (W_e - W_a)}{\text{Wt of sample taken (moisture and fat free)}}$$

3.3.5 Estimation of ash (AOAC , 1980)

Total ash was estimated by taking about 5g of the sample into a crucible (which has previously been heated to about 600°C and cooled). The crucible was placed on a clay pipe triangle and heated first over a low flame till all the material was completely charred followed by heating

in a muffle furnace for about four to five hours at about 600°C. It was then cooled and weighed. This was repeated till two consecutive weights were same and the ash was almost white or greyish white in colour.

$$\text{Ash content (g/100g sample)} = \frac{\text{Weight of the ash}}{\text{Weight of the sample}} \times 100$$

3.3.6 Computation of carbohydrate (AOAC, 1980)

Carbohydrate content was calculated by differential method.

Carbohydrate (g/100g) = 100 - [Protein (g) + Fat (g) + Fibre (g) + Ash (g) + Moisture (g)]

3.3.7 Computation of energy (AOAC, 1980)

Energy was computed as followed for all the samples.

Energy (kcal) = [Protein (g) x 4] + [Carbohydrate (g) x 4] + [Fat (g) x 9

3.3.8 Preparation of mineral solution

The mineral solution was prepared by dissolving the ash obtained after ashing (Annexure-XI) the samples in a muffle furnace in dilute hydrochloric acid.

3.3.9 Estimation of calcium (AOAC, 1980)

The calcium content was estimated by precipitating it as calcium oxalate and titrating the solution of oxalate in dilute acid against standard potassium permanganate (Annexure-XII).

3.3.10 Estimation of Phosphorus (AOAC, 1980)

Determination of phosphorus was carried out by measuring calorimetrically the blue colour formed when the ash solution was

treated with ammonium molybdate and thus phosphomolybdate formed was reduced (Annexure XIII).

3.3.11 Estimation of Iron (AOAC, 1980)

Iron was determined by calorimetric method. When potassium thiocyanate was added to the sample it turned red indicating the presence of iron in a sample. (Annexure XIV).

3.3.12 Estimation of β-carotene (Ranganna 2002)

β-Carotene was estimated by colourimetric method. The samples were first extracted with acetone, then by petroleum ether. The concentration of β-Carotene in the solution was determined by measuring the optical density of the solution in a 452nm (Annexure XV)

3.3.13 Estimation of total sugars (Dubois et al. 1956)

The total soluble sugar content in the sample was estimated by phenol-Sulphuric acid method. The concentration of the sugars was determined by measuring the optical density at 496nm (Annexure X)

3.3.14 Estimation of oxalates (AOAC, 1980)

Oxalic acid was estimated by precipitating calcium oxalate which was then titrated against standard potassium permanganate (Annexure XVI).

3.3.15 Estimation of Tannins (AOAC, 1980)

Tannin was estimated calorimetrically based on the measurement of blue colour formed by the reduction of phosphotungstomolybdate acid in alkali solution (Annexure XVII).

3.3.16 Colour (Ranganna, 2002):

Colour of different varieties of leaves was recorded by referring to Munsell colour dictionery for plant tissues as per the method described by Ranganna (2002).

3.4 In-vitro protein and carbohydrates digestibility

IVPD was obtained by calculating the difference between the amount of total nitrogen in the sample before and after in vitro digestion with (0.2 percent) pepsin and expressing it as percentage. Nitrogen was multiplied by the factor 6.25 to obtained crude protein (Anonymous), the procedure is included in (Annexure XVIII).

IVCD was obtained by *in vitro*-α-amylolysis using standard procedure (Raghuramulu, 2003). One unit of α-amylase activity was expressed as the reducing sugar value equivalent to 1 mg maltose released per mg of the enzyme in 3 minutes. (Annexure XIX).

3.5 Product development

Products such as papad, khakara, chapathi mix and chutney powder were developed. The methods of preparation are presented in Annexure I, II, III and IV. The mulberry and spinach leaf powders were incorporated at 2 and 5 % levels in the products. Thus in all, for each type of product, there were four variations and a control.

- Control (a standard traditional recipe)
- Variations (incorporation of leaf powders in standard recipe)
    - 2 % substitutions of spinach leaf powder
    - 5 % substitution of spinach leaf powder
    - 2 % substitution of mulberry leaf powder
    - 5 % substitution of mulberry leaf powder

3.6 Sensory evaluation of products developed

All the developed products were evaluated by a panel of semi trained judges (n=20). The products were evaluated for appearance, texture, aroma, taste and overall acceptability on a five point hedonic scale. Where 5 = excellent, 4 = good, 3 = neither good nor bad, 2 = poor, 1 = very poor. Score sheet used for the evaluation of products is included in Annexure-V (Amerine et. al., 1965).

3.7 Shelf life study of the products

Shelf life of four products namely papad, khakara, chutney powder and chapathi mix was evaluated. The products were stored up to two months. Two types of packaging materials were used. Each product was stored in low density polyethylene covers and laminated covers and kept at room temperature. The products were evaluated for sensory evaluation.

3.8 Microbial load of products

Initial and final microbial counts were carried out. The microbial contamination was estimated by analyzing microbial load in chapathi mix by using Nutrient Agar (NA) for bacteria and Martins Rose Bengal Agar (MRBA) for mould count, following the dilution pour plate method. $10^{-3,}$ 10^{-4} and $10^{-4,}$ 10^{-5} were the dilutions use for analyzing mould and bacteria respectively (Anon 1957, and Martin 1950).

3.9 Glycemic index of selected product

The glycemic index indicates the extent of rise in blood glucose in response to test food in comparison with the response to an equivalent amount of white bread. Product developed from mulberry leaf powder namely chapathi made out of chapathi mix was tested for its glycemic response on 10 healthy volunteers. They comprised of students of UAS

Bengaluru ranging between 20-35 years. All the volunteers were normal and leading sedentary life. The purpose of study was explained to each subject and consent taken for participation. The subjects were instructed to not to take any medication, requested to avoid physical exertion during the experimental period. The peripheral blood glucose was obtained using 28 G pricking lancets and the blood glucose in peripheral blood was estimated using the in vitro diagnostic kit (XCE 188-1311) of the Abbott Diabetes Care Inc. Almeda.CA 94502, USA. The Glycemic index was computed using the following formula. The protocol is included in Annexure XX.

$$\text{Glycemic index (GI)} = \frac{\text{Area under test food curve}}{\text{Area under reference food curve}} \times 100$$

3.10 Statistical analysis

Data was tabulated and subjected to statistical analysis. In order to obtain the significance of difference between varieties for nutrient composition and for different products analysis of variance test (F-test) was used. Three way analysis of variance was performed to know the effect of packaging and levels of incorporation in fresh and stored products.

RESULTS

IV. EXPERIMENTAL RESULTS

Mulberry leaves have been attributed with several nutritional and health benefits. However, they are not being consumed locally. The present study was undertaken to know the different characteristics of varieties of mulberry leaves. Spinach leaves were employed throughout the study as a check due to their wider use in food products. The results recorded during the study are presented under the following sub headings

4.1 Nutrient composition and digestibility of selected varieties of mulberry leaves

4.2 Chlorophyll content and colour of selected varieties of mulberry leaves.

4.3 Development of products from selected varieties of mulberry leaves and their sensory evaluation.

4.4 Nutrient composition of the developed products

4.5 Shelf life study

4.6 Glycemic index of product developed from mulberry leaf powder.

4.1 Nutrient composition and digestibility of selected varieties of mulberry leaves

Leaves from different varieties of mulberry namely RFS-175, S-13, S-41, S-30, S-34, DD-1, Mysore local, M5 were analysed (Plate 1). Values were compared with spinach. Results of macro nutrients, micro nutrients, antinutrients, *in vitro* digestibility of protein and carbohydrate is presented in tables 1 to 5 and are explained in the following paragraphs.

Plate 1 : View of leaves of different mulberry varieties used in the study

4.1.1 Macro Nutrient composition

Macronutrients such as moisture, energy, protein, fat, carbohydrate, crude ash and crude fibre were estimated. The results are presented in Table 1.

4.1.1.1 Moisture content

The moisture of fresh leaves of mulberry varieties ranged between 67.90 and 75.05 per 100g. The results exhibited statistically significant differences among varieties. Spinach had higher moisture per cent compared to others. Among mulberry varieties S34 had highest (75.05 per cent) followed by S30 (73.53 per cent) and RFS175 (73.00 per cent). The moisture content of dried samples of leaves ranged between 5.27g and 9.24g/100g. The results exhibited statistically significant differences among varieties.

4.1.1.2 Protein

The protein content of dried samples of mulberry leaves ranged from 20.50 to 30.87g/100g. RFS175 had highest protein content (30.87g/100g) S13 (28.07g/100g) and least was in S34 (20.50g/100g). The results showed a significant difference among varieties. Dried spinach powder had comparable and high protein content (30.06g/100g).

4.1.1.3 Total lipid

The total lipid content of dry powdered samples ranged from 2.03 to 4.10g/100g. The spinach powder had fat content of 4.10g/100g. The result showed a non significant difference among varieties.

4.1.1.4 Crude fibre

The fibre content ranged from 9.41g to 15.08g/100g of dried sample. Spinach leaf powder had fibre content of 10.72g/100g.

Table 1. Macro nutrient composition in leaves of different mulberry varieties (per 100 g dry weight basis)

Varieties	Moisture		Energy □	Protein	Lipids	Carbohydrate □	Crude fibre
	Fresh (%)	Dry (%)	(Kcal)	(g)	(g)	(g)	(g)
RFS-175	73.00^{c}	9.24^{a}	277.00^{h}	30.87^{a}	2.52	31.81^{d}	12.16^{b}
S-13	71.00^{d}	7.99^{ab}	288.98^{e}	28.07^{b}	2.03	40.42^{c}	9.41^{d}
S-41	67.90^{f}	8.11^{ab}	284.41^{g}	24.56^{d}	3.43	43.24^{b}	10.62^{cd}
S-30	73.53^{c}	5.27^{b}	294.06^{c}	26.23^{c}	3.43	40.69^{c}	13.08^{a}
S-34	75.05^{b}	7.27^{ab}	292.98^{d}	20.50^{f}	2.93	46.95^{a}	12.00^{bc}
DD1	70.93^{d}	6.16^{ab}	294.88^{b}	26.60^{c}	4.10	39.44^{c}	11.44^{bc}
Mysore local	70.11^{d}	8.23^{ab}	286.89^{f}	26.73^{c}	2.60	40.94^{c}	11.64^{bc}
M5	68.90^{e}	9.06^{a}	286.82^{f}	21.77^{e}	2.36	44.64^{b}	14.94^{a}
Spinach	89.94^{a}	6.97^{ab}	343.02^{a}	30.06^{a}	4.10	47.96^{a}	10.72^{bcd}
Significance of test at 5 %	*	*	*	*	NS	*	*
S.Em±	0.3433	0.9714	0.1045	0.4112	0.6009	04958	0.4639
CD	1.0201	2.8862	0.3105	1.2217	-	1.47	1.37

*Significant at 5 % level NS – Non significant □- computed values

(Within the column means with different superscript indicates significant difference)

The highest crude fibre content was found in S30 (15.08g/100g) followed by M5 (14.94g/100g) and RFS 175(12.16g/100g) varieties. The result exhibited a significant difference among varieties.

4.1.1.5 Carbohydrate

The carbohydrate content of varieties ranged from 31.81 to 46.95g/100g of dried sample powder. The spinach powder had highest carbohydrate content (47.96g/100g) compared to S34 (46.95g/100g) and M5 (44.64g/100g). The result exhibited a significant difference among varieties.

4.1.1.6 Energy

The energy value ranged from 277.06 to 294.88Kcal/100g. Spinach powder had highest energy value (343.02Kcal/100g) followed by DD1 (294.88 Kcal/100g) and S30 (294.06Kcal/100g). The results showed a significant difference among varieties.

4.1.2 Total ash and Micro nutrients

Total ash and micro nutrients such as calcium, phosphorous, iron, vitamin C, β- Carotene and total sugars were estimated. The results are presented in Table 2.

4.1.2.1 Total ash

Ash content ranged from 11.19 to 15.18g/100g of dried sample. M5 variety had highest ash content followed by DD1 (14.37g/100g) and RFS175 (14.39g/100g). The result exhibited significant difference among varieties.

Table 2 : Total ash and micronutrient composition in leaves of different mulberry varieties (per 100 g dry weight basis)

Varieties	Total ash	Calcium	Phosphorous	Iron	Ascorbic acid	β-carotene	
						Fresh	Dry
	(g)	(mg)	(mg)	(mg)	(mg)	(µg)	(µg)
RFS-175	14.39^{b}	561.66	6.40^{bc}	97.66^{d}	176.66^{c}	312.60^{h}	426.66^{h}
S-13	13.02^{c}	360.00	4.60^{bc}	86.33^{e}	176.66^{c}	363.16^{g}	650.00^{g}
S-41	13.00^{c}	243.33	7.83^{bc}	79.66^{f}	180.00^{b}	725.33^{b}	13354.09^{b}
S-30	11.37^{e}	424.33	9.36^{ab}	98.00^{d}	133.33^{b}	637.33^{c}	10799.96^{c}
S-34	11.19^{f}	393.00	12.90^{a}	98.33^{d}	216.66^{a}	200.00^{i}	757.33^{f}
DD-1	14.37^{b}	168.33	8.43^{bc}	72.33^{g}	180.00^{b}	562.66^{d}	774.00^{f}
Mysore local	11.55^{d}	384.33	8.23^{bc}	115.00 ^{c}	216.66^{a}	761.30^{a}	13515.66^{a}
M5	15.18^{a}	296.33	7.33^{bc}	150.00 ^{b}	180.00^{b}	375.00^{f}	2316.06^{e}
Spinach	12.04^{g}	200.00	4.50^{c}	175.00 ^{a}	176.66^{c}	512.33^{e}	2436.33 ^{d}
Significance of test at 5 %	*	NS	*	*	*	*	*
S.Em±	0.02	0.88	1.37	1.11	26.17	2.41	6.19
C D	0.07	2.62	4.08	3.31	77.77	7.17	18.40

*Significant at 5 % level NS-non significant

(Within the column means with different superscript indicates significant difference)

4.1.2.2 Calcium

Calcium content ranged from 168.33 to 561.66mg/100g content of dried leaf samples of mulberry. The result showed a significant difference among the samples analysed.

4.1.2.3 Phosphorous

The phosphorous content ranged from 4.60 to 12.90mg/100g of dried sample. S34 variety had highest phosphorous content (12.90mg/100g) followed by S30 (9.36mg/100g) and DD1 (8.43mg/100g). The result exhibited significant difference among varieties.

4.1.2.4 Iron

The iron content ranged from 72.33 to 150.00mg/100g of dried sample. M5 variety had highest iron content (150.00mg/100g) followed by Mysore local (115.00mg/100g) and spinach higher iron content than mulberry varieties (175.00mg/100g)

4.1.2.5 Ascorbic acid

The ascorbic acid content ranged from 133.33 to 216.66mg/100 in dry powder sample. Highest ascorbic acid was found in S34 and Mysore local and least in S30 variety. The result exhibited a significant difference among varieties.

4.1.2.6 β-carotene

The β-carotene content ranged from 200 to 761.30µg/100g (S-34 and Mysore local) of fresh sample. The result exhibited significant difference among varieties. In dry powder it ranged from 426.66 to 13515.66µg/100g of dried sample of S-13 and Mysore local varieties respectively.

4.1.2.7 Total sugars

Total sugar content of dry mulberry leaf powders ranged from 5.0 to 15.66per cent. The highest sugar content was found in S34 (15.66 per cent). There was no significant difference between varieties S13 to S41 and DD1 to RFS 175. The result revealed statistically significant differences among varieties. The total sugar content in leaves of different mulberry varieties is shown in Table 3.

4.1.2.8 *In-vitro* Protein Digestibility

In vitro protein digestibility was estimated and values ranged between 79.27 to 99.68 per cent. The values were expressed as per cent digestibility. The highest per cent digestibility was found in S13 and least was found in M5. Spinach leaf powder had protein digestibility of 58.86 per cent. The values are presented in Table 4. Significant differences were observed among samples.

4.1.2.9 *In-Vitro* α-amylolysis of selected varieties of mulberry leaves.

Carbohydrate digestibility at 3 minutes ranged between 9 to 26mg of maltose released per mg of enzyme. Milligrams of maltose released per mg of enzyme in every three minutes of time was 6 to 39mg after an elapse of 6 minutes of time and after 9 minutes of time it was 2 to 32mg. Carbohydrate digestibility is presented in Table 5.

4.1.2.10 Oxalate and Tannin content

The oxalate and tannin content of selected varieties of mulberry leaves is presented in Table 6. Significant differences were observed between the samples for both oxalates as well as tannin content.

Table 3: Total sugar content in leaves of different mulberry varieties (per 100 g dry weight basis)

Variety	Total sugar (%)
RFS-175	5.00 [d]
S-13	15.33 [a]
S-41	15.33 [b]
S-30	10.66 [c]
S-34	15.66 [a]
DD-1	5.00 [d]
Mysore local	8.33 [a]
M5	14.66 [e]
Spinach	3.66 [e]
Significance of test at 5 %	*
SEm±	0.675
CD	2.00

*significant at 5 % level

(Within the column means with different superscript indicates significant difference)

Table 4 : *In-vitro* digestibility of protein in leaves of different mulberry varieties (per 100 g dry weight basis)

Variety	Protein content		
	Before digestion	**After digestion**	**Per cent digestibility**
RFS-175	30.19	27.80[a]	92.08
S-13	27.04	26.95[a]	99.68
S-41	24.03	21.24[d]	90.92
S-30	26.46	17.61[f]	66.13
S-34	20.45	19.55[e]	95.92
DD1	26.36	25.33[b]	97.09
Mysore local	26.79	23.72[c]	88.56
M5	22.13	17.47[f]	79.27
Spinach	30.59	17.50[f]	58.86
Significance of test at 5%	-	*	-
S.Em±	0.58	0.66	0.66
C D	1.74	1.97	1.97

*****significant at 5 % level

(Within the column means with different superscript indicates significant difference)

Table 5 : ***In-vitro*** **α-amylolysis in leaves of different mulberry varieties (per 100 g dry weight basis)**

Varieties	(0- 3) minutes	(3-6) minutes	(6-9) minutes
	mg of maltose released per mg of enzyme		
RFS-175	9	8	2
S-13	26	25	24
S-41	20	25	13
S-30	9	6	4
S-34	14	6	21
DD1	31	25	32
Mysore local	22	26	6
M5	10	39	22
Spinach	20	27	20

Table 6 : Oxalate and tannic acid content in leaves of different mulberry varieties (per 100 g dry weight basis)

Variety	Oxalate (mg/100g)	Tannin (mg/100g)
RFS -175	32.24^{bcd}	4.99^{h}
S-13	27.15^{d}	14.42^{b}
S-41	35.17^{abc}	5.59^{f}
S-30	28.67^{cd}	15.18^{a}
S-34	27.67^{d}	10.79^{c}
DD-1	29.39^{bcd}	6.98^{e}
Mysore local	39.95^{a}	5.19^{g}
M5	36.15^{ab}	7.96^{d}
Spinach	35.61^{ab}	4.99^{h}
Significance of test at 5%	*	*
S.Em±	2.12	0.0147
CD	6.311	0.0437

*Significant at 5 % level

(Within the column means with different superscript indicates significant difference)

Oxalate content

The average oxalate content ranged from 27.15 to 39.95mg/100g of dried sample of leaf powders. The highest oxalate content was found in Mysore local (39.95mg/100g) followed by M5 (36.15mg/100g) and spinach (35.61mg/100g). There were significant differences among varieties.

Tannin content

The average tannin content ranged from 4.99 to 15.18mg/100g of dried sample. The highest tannin content was found in S30 (15.18µg/100g) followed by S13 (14.42µg/100g) and there was no significant difference between RFS175 and spinach. The result exhibited a significant difference among varieties.

4.2 Chlorophyll content and colour of selected varieties of mulberry leaves

4.2.1 Chlorophyll

Chlorophyll 'a', chlorophyll 'b' and total chlorophyll content of the leaves is shown in Table 7.

Chlorophyll 'a'

The chlorophyll 'a'content ranged from 0.30 to 6.70 mg/g in all the fresh samples. The highest was found in RFS 175 (6.70mg/g) followed by S34 (4.72 mg/g) and there was no significant difference between S13 and Mysore local. The result showed significant differences among varieties.

Table 7: Total chlorophyll content in leaves of different mulberry varieties (dry weight basis)

Variety	Chlorophyll (mg/g)		
	a	**b**	**Total**
RFS-175	6.70[a]	3.53[a]	10.23[a]
S-13	3.35[c]	2.48[c]	5.44[bc]
S-34	4.72[b]	2.99[b]	7.42[ab]
S-41	2.66[e]	1.12[e]	4.03[bcd]
S-30	3.10[d]	1.98[d]	4.94[bc]
DD-1	2.36[f]	1.98[d]	4.15[bcd]
Mysore local	3.51[c]	2.46[c]	5.75[bc]
M5	1.85[g]	1.18[f]	2.94[cd]
Spinach	0.30[h]	0.35[f]	0.65[d]
Significance of test at 5 %	*	*	*
SE±m	0.04	0.01	0.64
CD	0.13	0.05	1.91

***-**Significant

(Within the column means with different superscript indicates significant difference)

Chlorophyll 'b'

The chlorophyll 'b' content ranged from 0.35 to 3.53mg/g in all the fresh samples. The highest was found in RFS175 (3.53mg/g) followed by S34 (2.99mg/g) and there was no significant difference between S13, Mysore local, between S30 and DD1 and between M5 and spinach. The result showed a significant difference among varieties.

Total chlorophyll

Total chlorophyll content ranged from 0.65 to 10.23mg/g. The highest total chlorophyll content was recorded in RFS175 (10.23mg/g) followed by S34 (7.42mg/g). The result showed a significant difference among varieties

4.2.2 Colour of the leaves

There were some differences between varieties for the attribute colour. Spinach leaves, DD-1 and S-30 had pale green colour and rest of the varieties had dark green colour. Similarly some variations were observed in the hue, value and chroma values. The Results are shown in Table 8.

4.3 Development of products from selected varieties of mulberry leaves and their sensory evaluation.

The preliminary test of development of value added products from mulberry leaf powder was carried out in the Department of Food Science and Nutrition, GKVK, UAS, Bengaluru (Joshi *et al* 2008). The result showed mulberry leaf powder incorporation up to 5 per cent level was acceptable. Four products developed from spinach and M5 leaf powder were chapathi mix, papad, chutney powder and khakra. Acceptability and difference in sensory attributes were tested. The results are depicted in Tables 9 to 12 and are explained below.

Table 8 : Hue, value and chroma values of selected varieties of mulberry leaves

Variety	Hue name	Hue scale	Value	Chroma
RFS-175	OG	5	4	4
S-13	OG	5	3	4
S-34	OG	5	3	4
S-41	OG	5	3	4
S-30	GY	2.5	6	8
DD-1	GY	2.5	6	8
Mysore local	OG	5	4	4
M5	OG	5	3	4
Spinach	GY	2.5	6	8

OG-Dark Green

GY- Pale Green

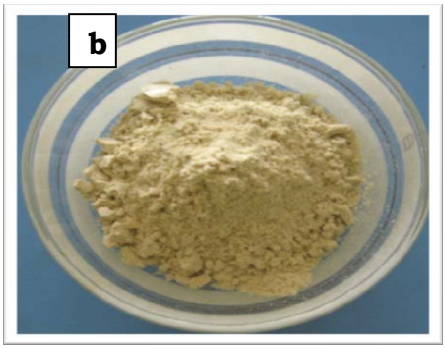


Plate 2 : Product chapathi mix developed from mulberry and spinach leaf powder incorporation (a) control (b) 2 % mulberry (c) 2 % spinach (d) 5 % mulberry (e) 5 % spinach

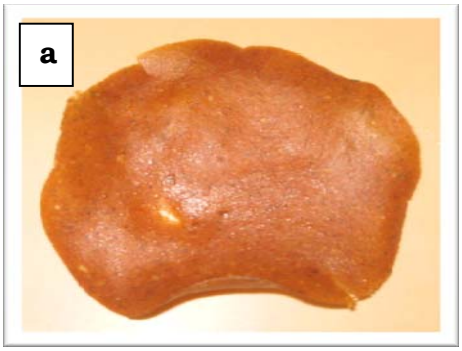
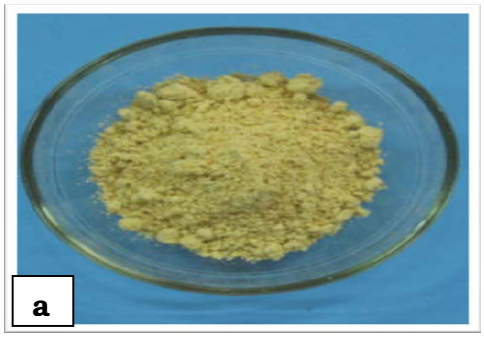
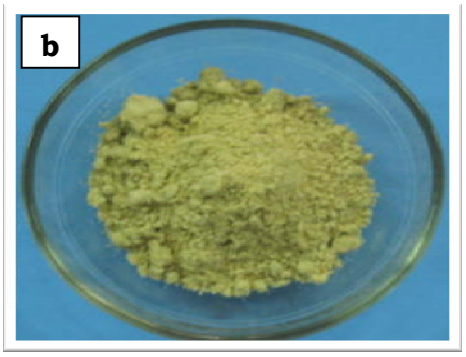
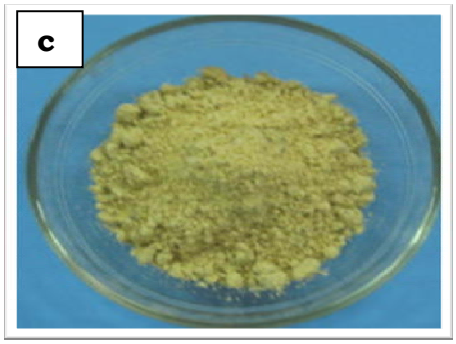


Plate 3 : Product papad developed from mulberry and spinach leaf powder incorporation (a) control (b) 2 % spinach (c) 2 % mulberry (d) 5 % spinach (e) 5 % mulberry

Plate 4 : Product khakra developed from mulberry and spinach leaf powder (a) control (b) 2 % spinach (c) 2 % mulberry (d) 5% spinach (e) 5 % mulberry







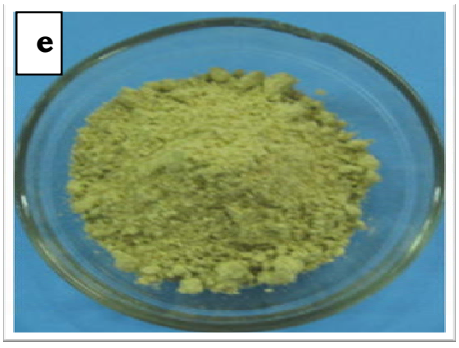


Plate 5 : Product chutney powder developed from mulberry and spinach leaf powder (a) control (b) 2 % spinach (c) 2% mulberry (d) 5%spianch (e) 5 % mulberry

4.3.1 Mean scores for sensory characteristics of chapathi mix.

The mean scores of sensory evaluation of chapathi mix are given in the Figure 1 and Table 9. Control showed a high for all the characters. However, there was no significant difference between the variations of chapathi mix developed from mulberry and spinach leaf powder for all the sensory characters.

4.3.2 Mean scores for sensory characteristics of papad.

The mean scores of sensory evaluation of papad are given in Figure 2 and Table 10. Control showed slightly high sensory scores compared to other variations. Significant difference was found for the sensory characters such as appearance, colour and overall acceptability between papad developed from mulberry and spinach leaf powder.

4.3.3 Mean scores for sensory characteristics of chutney powder.

The results of the sensory evaluation of chutney powder are given in Figure 3 and Table 11. The results revealed that there was no significant difference for the sensory characteristics of chutney powder developed from mulberry and spinach leaf powder.

4.3.4 Mean scores for sensory characteristics of khakra.

The mean scores of sensory evaluation of khakra are given in the Figure 4 and Table 12. Results showed that there was no significant difference between mulberry and spinach leaf powder incorporated khakra.

4.4 Nutrient composition of the developed products

Macro nutrients such as moisture, energy, fat, protein, carbohydrate and fibre were computed for all the four products chapathi mix, papad, chutney powder and khakra. Values for micro nutrients such as calcium, phosphorous and β-carotene were computed. The nutrient compositions of products are presented in Table 13.

Table 9 : Mean sensory scores for chapathi prepared from poushtik chapathi mix

	Appearance	**Texture**	**Colour**	**Taste**	**Overall acceptability**
Control	4.0	3.85	3.85	4.10	4.0
Mulberry leaf powder (Incorporation levels)					
2%	3.65	3.90	3.50	3.65	3.75
5%	4.05	3.80	4.0	4.05	3.85
Spinach leaf powder (Incorporation levels)					
2%	3.75	3.80	3.80	3.70	3.95
5%	3.85	3.95	3.85	3.75	3.80
Significance of test at 5 %	NS	NS	NS	NS	NS
S.Em±	0.183	0.182	0.157	0.193	0.159
C D	-	-	-	-	-

NS- non significant

(Within the column means with different superscript indicates significant difference)

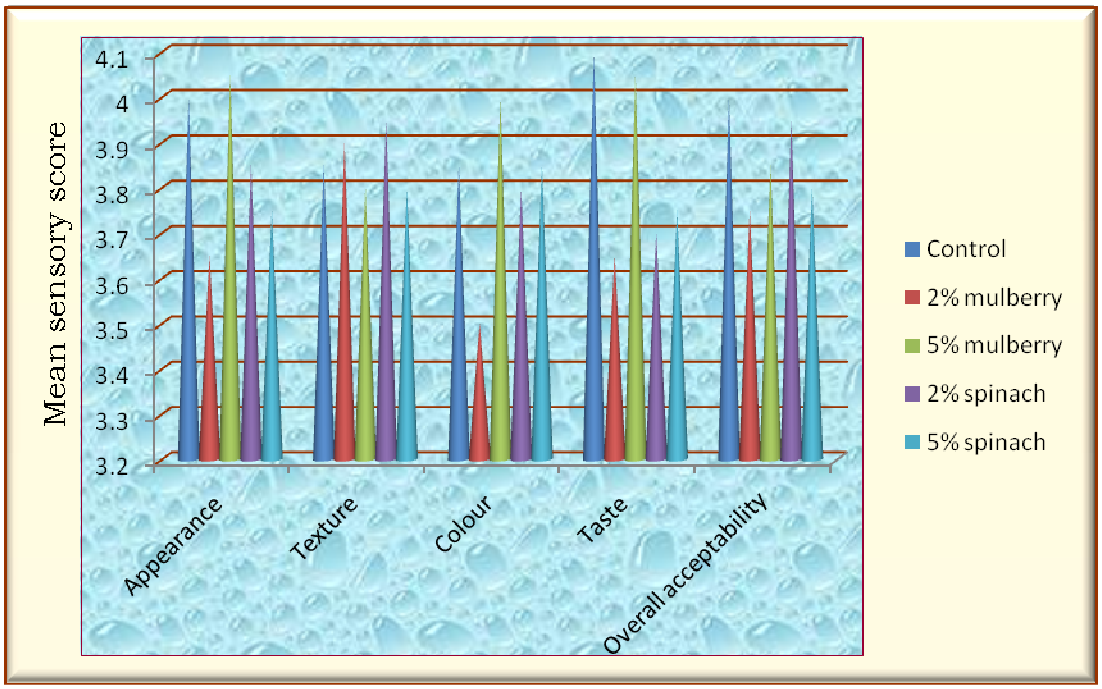


Fig. 1 : Mean sensory scores for chapathi developed from chapathi mix

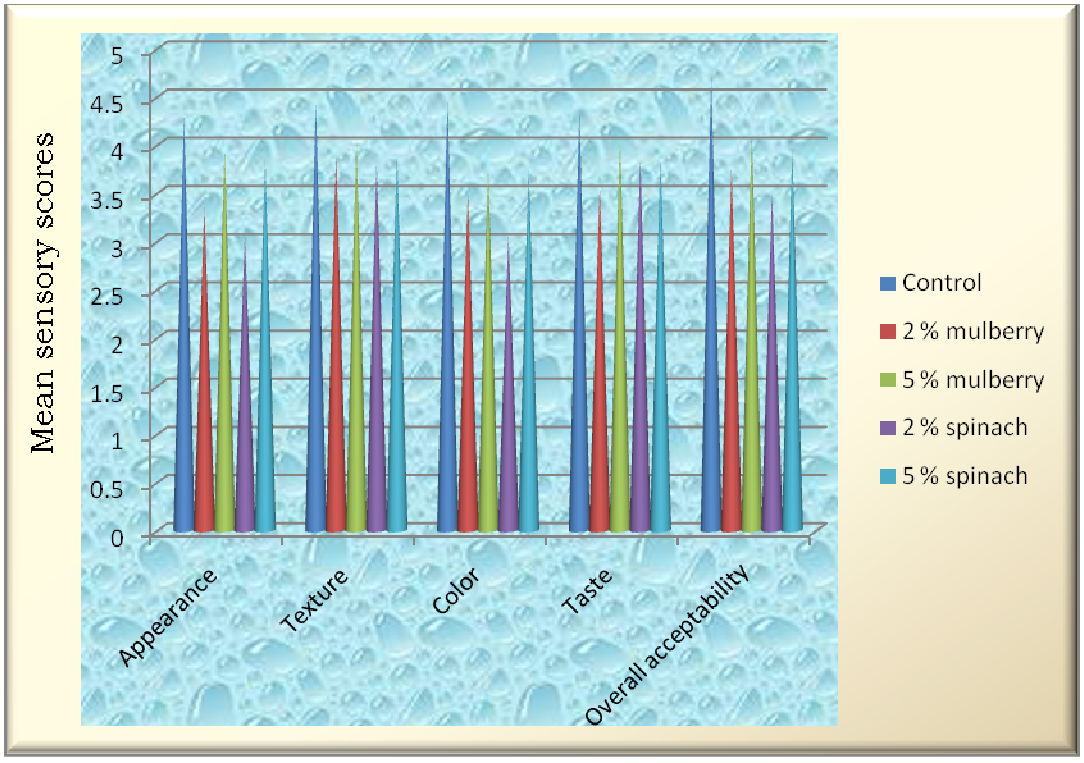


Fig. 2 : Mean sensory scores for papad

Table 10 : Mean sensory scores for product papad

Product	Appearance	Texture	Color	Taste	Overall acceptability
Control	4.30[a]	4.45	4.45[a]	4.35	4.70[a]
Mulberry leaf powder (Incorporation levels)					
2 %	3.35[d]	3.90	3.50[d]	3.55	3.75[c]
5 %	3.95[b]	4.0	3.75[b]	4.05	4.10[b]
Spinach leaf powder (Incorporation levels)					
2 %	3.05[e]	3.80	3.05[e]	3.85	3.50[e]
5 %	3.85[c]	3.90	3.75[b]	3.85	3.95[c]
Significance of test at 5%	*	NS	*	NS	*
S.Em±	0.154	0.158	0.166	0.174	0.148
C D	0.433	-	0.466	-	0.416

*****- significant NS- non significant

(Within the column means with different superscript indicates significant difference)

Table 11 : Mean sensory Score for product chutney powder

Product	Appearance	Texture	Color	Taste	Overall acceptability
Control	4.40	4.40	4.45	4.25	4.45
Mulberry leaf powder (Incorporation levels)					
2 %	3.85	3.90	3.95	4.15	3.90
5 %	4.35	4.15	4.20	4.30	4.40
Spinach leaf powder (Incorporation levels)					
2 %	4.05	4.15	4.0	4.05	4.15
5 %	4.10	4.0	4.0	3.80	4.0
Significance of test at 5%	NS	NS	NS	NS	NS
S.Em±	0.131	0.148	0.160	140.153	0.148
C D	-	-	-	-	-

NS- non significant

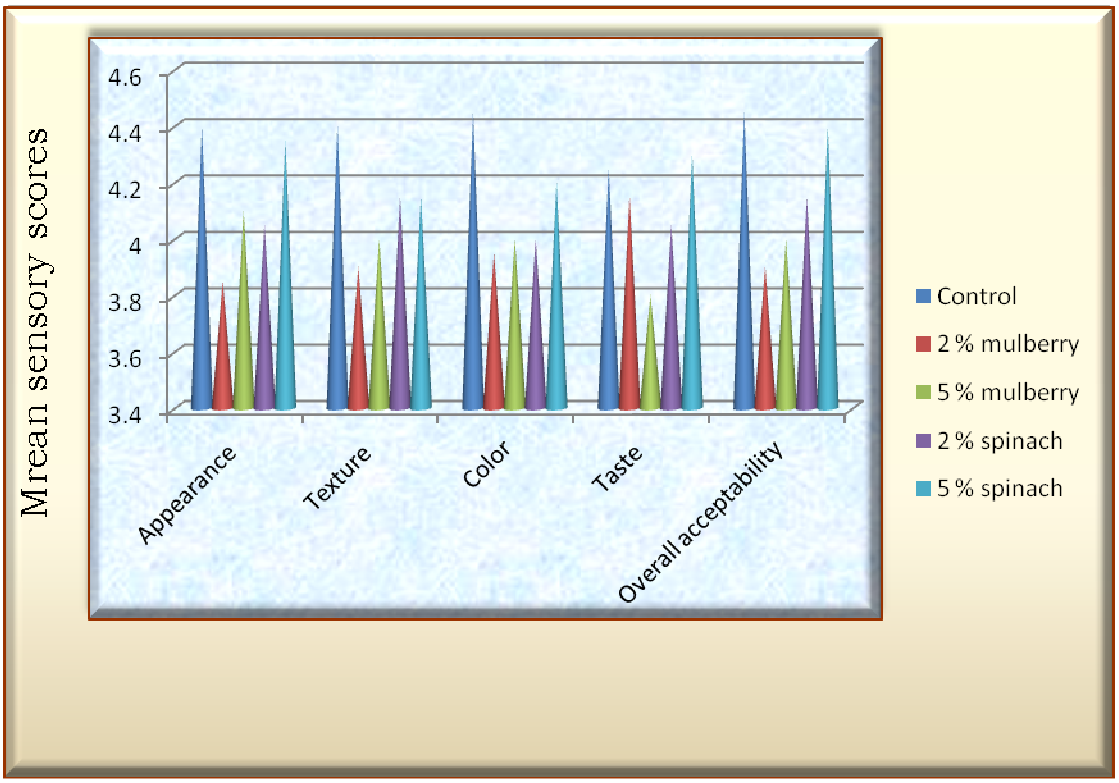


Fig. 3 : Mean sensory scores for chutney powder

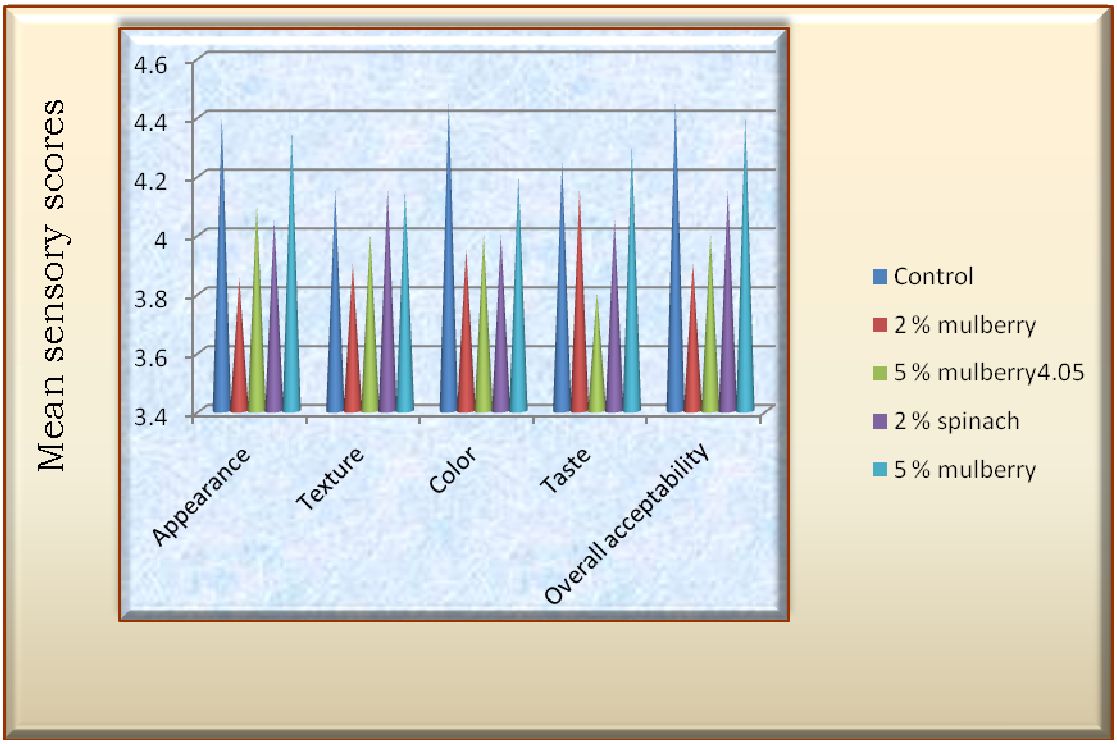


Fig. 4 : Mean sensory scores for khakra

Table 12 : Mean sensory Score for product khakra

Product	Appearance	Texture	Colour	Taste	Overall acceptability
Control	4.40	4.15	4.45	4.25	4.45
Mulberry leaf powder (Incorporation levels)					
2 %	3.85	3.90	3.95	4.15	3.90
5 %	4.35	4.15	4.20	4.30	4.40
Spinach leaf powder (Incorporation levels)					
2 %	4.05	4.15	4.0	4.05	4.15
5 %	4.10	4.0	4.0	3.80	4.0
Significance of test at 5%	NS	NS	NS	NS	NS
S.Em±	0.131	0.161	0.160	0.153	0.148
C D	-	-	-	-	-

NS- non significant

Table 13 : Nutrient composition of products developed from mulberry and spinach leaf powder (computed)

Level of incorporation	Moisture	Energy	Protein	Fat	Carbohydrate	Fiber	Calcium	Phosphorous	Iron	β-carotene
	(g)	Kcal	(g)	(g)	(g)	(g0	(mg)	(mg)	(mg)	(µg)
Chapathi mix										
Control	14.45	392	16.31	3.38	75.54	1.58	51.00	315	6.11	36.40
2% mulberry	14.48	393	16.74	3.42	76.43	1.87	57.00	317	9.11	82.72
5% mulberry	14.54	406	17.39	3.49	77.77	2.32	66.00	319	13.61	152.20
2% spinach	14.48	399	16.35	3.39	75.54	1.79	53.00	316	9.53	85.10
5% spinach	14.88	409	17.84	3.58	77.93	2.11	60.00	317	14.86	158.20
Papad										
Control	6.14	158	2.71	0,25	36.13	0.37	6.00	62	1.60	6.90
2% mulberry	6.17	163	3.14	0.29	37.02	0.66	12.00	64	4.60	53.22
5% mulberry	6.23	172	3.79	0.36	38.36	1.11	21.00	67	9.10	122.70
2% spinach	6.27	164	3.31	0.33	37.08	0.58	10.00	62	5.10	55.62
5% spinach	6.48	175	4.21	0.45	38.52	0.90	16.00	63	10.35	127.80

Khakra										
Control	14.25	331	10.95	0.86	70.26	0.41	23.00	116	2.60	27.25
2% mulberry	14.28	336	11.38	0.90	71.45	0.71	29.00	117	5.60	73.57
5% mulberry	14.34	345	12.03	0.97	72.49	1.16	37.00	120	10.10	143.05
2% spinach	14.38	338	11.55	0.94	71.21	0.63	26.00	117	6.10	75.92
5% spinach	14.59	348	12.45	1.06	72.65	0.95	32.00	118	11.35	149.00
Chutney powder										
Control	16.27	609	47.47	17.92	64.14	6.30	228.00	873	27.57	243.50
2% mulberry	16.30	615	47.90	17.96	65.03	6.59	234.00	874	57.74	290.12
5% mulberry	16.36	623	48.55	18.07	66.37	7.04	243.00	878	35.05	359.60
2% spinach	16.46	616	48.00	18.02	65.00	6.51	232.00	874	31.07	292.52
5% spinach	16.61	626	48.97	18.12	66.50	6.80	238.00	875	36.32	365.60

Moisture

The moisture content chapathi mix developed from mulberry and spinach leaf powder ranged between 14.45 and 14.88 per cent. For the product papad, it was ranged between 6.41 and 6.48 per cent, in product khakra and chuney powder it ranged between 14.25 and 14.59, 16.27 and 16.61 per cent respectively.

Energy

Energy content of chapathi mix ranged from 392 to 409Kcal/100g. For the product papad, it ranged between 158 and 175Kcal/100g. In the product khakra energy ranged between 331 to 348Kcal/100g and product chutney powder it was 609 to 623Kcal/100g of product.

Protein

Protein content of chapathi mix developed from mulberry and spinach leaf powders ranged between 16.31 and 17.84g/100g. In product papad it was ranged between 2.71 and 4.12g/100g. Khakara contained a protein content ranging between 10.95 and 12.45g/100g. In the product chutney powder, the protein content was between 47.47 and 48.97g/100g of product.

Fat

The fat content in chapathi mix ranged from 3.38 to 3.58g/100g. For the product papad, it was 0.25 to 0.45g/100g. In the product khakra fat content ranged from 0.86 to 1.06g/100g and in product chutney powder, it ranged from 17.92 to 18.12g/100g.

Carbohydrate

The chapathi mix developed had carbohydrate content ranging between 75.54 and 77.93g/100g. For the product papad, it ranged from

36.13 to 38.52g/100g. In the product kahkra it ranged from 70.26 to 72.65g/100g and in chutney powder it was from 64.14 to 66.50g/100g of product.

Fibre

The chapathi mix developed contained fibre ranging from 1.58 to 2.11g/100g and in product papad, it was from 0.37 to 0.90g/100g. In the product khakra fibre content ranged from 0.41 to 0.95g/100g. Chutney powder had fibre content ranging from 6.30 to 6.80g/100g.

Calcium

The calcium content in chapathi mix ranged from 51 to 66mg/100g. For the product papad, it ranged from 6 to 21mg/100g. In the product khakra calcium content ranged from 23 to 37mg/100g and in chutney powder, it ranged from 228 to 243mg/100g of product.

Phosphorous

The phosphorous content in chapathi mix ranged from 315 to 319 mg/100g and in product papad, it ranged from 62 to 67mg/100g. The phosphorous content in khakra ranged from 116 to 120mg/100g and in chutney powder it was from 873 to 878mg/100g of product.

Iron

The iron content of chapathi mix ranged from 6.11 to 14.86mg/100g. For the product papad it was 1.60 to 10.35mg/100g. The iron content of khakra ranged from 2.60 to 11.35mg/100 g and in chutney powder it was 27.57 to 36.32mg/100g.

β-carotene

The β-carotene content in chapathi mix ranged from 36.40 to 158µg/100g. Papad contained β-carotene ranged from 6.90 to

127.80µg/100g. The β-carotene content of khakra ranged from 27.25 to 149µg/100g and chutney powder it was ranging between 243.8 and 365.6µg/100g.

4.5 Shelf life study

4.5.1 Sensory evaluation of stored products developed from mulberry and spinach leaf powder

Sensory evaluation of stored products namely chapathi mix, khakra, papad and chutney developed from mulberry and spinach leaf powders was performed. The mean sensory scores are shown in Table 14, 15, 16, 17.

Effect of packaging material

Two types packaging material – low density polyethylene and laminated foil pouches were used. In the product chapathi mix, except for the sensory attribute appearance and texture a significant difference was found for other attributes between packaging materials studied. In the product papad, except for texture and colour significant difference was observed for other sensory characters. For the product chutney powder, there was a significant difference for all the sensory characters. For the product khakra, except colour and overall acceptability a non significant difference was found.

Effect of storage

The products were evaluated for sensory attributes when they were fresh (0 days) and after 60 days of storage. In product chapathi mix, except appearance and overall acceptability a non significant difference was found between two storage periods. For the product papad, there was a significant difference for all the sensory characters. For the product chutney powder, except colour significant difference was found

Plate 7 : Packaging materials used for storage of products (Low density polyethylene covers and laminated pouches)

between the storage periods. In the product khakra, a non significant difference was found for all sensory characters.

Effect of levels of incorporation of leaf powders

Two levels of incorporation two and five per cent were evaluated. In product chapathi mix, except for appearance a non significant difference was found among other sensory characters. For the product papad, except for taste and overall acceptability a significant difference was found. For the product chutney powder a non significant difference was observed for all the sensory characters. In the product khakra, except colour a non significant difference was found between the levels of incorporation of leaf powders for all the attributes.

Interaction between packaging materials, storage periods and levels of incorporation of leaf powders

Three way interactions between packaging materials, storage periods and levels of incorporation of leaf powders was statistically tested to find the source of difference for the different sensory attributes that were studied. For the product chapathi mix, except appearance and texture a non significant difference was found for other sensory characters. For the product papad and khakra a non significant difference was found for all the sensory characters. For the product chutney powder, except overall acceptability a non significant difference was found for other sensory characters.

4.5.2 Microbial count

Microbial count included the bacterial and fungi. The microbial load of chapathi mix was measured and compared with fresh sample. The microbial loads were found to be low. Results are depicted in table 18.

Table 14 : Mean sensory scores for stored products developed from mulberry and spinach leaf powder- chapathi mix

Levels of incorporation	Packaging materials	Storage period	Appearance	Texture	Color	Taste	Overall acceptability
Control	LDPE	0 days 60 days	4.00 3.65	3.85 3.70	3.85 3.55	4.10 3.60	4.00 3.90
	Laminated pouches	0 days 60 days	4.00 3.65	3.85 3.65	3.85 3.65	4.10 3.90	4.00 4.05
2 % mulberry	LDPE	0 days 60 days	3.65 3.60	3.90 3.55	3.50 3.30	3.85 3.65	3.75 3.30
	Laminated pouches	0 days 60 days	3.65 3.80	3.90 3.80	3.50 3.50	3.85 3.80	3.75 3.60
5%mulberry	LDPE	0 days 60 days	4.05 3.75	3.80 3.70	4.00 3.85	4.05 3.65	3.85 3.90
	Laminated pouches	0 days 60 days	4.05 3.90	3.80 3.80	4.00 3.70	4.05 3.85	3.85 3.80
2%spinach	LDPE	0 days 60 days	3.75 3.75	3.80 3.70	3.80 3.65	3.70 3.75	3.95 3.90
	Laminated pouches	0 days 60 days	3.75 3.60	3.80 3.65	3.80 3.60	3.70 3.70	3.95 3.70
5 % spinach	LDPE	0 days 60 days	3.85 3.35	3.95 3.75	3.85 3.85	3.75 3.90	3.80 3.80
	Laminated pouches	0days 60 days	3.85 3.55	3.95 3.45	3.85 3.60	3.75 3.70	3.80 3.75
	Between packaging(A)	F-value SE±m CD	2.99* 0.04 0.13	7.33* 0.05 0.15	NS - -	NS - -	NS - -
	Between storage(B)	F-value SE±m CD	3.51* 0.04 0.13	NS - -	NS - -	NS - -	2.90* 0.04 0.13
	Between levels of incorporation (C)	F-value SE±m CD	5.05* 0.07 0.21	NS - -	NS - -	Ns - -	NS - -
	Between A×B×C	F-value SE±m CD	3.40* 0.11 0.30	2.45* 0.12 0.34	NS - -	NS - -	NS - -

*-significant at 5%level NS-Non significant

Table 15 : Mean sensory scores for stored products developed from mulberry and spinach leaf powder- papad.

Levels of incorporation	Packaging materials	Storage period	Appearance	Texture	Color	Taste	Overall acceptability
Control	LDPE	0 days	4.30	4.45	4.45	4.35	4.70
		60 days	4.25	4.25	4.00	4.00	4.40
	Laminated pouches	0 days	4.30	4.45	4.45	4.35	4.70
		60 days	4.20	4.20	4.25	4.10	4.35
2 % mulberry	LDPE	0 days	3.35	3.90	3.50	3.55	3.75
		60 days	3.80	3.95	3.40	3.60	3.85
	Laminated pouches	0 days	3.35	3.90	3.50	3.55	3.75
		60 days	3.20	3.80	3.45	3.50	3.70
5%mulberry	LDPE	0 days	3.95	4.00	3.75	4.05	4.10
		60 days	3.80	3.95	3.70	4.00	3.90
	Laminated pouches	0 days	3.95	4.00	3.75	4.05	4.10
		60 days	3.70	3.80	3.60	3.95	3.95
2%spinach	LDPE	days	3.05	4.00	3.05	3.85	3.50
		60 days	3.00	3.90	3.15	3.90	3.70
	Laminated pouches	0 days	3.05	4.00	3.05	3.85	3.50
		60 days	3.05	3.80	3.20	3.80	3.60
5 % spinach	LDPE	0 days	3.85	3.80	3.75	3.85	3.95
		60 days	3.50	3.90	3.75	3.75	3.85
	Laminated pouches	0days	3.85	3.80	3.75	3.85	3.95
		60 days	3.50	3.90	3.80	3.70	3.75
	Between packaging(A)	F-value	3.10*	NS	NS	3.10*	5.19*
		SE±m	0.05	-	-	0.05	0.05
		CD	0.15	-	-	0.15	0.15
	Between storage(B)	F-value	8.36*	8.36*	2.78*	12.92*	4.64*
		SE±m	0.05	0.05	0.05	0.05	0.05
		CD	0.15	0.15	0.16	0.15	0.15
	Between levels of incorporation (C)	F-value	3.83*	3.83*	3.36*	NS	NS
		SE±m	0.08	0.08	0.09	-	-
		CD	0.24	0.24	0.26	-	-
	Between A×B×C	F-value	NS	NS	NS	NS	NS
		SE±m	-	-	-	-	-
		CD	-	-	-	-	-

*-significant at 5%level NS-Non significant

Table 16 : Mean sensory scores for stored products developed from mulberry and spinach leaf powder- chutney powder

Levels of incorporation	Packaging materials	Storage period	Appearance	Texture	Color	Taste	Overall acceptability
Control	LDPE	0 days	4.40	4.40	4.45	4.25	4.45
		60 days	4.20	4.20	4.25	4.05	4.25
	Laminated pouches	0 days	4.40	4.40	4.45	4.25	4.45
		60 days	3.85	4.25	3.90	3.95	4.00
2 % mulberry	LDPE	0 days	3.85	3.90	3.95	4.15	3.90
		60 days	3.80	3.60	3.85	3.90	3.95
	Laminated pouches	0 days	3.85	3.90	3.95	4.15	3.90
		60 days	3.75	3.75	3.70	3.85	3.70
5%mulberry	LDPE	0 days	4.35	4.15	4.20	4.30	4.40
		60 days	4.30	3.90	4.05	3.75	4.00
	Laminated pouches	0 days	4.35	4.15	4.20	4.30	4.40
		60 days	3.95	3.85	4.00	3.90	3.95
2%spinach	LDPE	0days	4.05	4.15	4.00	4.05	4.15
		60 days	4.10	4.00	3.90	3.90	3.90
	Laminated pouches	0 days	4.05	4.15	4.00	4.05	4.15
		60 days	3.95	3.75	3.85	3.95	4.00
5 % spinach	LDPE	0 days	4.10	4.00	4.00	3.80	4.00
		60 days	3.95	3.90	3.75	3.75	3.80
	Laminated pouches	0days	4.10	4.00	4.00	3.80	4.00
		60 days	3.80	3.70	3.70	3.60	3.75
	Between packaging(A)	F-value	9.65*	33.70*	12.78*	11.28*	18.49*
		SE±m	0.04	0.04	0.05	0.04	0.04
		CD	0.13	0.12	0.14	0.13	0.12
	Between storage(B)	F-value	4.29*	6.60*	10.85*	NS	14.74*
		SE±m	0.04	0.04	0.05	-	0.04
		CD	0.13	0.12	0,14	-	0.12
	Between levels of incorporation (C)	F-value	NS	NS	NS	NS	NS
		SE±m	-	-	-	-	-
		CD	-	-	-	-	-
	Between A×B×C	F-value	NS	NS	NS	NS	2.81*
		SE±m	-	-	-	-	0.10
		CD	-	-	-	-	0.28

*-significant at 5%level NS-Non significant

Table 17 : Mean sensory scores for stored products developed from mulberry and spinach leaf powder- khakra

Levels of incorporation	Packaging materials	Storage period	Appearance	Texture	Color	Taste	Overall acceptability
Control	LDPE	0 days 60 days	4.40 3.80	4.15 3.75	4.45 4.10	4.25 3.90	4.45 3.95
	Laminated pouches	0 days 60 days	4.40 3.75	4.15 3.75	4.45 4.25	4.25 3.85	4.45 3.90
2 % mulberry	LDPE	0 days 60 days	3.85 3.75	3.90 3.70	3.95 3.75	4.15 3.80	3.90 3.70
	Laminated pouches	0 days 60 days	3.85 3.55	3.90 3.75	3.95 3.75	4.15 3.85	3.90 3.75
5%mulberry	LDPE	0 days 60 days	4.35 3.90	4.15 3.90	4.20 3.90	4.30 3.95	4.45 4.05
	Laminated pouches	0 days 60 days	4.35 3.90	4.15 3.95	4.20 3.85	4.30 3.85	4..45 4.05
2%spinach	LDPE	0days 60 days	4.05 3.95	4.00 4.10	4.00 4.10	4.05 3.90	4.15 4.05
	Laminated pouches	0 days 60 days	4.05 3.90	4.00 3.70	4.00 3.60	4.05 3.70	4.15 3.95
5 % spinach	LDPE	0 days 60 days	4.10 3.75	4.00 3.60	4.00 3.95	3.80 3.70	4.00 3.85
	Laminated pouches	0days 60 days	4.10 3.75	4.00 3.75	4.00 3.90	3.80 3.65	4.00 3.95
	Between packaging(A)	F-value SE±m CD	NS - -	NS - -	NS - -	2.84* 0.04 0.13	11.03* 0.05 0.14
	Between storage(B)	F-value SE±m CD	NS - -	NS - -	NS - -	NS - -	NS - -
	Between levels of incorporation (C)	F-value SE±m CD	NS - -	NS - -	3.34* 0.08 0.23	NS - -	NS - -
	Between A×B×C	F-value SE±m CD	NS - -	NS - -		NS - -	NS - -

*-significant at 5%level NS-Non significant

Table 18 : Microbial load of chapathi mix

Sample	Packaging material	Duration (days)	TBC ($X10^5$ CFU)	Fungi ($X10^3$ CFU)
Chapathi mix (control)	LDPE	O days 60 days	2 5	3 5
	Laminated pouch	O days 60 days	3 5	3 4
Chapathi mix 2% mulberry	LDPE	0 days 60 days	0 2	0 7
	Laminated pouch	0 days 60 days	0 2	0 5
Chapathi mix 5% mulberry	LDPE	0 days 60 days	2 5	2 7
	Laminated pouch	0 days 60 days	0 4	0 11
Chapathi mix 2 % spinach	LDPE	0 days 60 days	1 0	0 4
	Laminated pouch	0 days 60 days	0 1	0 2
Chapathi mix 5% spinach	LDPE	0 days 60 days	0 2	0 6
	Laminated pouch	0 days 60 days	0 1	1 3

CFU- colony forming unit. TBC- total bacterial count.

(Safety range for bacteria 30-300 CFU $\times 10^5$)

Safety range for fungi 25-2500 CFU $\times 10^3$)

4.6 Glycemic index of product developed from mulberry leaf powder

The blood glucose response for white bread and mulberry incorporated chapathi mix is given in Tables 19 and 20 respectively. The glycemic index and calculated area under curve for blood glucose responses after ingestion of white bread and mulberry leaf powder incorporated chapathi mix on separate days is given in Table 21. A non significant difference was found for the mean blood glucose (mg/dl) levels at 0, 30, 60, 90 and 120 minutes of time interval beteween control and mulberry incorporated chapthi mix of subjects. Although the GI of mulberry was low (93.66) compared to white braed (100). The differences in total area under blood glucose curve between white bread and mulberry incorporated chapthi was found to be non significant. At the levels of incorporation of leaf powder and the product (i.e. food matrix) studied mulberry leaf exhibited a non significant hypoglycaemic effect.

Table 19 : Blood glucose levels (mg/dl) after consumption of white bread (50g carbohydrate equiv.) for individual subjects

Subjects	Fasting	30 min	60 min	90 min	120 min
1	109	153	143	100	105
2	98	152	126	110	98
3	101	118	124	108	104
4	90	103	123	96	86
5	93	124	132	97	106
6	100	116	122	102	98
7	98	134	149	124	109
8	104	131	140	98	116
9	90	129	112	102	87
10	102	139	117	99	95
Mean	98.5	129.9	128.8	103.8	100.4

Table 20 : Blood glucose levels (mg/dl) after consumption of mulberry incorporated chapathi (50g carbohydrate equiv.) for individual subjects

Subjects	Fasting	30 min	60 min	90 min	120 min
1	89	110	121	103	105
2	78	114	116	109	110
3	92	115	101	97	96
4	84	102	108	104	101
5	90	118	132	95	102
6	86	109	110	103	104
7	107	122	127	108	102
8	105	106	129	106	114
9	93	112	122	110	84
10	86	94	113	103	104
Mean	91	110.2	117.9	103.6	102.2

Table 21 : Glycemic index (GI) and Mean area under curve for test products

	Blood glucose (mg/dl)					Area under curve (AUC)					
	0 min	**30 min**	**60 min**	**90 min**	**120 min**	**AUC 1**	**AUC 2**	**AUC 3**	**AUC 4**	**TOTAL AUC**	**GI**
White bread											
Mean	98.50	129.90	128.80	103.80	100.40	471.00	925.50	535.11	145.90	2077.51	
SE	1.93	4.96	3.77	2.68	3.12	62.94	83.15	81.04	65.16	237.65	
SD	6.11	15.60	11.91	8.49	9.86	198.90	262.70	256.10	205.92	750.99	100
Mulberry chapathi mix											
Mean	91.00	110.20	117.90	103.60	102.2	288.00	691.5	595.50	370.92	1945.92	93.66
SE±m	2.85	2.56	3.17	1.54	2.55	46.60	76.03	75.15	89.04	252.02	
SD	9.00	8.09	10.02	4.87	8.06	147.27	240.25	237.49	281.36	796.38	
|t-value|	NS	NS	NS	NS	NS					NS	

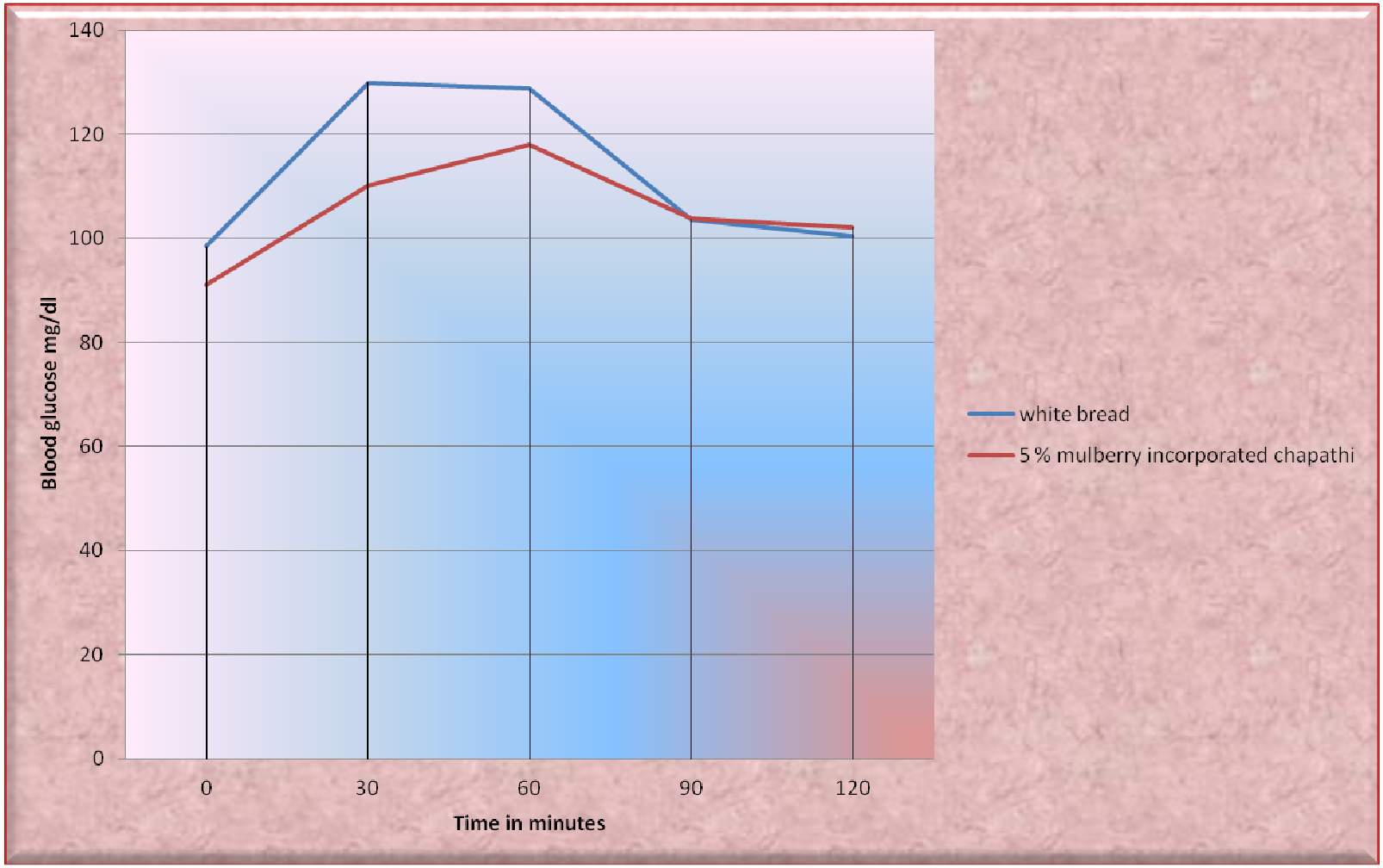


Fig. 5 : Mean area under blood glucose curve for white bread and mulberry incoprorated chapthi

Plate 8: Testing of blood glucose in subjects

DISCUSSION

V. DISCUSSION

Mulberry tree is widely distributed in India. Mulberry leaf is rich in protein, calcium, iron, phosphorous, carotene and essential amino acids. The leaves and leaf powders are being utilized in food industry to supplement human diet which is well documented (Chawdary *et al 2009).* However, limited use of mulberry leaves in daily diet of local population may be due to lack of awareness and popularization of technologies for its utilization. Recognising this need, the present study was undertaken to analyze the nutrient composition, digestibility, product development, organoleptic evaluation of value added products and glycemic index of the same. The outcome of the results is discussed hereunder.

5.1 Nutrient composition and digestibility of selected varieties of mulberry leaves

5.2 Chlorophyll and colour of the leaves of selected varieties of mulberry.

5.3 Product development and sensory evaluation

5.4 Nutrient composition of products developed

5.5 Shelf life study

5.6 Glycemic index of product developed from mulberry leaf powder.

5.1 Nutrient composition and digestibility of selected varieties of mulberry leaves

5.1.1 Macro nutrient composition

Macro nutrients such as moisture, energy, protein, fat, crude fibre, carbohydrate and energy were estimated for all the varieties. The results are discussed below.

5.1.1.1 Moisture content:

Moisture content is usually high in all green leafy vegetables *(Gopalan et al, 2004).* Fairly high moisture content, ranging between

68.90 and 75.05 of per cent in fresh leaves, was observed. After drying obviously the moisture content values were found to be lower and they ranged between 6.97 to 9.24g/100g for dried sample. It was observed that the moisture content of different varieties of mulberry leaves were slightly lower when compared to value reported for other greens. Higher moisture content in fresh leaves of amaranth, gogu and mint leaves were reported by Laxmi and Vimala (2000) as 88, 87 and 87 per cent respectively. A physical examination by the investigator confirms this aspect. The mulberry leaves appeared to be dry visually and texturally. The variety S34 had highest moisture content (75.05 per cent), and the least was in S41 (68.05 per cent). Spinach leaves had high moisture content of 89.94 per cent when compared to all varieties of mulberry. Compared to other green leafy vegetables mulberry leaves are less juicy and this contributes to higher concentration of nutrients.

In the present study, dried sample of mulberry, moisture was found to be highest in RFS175 (9.24%) and least was found in S30 (5.27%), lower than the values reported for green leaf powder Laxmi and Vimala (2000). They reported moisture content of dried leaves for amaranth, curry leaves, gogu and mint leaves as 9, 3.9, 11.3 and 9.6 % respectively. Spinach leaf powder had moisture content of 6.97g/100g. The lower moisture content of leaf powder in the present study indicates that these samples may have improved keeping quality.

5.1.1.2 Protein content (g/100g)

Sanchez (2008) reported that the protein content of mulberry leaves varied from 15 to 22.1 per cent on dry weight basis. Kitahara *et al.* (2000) found protein content of mulberry leaves to be 25.8 per cent. In the present study protein content of varieties ranged from 20.50 to 30.87g/100g of dried samples. Bongale *et al.* (1991) reported that the soluble protein content of four varieties of mulberry leaves varied from

27.1 to 32.7 per cent. In the present study, spinach had protein content of 30.06g/100g in dried powder. RFS-175 had highest content of protein of 30.87g/100g and least was found in S-34 (20.50g) in dry powder. Due to their high protein content, mulberry leaf powders can be recommended for improving the diets of poor.

5.1.1.3 Total lipid content (g/100g):

Kowsalya *et al.* (1999) reported the fat content of cauliflower leaves as 1.53%. Gautham *et al.* (2007) found 2.2 % of crude fat in aloevera powder. Shanthala *et al. (2005)* found that the fat content of curry leaf powder was 5.4 per cent. In the present study, the total lipid content of dry powdered sample ranged from 2.03g to 4.10g/100g. The values were found within the range of values reported for other green leaf powders. The spinach powder had fat content of 4.10g. Due to lower fat content leaf powder can be advised for diabetic, hyperlipidemic subjects as well as used in low calorie diets.

5.1.1.4 Crude fibre (g/100g):

In the present study the fibre content ranged from 15.08g to 9.41g/100g of dried sample of mulberry leaves and 10.72g/100g in spinach leaf powder. Similar values for crude fibre have been reported by Sanchez (2008). He reported that the fibre content of mulberry leaves ranged from 5.9 to 15.3 per cent. Kantwa *et al.* (2006) found 9.85% of crude fibre in mulberry leaves. While Laxmi *et al.* (2000) found fibre content of 6.8, 13.97, 11.30 and 12.60 per cent for amaranth, curry leaves, gogu and mint leaves dried leaves respectively Values in the present study were similar to what was reported earlier. Thus, mulberry leaf powder is found to be a significant source of fibre and can be added to functional food products. It can find application in the therapeutic diets. Fibre is that part of food that is not digested by the gut. Diets

containing high dietary fibre can reduce blood glucose and serum cholesterol *(*Raghuram *et al,* 1997*).*

5.1.1.5 Carbohydrate (g/100g)

In the present study, the carbohydrate content of varieties ranged from 31.81 to 46.95g/100g of dried leaf powder. The spinach leaf powder had a slightly higher carbohydrate content of 47.96g/100g. In some varieties carbohydrate content was considerably lower than spinach powder. Gautham *et al. (*2007*)* reported a value 48 per cent of carbohydrate in aloevera powder. Low carbohydrate ingredients are important in some disease conditions such as diabetes and obesity low carbohydrates are recommended *(*Srilakshmi, 2007*).* Thus mulberry leaf powder can be a good health adjuvant.

5.1.1.6 Energy(Kcal/100g)

In the present study, the energy value ranged from 294.88 to 277.06Kcal/100g. Spinach powder had a significantly higher energy value of 343.02Kcal/100g. In mulberry leaf powder almost similar values were found in dried samples of mulberry leaves. Gautam *et al.* (2007), found 231 Kcal for aloevera powder.The energy values were observed to be lower in mulberry leaf powder compared to spinach but slightly higher than that of aloevera leaf powder. Energy intakes need to be well regulated in several conditions *(*Srilakshmi, 2007*).* Lower energy values combined with higher protein values makes mulberry leaf powder a useful ingredient in managing several nutritionally challenging situations.

5.1.2 Total ash and micronutrient composition

Total ash and micronutrients such as calcium, phosphorous, iron, ascorbic acid, beta carotene were estimated and results are discussed below.

5.1.2.1 Total ash (g/100g)

Ash content which reflects the mineral content ranged from 15.18 to 11.19g/100g of dried sample of mulberry. Ash content was highest in M5 (15.18g) least was found in S-34 (11.19g). In present study spinach powder had ash content of 12.4g/100g. Sanchez (2008) has also analyzed the ash content in mulberry leaves. The reported values ranged between 4.5 to 17.3 per cent. Kantwa e*t al.* (2006) reported a value of 12 per cent of ash in mulberry leaves. Shantala *et al.* (2005) reported 9.7 per cent of total ash in curry leaf powder. From the results obtained it can be stated that mulberry is a rich source of minerals.

5.1.2.2 Calcium (mg/100g)

Srivatsava *et al.* (2009) reported 786mg/100g of calcium content in fresh mulberry leaves. Laxmi *et al.* (2000) found calcium content of 335 mg, 209mg, 34.51mg and 30.42mg for amaranth, curry leaves, gogu and mint in fresh respectively. In the present study the higher calcium content were observed and ranged from 561.66 to 168.33mg/100g of dried leaf samples. The highest calcium content was found in RFS-175 (561.66mg) least was found in DD-1 (168mg). Spinach had calcium content of 200mg/100g. Thus mulberry leaf powder can be considered as a rich source of calcium. Calcium is the most abundant mineral in the body and essential for all body processes.

5.1.2.3 Phosphorous

Nambiar *et al.* (2007), reported that the phosphorous content ranged from 2 to 11mg in uncommon green leafy vegetables of western India. In the present study the phosphorous content ranged from 6.40 to 12.96mg/100g in dried sample. There were large variations. The highest phosphorous content was found in S-34 (12.96mg) and least was found in RFS-175 (6.40mg). Thus the mulberry leaves are poor source of

phosphorus. However, this deficiency can be easily overcome by inclusion of other phosphorous rich foods to get a desirable Ca:P ratio.

5.1.2.4 Iron

Laxmi and Vimala (2000) have reported that the iron content of amaranth, curry leaves, gogu and mint leaves was 71.27, 29.69, 38.35 and 55.88mg/100g on dry weight basis. Nambiar *et al.* (2007) found that the iron content of un common green leaves varied from 0.2 to 2.2mg/g dry weight with an exception in an *Amaranthus Spp*. and *Leucas aspera* in which the iron content was high as 1.4mg/g and 2.2mg/g dry weight. In the present study, the values ranged between 150mg/100g and 72.33mg/100g. The spinach leaf powder had iron content of 175mg. Thus mulberry leaves can be considered as a very rich source of iron and can be utilised for supplementation of Indian diets which are poor source. It's use by the poor could help reduce the micro nutrient deficiency among vulnerable populations.

5.1.2.5 Ascorbic acid:

Srivatsava *et al.* (2009) reported ascorbic acid value of 230mg/100g of fresh mulberry leaves. In the present study ascorbic acid content ranged from 133.33 to 216.66mg/100g of dry sample. Spinach powder had ascorbic acid content of 176.66mg/100g on dry weight basis. Laxmi and Vimala (2000) found 67.50, 293.0, 60.72 and 42.28mg/100g of fresh amaranth, curry leaves, gogu and mint leaves respectively. Thus mulberry is considered as good source for ascorbic acid and it also contributes to the antioxidant activity of leaves.

5.1.2.6 Beta carotene:

In the present study the β-carotene content ranged from 200 to 761.30µg/100g in fresh and 426.66 to 13515µg/100g of dry sample. Spinach had β-carotene content of 512.33µg/100g of fresh sample and

2436.33µg/100g of dried sample. Srivatsava *et al.* (2009) found 13125µg of carotene in mulberry leaves. Gautham *et al.* (2007) reported values of 335.8mg/100g of aloevera powder. Rahman *et al.* (1990) studied the beta carotene content for commonly consumed green leafy vegetables in Bangladesh. The Palang sak (spinach) had 6400µg/100g of fresh leaves. Dried powder of mulberry leaves has a high retention of beta carotene and can be considered as an excellent source.

5.1.2.7 Total sugars:

Total sugar content of dried samples of mulberry leaves ranged from 5.0 to 15.66 per cent. The highest total sugar content was found in S-34 (15.66%) and least was found in DD-1and RFS-175 (5.0%). The spinach sample had a considerably lower total sugar content of 3.66 per cent on dry weight basis. Chaluvachari *et al.* (1991) reported total sugar content of four varieties of mulberry leaves namely Mysore local, Kanva 2, S36 and S41 which ranged between 11.1 to 15.2 per cent at 45 days of maturity. Some mulberry leaf samples had a higher sugar content compared to spinach leaves. There is a large variation in the sugar content. This has implications in the digestibility of the samples. It is presumed that higher the sugar higher might be the digestibility.

5.1.2.8 *In vitro* digestibility of protein (IVPD) of selected varieties of mulberry leaves.

Sanchez (2008), found 78.4 to 95 per cent of digestibility of fresh leaves of mulberry in goats. In present study the in vitro protein digestibility of dry leaf powder ranged from 58.86 to 99.86 per cent. The spinach sample showed a lower IVPD (58.86%). Thus the *in- vitro* protein digestibility of all varieties of mulberry leaves was superior to that of spinach leaf powder. Higher IVPD combined with higher protein content indicates that the mulberry leaf powder is not only a rich but also a digestible source of protein.

5.1.2.9 In-vitro α-amylolysis of selected varieties of mulberry leaves

The *In vitro* α-amylolysis was observed in intervals of three minutes. The *in vitro* α-amylolysis of mulberry varieties at first 3 minutes interval ranged from 9 to 26mg of maltose released per mg of enzyme. In next three minutes interval it ranged from 6 to 39 and at subsequent three minutes interval it ranged from 2 to 32mg of maltose released per mg of enzyme. This shows the slow digestibility of carbohydrates and has implications in diabetes and other chronic disorders.

5.1.2.10 Oxalate and tannin content

In the present study the average oxalate content ranged from 27.15 to 39.95mg/100g of dried sample powder. Spinach had a low oxalate content of 35.61mg/100g of dried sample. The mulberry leaf powders had similar oxalate content as spinach. Kawsalya *et al.* (1999) reported values of 26 and 1.6mg/100g oxalate and tannins of fresh cauliflower leaves respectively. The oxalate content in fresh green leaves range between 30 to 658mg/100g as reported by Gopalan *et al.* (2004).

The average tannin content ranged from 4.99 to 15.18mg/100g of dried sample. The spinach powder had the tannin content of 4.99mg/100g. When compared to other green leafy vegetables mulberry leaf powder was found to contain lower values of tannin. Gupta et al, (2004) reported tannin content of 13 underutilized green leafy vegetables. His values ranged between 61 to 205mg/100g of dried samples. Tannins inhibit the absorption of minerals from the gut. Thus lower tannin content in mulberry leaf powder helps in better mineral absorption. It must be noted that mulberry leaf powder is a rich source of minerals.

5.2 Chlorophyll and colour of the leaves of selected varieties of mulberry leaves

5.2.1 Chlorophyll 'a'

Bongale *et al.* (1991) reported that the chlorophyll 'a' content ranged from 2.56 to 3.58mg/g of dry sample in varieties namely M5, DD, S34 and TG respectively. Chaluvachari *et al.* (1993) found it to be ranging from 1.66 to 2.01mg/g on dry weight basis of eight varieties namely Mysore local, S1, kanva 2, S36, DD, RFS-175, KNG and English black. In the present study chlorophyll 'a' content ranged from 0.30 to 6.70mg/g in all the fresh samples. The highest chlorophyll 'a' content was found in RFS-175 (6.70mg/g) and least was found in M5 (0.30mg/g). The control spinach sample had chlorophyll "a" content of 0.30mg/100g of fresh sample.

5.2.2 Chlorophyll 'b'

Chaluvachari *et al.* (1993) reported chlorophyll 'b' content ranged between 0.76 to 1.14mg/g of eight varieties. In the present study, chlorophyll 'b' content ranged from 0.35 to 3.53mg/g in all the fresh samples. The control sample spinach had chlorophyll b content of 0.35 mg/g of fresh sample.

5.2.3 Total chlorophyll

Total chlorophyll content ranged from 0.65 to 10.23mg/g. The highest total chlorophyll content was found in RFS-175 (10.23mg/g) and least was found in M5 (0.65mg/g). The control sample spinach had 0.65mg/g of fresh sample. The high chlorophyll content of leaves may acts as antioxidants and support health of human beings.

5.2.4 Colour

In the present study, spinach leaves, DD1 and S30 have pale green colour and rest of varieties had dark green colour. The colour of DD1 and S30 were found to be similar to spinach according to the Munsell colour chart *(*Munsell*,* 1952*).*

5.3 Product development and sensory evaluation of products

Mean sensory scores for sensory characteristics of different products developed from mulberry leaves are discussed below. The M5 variety was selected for the product development. The spinach leaves were used as reference throughout the study. Both these were incorporated at 2 and 5 per cent levels. A traditional recipe without leaf powder served as control.

5.3.1 Chapathi mix

Chawdary *et al.* (2009) reviewed the use of mulberry leaves in preparations like chapathi and paratha. They stated that acceptable products could be prepared using mulberry leaf powder. Kawsalya *et al.* (1999) studied the acceptability of cauliflower leaf powder incorporated in chapthi at 10 and 20 per cent levels. They found that the 10 per cent incorporation in chapathi scored highest level of preference. In the present study the control (without incorporation of leaf powders) had highest acceptability. However, the mean scores for all characteristics and all levels of incorporation did not differ significantly from the control. Thus, even at five per cent level of incorporation control and mixes were judged to be highly acceptable.

5.3.2 Papad

Joshi *et al.* (2008) developed papad incorporating mulberry leaf powder and products were found to be acceptable. In the present study

control showed highest sensory scores for appearance, colour and overall eating quality. Colour of incorporated leaf powders seems to have influenced the appearance and overall acceptability. However, it must be noted that papad is consumed for its unique texture i.e. crispiness and its good taste. It is noticed that the sensory scores for texture and taste of papad with leaf powder incorporation remain on par with the control. Thus, it is safe to say that leaf powders can be easily incorporated into this product.

5.3.3 Chutney powder

Shanthala *et al.* (2005) studied the acceptability of curry leaf powder in spice mixture and found to be highly acceptable. In the present study the control product showed highest scores for all sensory characters. Chutney powder is consumed for its unique taste along with other dishes for its taste. In this study it is found that the mean scores for all characteristics and at all levels of incorporation did not differ significantly from control. Thus the leaf powders can be easily incorporated into chutney powder.

5.3.4 Khakra

Joshi *et al.* (2008*)* developed khakra incorporating mulberry leaves and products were found to be acceptable. Khakra is a North Indian recipe which is unique in its texture and taste. In the present study, the mean sensory scores for all characteristics and at all the levels studied did not differ from control. Hence leaf powders can be incorporated to improve nutritional and sensory characteristics of the Khakra.

5.4 Nutrient composition of products developed

The products like chapathi mix, papad, chutney powder and khakra were developed for which the values for nutrients were calculated. It is important to know the nutrient composition of the

products for the conformity of their richness in terms of nutrition and to incorporate them in our daily diet and value addition in particular product preparation.

The highest protein, fat and carbohydrate content were highest in five per cent spinach leaf powder incorporated chapathi mix (17.84g/100g, 3.58g/100g and 77.93g/100g of chapathi mix) respectively. The fibre content was highest in five per cent mulberry leaf powder incorporated chapthi mix (2.32g/100g). This may be due to high fibre content of mulberry leaf powder. Calcium content was found to be highest both in mulberry and spinach leaf powder incorporated chapathi mix at five per cent level (66mg/100g). The phosphorous content was highest in five per cent incorporated mulberry chapathi mix (319mg/100g). Iron and β- carotene content was highest in five per cent spinach incorporated chapathi mix (14.86mg/100g and 158.20µg/100g respectively) and lower values were found in control product. The highest energy values were found in five per cent spinach leaf powder incorporated chapathi mix (409Kcal/100g) this may be due to higher values of protein, lipids and carbohydrate content of spinach leaf powder. Thus the incorporation of the leaf powders resulted in a substantial improvement in the nutritive value of chapathi.

The highest energy, protein, lipid and carbohydrate content was found in five per cent spinach leaf powder incorporated papad (175Kcal/100g, 4.21g/100g, 0.45g/100g and 38.52g/100g) respectively. The lower values were found in control product. The fibre, calcium and phosphorous were found to be high in five per cent mulberry incorporated papad (1.11g/100g, 21mg/100g and 67mg/100 g respectively among the variations) and least was found in control product. Iron and ß-carotene content was found to be high in five per cent mulberry leaf powder incorporated papad (10.35mg/100g and

127.80µg/100g respectively among variations). Incorporation of the leaf powders resulted in a substantial improvement in the nutritive value of papad.

Energy, protein, lipid and carbohydrate content of khakra was high in five per cent incorporation of spinach leaf powder (348Kcal/100g, 12.45g/100g, 1.06g/100g and 72.65g/100g respectively among variations) and lower values were found in control product. The five per cent mulberry leaf powder incorporated khakra had higher values of fibre, calcium and phosphorous (1.16g/100g, 37mg/100g and 120mg/100g respectively) compared to control product. The five per cent spinach leaf powder incorporated khakra had higher values of iron and β-carotene content (11.35mg/100g and 149.00µg/100g respectively) compared to other variations of product. Incorporation of the leaf powders resulted in a substantial improvement in the nutritive value of khakra.

Energy, protein, lipid and carbohydrate content was high in five per cent spinach incorporated chutney powder (626kcal/100g, 48.97g/100g, 18.12g/100g and 66.50g/100g respectively) compared to other variations. The fibre, calcium and phosphorous content were high in five per cent mulberry incorporated chutney powder (7.04g/100g, 243 mg/100g and 878mg/100g respectively). Iron and β-carotene content was found to be high in five per cent spinach incorporated chutney powder. Incorporation of the leaf powders resulted in a substantial improvement in the nutritive value of chutney powder.

5.5 Shelf life study

Shelf life of four products namely papad, khakara, chutney powder and chapathi mix was evaluated. The products were stored up to two months. Two types of packaging materials were used. These were stored

in low density polyethylene and laminated pouches. The samples were stored at room temperature. The ambient temperature during the storage period ranged between 25°C and 30°C and the RH 40 per cent and 60 per cent.

5.5.1 Mean sensory scores for shelf life of chapathi mix.

A perusal of table 17 reveals that in chapathi mix for sensory attribute appearance there was significant difference between storage periods, packaging materials, variations tried. Significant differences also existed when the interaction of all three parameters were considered i.e. between storage, packaging and variations of products. For the attribute texture there was significant difference between packaging materials and also for the three way interaction of packaging materials, storage periods and variations of product. For color and taste there was no significant difference for packaging material, storage period, variations and between three way interaction of storage, packaging material and variations among products. For overall acceptability there was significant difference between storage periods. At 2 and 5 per cent level of incorporation of spinach leaf powder at 60 days of storage in laminated pouches showed a slightly higher score this may be due to some mellowing with age product may have developed some aroma and taste.

5.5.2 Mean sensory scores for shelf life of papad

In product papad for sensory attribute appearance there was significant difference between packaging materials, storage periods and variations among products. For the attribute texture and color there was significant difference between storage periods and levels of incorporation. For taste and overall acceptability there was a significant difference between packaging materials and storage periods. There was no significant difference when three way interaction between packaging

materials, storage periods and variations among products was observed. Hence the product papad can be stored up to two months.

5.5.3 Mean sensory scores for shelf life of chutney powder.

In chutney powder, between packaging materials and storage periods for all sensory attributes there was a significant difference. However, between the levels of variation there was non significant difference for the attributes studied. For the attribute overall acceptability there was a significant difference when statistical test was applied to study a three way interaction of packaging materials, storage periods and levels of incorporation, indicating that the product can be stored upto 2 months.

5.5.4 Mean sensory scores for shelf life of khakra

In the product khakra for the sensory attributes appearance and texture there was no significant difference between packaging materials, storage periods, levels of incorporation and between all the three. For the attribute color there was significant difference between levels of incorporation. For sensory attributes colour and overall acceptability there was significant difference between packaging materials and storage periods, indicating that the product can be stored upto 2 months.

5.6 Microbial count

Microbial count for chapathi mix (Bacteria and Fungi) was estimated before and after storage. In all the variations of chapathi mix a lower microbial count was observed. This may be due to presence of turmeric which possesses antibiotic property and mulberry is also having anti microbial property. Rattanasiri (2004) reported that *Curcuma aromatica L.* extract and *Morus alba L.* extract were had antimicrobial efficacy against *Propionibacterium acnes*, *staphylococcus epidermis* and *staphylococcus aureus* in pharmaceutical products. Rizk and Ebid

(1989), reported lower values for total aerobic bacteria in seven different flours were 3.0 x 10^6 to 9.1 x 10^7. In the present study the colony forming units (c.f.u) at 0 days for product chapathi mix (control) for low density polyethelene pouches (LDPE) were 2 x 10^5 to 3 x 10^5 for bacteria and 3 x 10^3 fungi respectively and for laminated pouches 5 x 10^5 for bacteria and 4 x 10^5 to 5 x 10^5 fungi respctively. In the other variations of chapathi mix for LDPE pouches bacterial count was 1 x 10^5 and for fungi 1 x 10^3 and 2 x 10^3 and for laminated pouches bacterial count was 1 x 10^5 to 5 x 10^5 and for fungi 2 x 10^3 to 11 x 10^3 repsectively. Microbial count after storage period of two months in laminated pouch and low density polyethelene pouches showed less cfu. The results indicated that chapathi mix contains less number of colony forming unit of bacteria and fungi and can be stored for a longer period in both packaging materials and can be used whenever required. The general ranges in common acceptance for countable number of colonies on a plate are 30 to 300 and 25 to 250 for total bacteria and fungi respectively *(*Breed and Dotterrer*,* 1916*).* Hence the total bacterial count and fungal count were in safe limits. The product can be stored safely for a period of 2 months.

5.7 Glycemic index of product developed from mulberry powder.

Product developed from five per cent incorporation of mulberry leaf powder namely chapathi made out of chapathi mix was tested for its glycemic response on 10 healthy volunteers.

Glycemic index (GI) can be used in conjunction with information about food composition to guide food choices. The glycemic response for different foods are markedly is different in diabetic and normal subjects *(Anon 1998).* Several factors influence glycemic responses. There are choice of standard food, methodology of testing, subject's characteristics, amount of carbohydrate, nature of the monosaccharide components

(glucose, fructose and galactose), nature of starch, cooking or food processing and other food components like fat and protein, dietary protein, anti nutrients and organic acids. Meals containing low GI foods reduce both postprandial blood glucose and insulin responses. Clinical trials in normal, diabetic and hyperlipidemic subjects show that low GI diet reduces mean blood glucose concentrations reduce insulin secretion and reduce serum triglycerides in individuals with hyperglyceridemia *(*Cherbut *et al.* 1995*,* Cummings *et al.* 1993 and Salvador *et al.* 1992*).* In addition the digestibility of the carbohydrate in low GI foods is generally less than that of high GI foods. Thus, low GI foods increase the amount of carbohydrate entering the colon and increase colonic fermentation and short chain fatty acid production. This has implications for local events within the colon *(*Anon *1998).* In the present study the GI was measured in capillary whole blood. Normally GI is measured in capillary blood and capillary blood is preferred over venous plasma because it is easier to obtain and the rise in blood glucose is greater than in venous plasma. Results for capillary blood glucose are less variable than those for venous plasma glucose. The differences between foods are larger and easier to detect statistically using capillary blood glucose *(*Gibson *et al,* 1995*)*

In the present study GI response to the mulberry leaf incorporated chapathi was slightly lower (93.66) compared to white bread. Mulberry leaves are high in mucilaginous substance *(*Gururaj*, 2009).* Similar mucilaginous substance is present in aloe vera and this has been reported to be source of the bioactive polysaccharide gluco mannan. When Eberndu *et al* conducted the quantitative analysis of these polysaccharides, they found that gluco mannan underwent rapid degradation to sugars mannan and glucose. There are no reports about the stability of the polysaccharides in mulberry leaves especially in response to processing. The fresh leaves contain around 12 to 13 per cent sugars (Chaluvachari and Bongale, 1993). In the present study the

mulberry leaves variety M5 was used for testing the glycemic index. Total sugars in the M5 variety was 14.66 per cent. Higher glucose levels coupled with the decomposition of mucilaginous substance and other hypotheticated hypoglycemic substances may have resulted in higher than expected glycemic index.

The present study indicated that although large amounts of variations were observed between the varieties for most nutrients, mulberry leaf powders were found to be the good source of protein (25.66g/100g), minerals (13g/100g), calcium (353.9mg/100g), iron (99.66mg/100g), ascorbic acid (182.4mg/100g), β-carotene (5324.2µg/100g), total sugars (11.24 per cent). Conversely the varieties of mulberry leaf powder that were studied had lower amounts of fat (2.92g/100g), carbohydrate (41.01g/100g), energy (288.25Kcal/100g) and phosphorous (7.12mg/100g). Thus these samples can be considered to be a nutrient dense but low energy, low carbohydrate and low fat food item. The digestibility with respect to protein was excellent. The mulberry powders lend themselves well in the different products that were tried and number of acceptable value added products could be made. The incorporation of mulberry leaf powder helped in improving the nutritive value of value added products better than that was observed for spinach leaf powder. The shelf life of mulberry incorporated product was high. The GI values of mulberry leaf incorporated product were higher than expected. There are no cases of toxicity of mulberry leaf powder and hence it is safe. Thus from the results of this study, it may be concluded that the mulberry leaf powder is the excellent source of nutrients and can be used as a low cost nutritional adjuvant in daily diets.

SUMMARY

VI. SUMMARY

Mulberry is being grown throughout India and leaves are good sources of protein, fibre and micro nutrients. Green leafy vegetables which constitute important part of the diet contribute the substantial quantities of key nutrients to the diet. Mulberry leaves are available abundantly but they are not used in the local diets. Thus, there is a need to evaluate the locally available cultivars of mulberry leaves for food purpose. The main aim of present study was the nutritional evaluation of selected varieties of mulberry leaves, development of value added products and evaluation of developed products glycemic index of a selected product was assessed. The summary of the study entitled "Nutritional evaluation and glycemic index of selected varieties of mulberry leaves is presented here.

The salient findings of the study are summarised as follows:

- The nutrient composition of fresh and dried sample was estimated. The mean moisture content for varieties was 71.30g/100g in fresh leaves and 7.66g/100g in dried leaf powder. The mean protein content of leaves for all the varieties was 25.66g/100g (20.50 to 30.87 g/100g). The mean fat content for all varieties was 2.92g/100g (2.03 to 3.43g/100g) in samples of mulberry leaf powder. The mean crude fibre was 11.91g/100g (9.41 to 14.94g/100g) of mulberry leaf powder. Mean carbohydrate content of mulberry varieties was 41.01g/100g (31.81 to 46.95g/100g) of mulberry leaf powder. The mean energy value was found to be 288.25Kcal/100g (277 to 294.88Kcal/100g) of powder.
- The mean ash content of mulberry varieties was found to be 13.00 g/100g (11.19 to 15.18g/100g) in leaf powder. Mean calcium content was 353.91mg/100g (168.33 to 561.66mg/100g). The mean phosphorous content was 8.13mg/100g (4.6 to 12.90mg/100g). The

mean ascorbic acid content was 182.47mg/100g (133.33 to 216.66 mg/100g) in dried leaves. The mean β-carotene content of different mulberry varieties was 5324µg/100g of dried sample. The mean total sugar content was 11.24g/100g (5.0 to 15.66g/100g) of dried sample. The mean oxalate and tannin contents were 32.04 mg/100g (27.15 to 39.95mg/100g) and 8.87mg/100g (4.99 to 15.18mg/100g) of powder respectively.

- The mean *in vitro* protein digestibility (IVPD) of mulberry leaf powder samples was 88.70 percent (66.13 to 99.68 percent). IVPD of spinach leaf powder was significantly lower i.e. 58.86 percent. *in vitro* carbohydrate digestibility (IVCD) of different varieties was quite different.
- Leaves of mulberry varieties DD-1 and S-30 had got pale green colour similar to spinach. Other varieties had dark green colour. The mean chlorophyll 'a' and chlorophyll 'b' content for the varieties were 3.53 mg/g and 2.21mg/g respectively. The mean total chlorophyll content of all varieties ranged was 5.61mg/g (2.94 to 10.23mg/g).
- Four products were developed namely, chapathi mix, papad, khakra and chutney powder. The leaf powder of mulberry and spinach (control) was incorporated at two and five percent level. Sensory scores of all the products indicated that products were well accepted. Chutney powder was highest accepted product among all the products.
- The nutrient composition of products was computed and there was difference between products. Chutney powder had more nutrients compared to other products at five percent level of incorporation.
- Microbial load for chapathi mix was low. Upon storage up to two months sensory characters did not show any deterioration.

- Glycemic index of chapathi mix with mulberry leaf powder incorporation at 5 percent was estimated in comparison with white bread. The incorporation of leaf powder at five percent level did not lower glycemic index appreciably (GI= 93.66)

Future line of work

- Bio availability studies for nutrients as a function of specific food matrix could be carried out.
- Identification, characterization and extraction of the bioactive principles in different medium can be studied.
- Glycemic index of foods with higher levels of incorporation needs to be assessed.
- Long term effect of feeding mulberry leaves can be undertaken on suitable animal models.

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APPENDICES

ANNEXURE –I

RECIPES

Poushtik chapathi mix

Ingredients	Quantity
Wheat flour	80g
Gram flour(basan)	10g
Mulberry leaf powder	5g
Turmeric	1g

Method

- Mix all the ingredients and store in air tight containers in a cool and dry place

ANNEXURE–II

Papad

Ingredients	Quantity
Rice flour (powder)	37.5g
Sago powder	7.5g
Mulberry leaf powder	5g
Salt	1g
Red chilli powder	2g
Cumin powder	1g
Pepper powder	0.2g
Papad khara	3g

Method

- Mix all ingredients.
- Boil ½ cup of water and stir mixture and keep stirring till a soft dough is formed.
- Divide the dough into small bolls and press in a papad press to get a thin circular disc of about 1mm thick
- Dry the papads and deep fry before use.

ANNEXURE–III

Mulberry khakra

Ingredients	Quantity
Maida flour	95g
Mulberry leaf powder	5g
Salt	2g
Green chilli powder	1g
Hing	0.1g
Methi powder	1g
Jeera powder	0.25g
Garlic powder	0.25g
Oil	5g

Method

- Mix all the ingredients except oil.
- Make a soft dough using water.
- Divide the dough into small bolls and press in a papad press to get a thin circular disc of about 1mm thickness.
- Roast the khakra on a griddle, till color changes
- Remove and spread on a plate to dry for 15 minutes, smear oil on the khakra.
- Once again roast till it is dry.

ANNEXURE–IV

Mulberry chutney powder

Ingredients	Quantity
Roasted Bengal gram	70g
Soyabean dal	30g
Mulberry leaf powder	5g
Chilli powder (red)	10g
Garlic powder	3g
Curry leaves	1g
Tamarind	4g
Jaggery	5g
Salt	4g

Method : Grind all the ingredients into a fine powder

ANNEXURE–V

Score Sheet for Sensory Evaluation of Value added products from mulberry leaf powder and spinach leaf powder

Name:
Date:

Name of product:

Directions

- Rinse the mouth between samples
- Place the numerical score in the space provided
- Comments should justify the numerical score, comments must be brief
- Evaluation of the products must be on individual basis

Score system:

Excellent-5, Very good-4, Good-3, Fair-2, Poor-1

Quality characters	XYA	XYB	XYC	XYD	XYE
Appearance					
Texture					
Aroma					
Taste					
Overall acceptability					

Comments/Suggestions
Signature

ANNEXURE–VI

Estimation of protein

Principle

Organic nitrogen digested with sulphuric acid in the presence of catalyst is converted to ammonium sulphate. Ammonium liberated by making the solution alkaline is distilled into a known volume of standard acid, which is then back titrated. Protein per cent was calculated by multiplying the nitrogen presented the factor 6.25.

Reagents

1. 2% boric acid solution: 20g of boric acid was dissolved in some distilled water. The solution was then transferred to a 1000 ml volumetric flask and made up to the mark.
2. 40% NaOH (W/V).
3. 0.1 N HCl: 8.33 ml of fuming HCl was dissolved in 1000ml distilled water.
4. Mixed indicator: Was made by mixing methyl red (0.2%) and Bromocresol green (0.2%) in a 1:2 ratio (v/v) respectively.
5. Digestion mixture: Anhydrous sodium sulphate and copper sulphate
6. Concentrated sulphuric acid (H2SO4).

Procedure

Digestion: 0.5 g of the sample was weighed into the digestion tubes of the Gerhardt digester in duplicate and two heaped spatulas each of sodium and copper sulphate were added to each tube. 25 ml of concentrated sulphuric was also added and samples digested until the contents of the tubes were sea green in color. Each of the digested materials was dissolved in distilled water and transferred into a 100 ml volumetric flask and then brought to the mark.

Distillation: 10ml of each samples was transferred into the distillation tube of the automatic Gerhardt unit and 20ml of 2 per cent boric acid to which was added 3-4 drops of the mixed indicator was placed in the collecting conical flask to trap the liberated ammonia. The unit was furnished with 40 per cent NaOH and distilled water to facilitate operation. Distillation was done for 5 minutes and the ammonia collected and trapped by the boric acid. In between the distillation of samples, the unit was rinsed with distilled water for 2.5 minutes. The boric acid turned from reddish pink to green as it collected the ammonia.

Titration: The green colored boric acid was titrated against the 0.1NHCl until its color turned to pink. A blank was run simultaneously. The titer values obtained were incorporated in the equation below to obtain the per cent nitrogen present in the sample which, in turn, was multiplied by the factor 6.25 to obtain the per cent protein.

$$\text{Per cent nitrogen (\%N)} = (VA - VB) \times 0.0014 \times \frac{V1}{V2} \times \frac{100}{W}$$

Where:

VA = Titre value of sample

VB = Titre value of blank

V1 = Volume to which digested sample was made up (100 ml)

V2 = Volume to aliquot used in distillation

W = Weight of samples taken for digestion

ANNEXURE-VII

Estimation of total lipids (Bligh and dyer method)

In this method, a mixture of chloroform and methanol (2:1V/V) was used. The tissue (about 1 g wet weight) was first ground in a pestle and mortar with about 10 ml of distilled water. The pulp was transferred to a conical flask (250ml capacity) and 30ml of chloroform – methanol mixture was added and mixed well.

For complete extraction, it was kept overnight at room temperature, and in dark. At the end of the period, 20 ml chloroform and 20 ml of water was added. The resulting solution was subjected to centrifugation, and three 3 layers were seen. A clear lower layer of chloroform containing all the lipids, a colored aqueous layer of methanol with all water soluble material and a thick pasty interface were seen.

The methanol layer was discarded and the lower layer was carefully collected by filtering through glass wool. The organic layer was taken in a pre- weighed beaker or vial and carefully evaporated. The sample was kept in warm water (around 50°C).

When the solution was free of organic solvents, the weight was taken again. The difference in weight gives the weight of the lipids. The results were expressed in terms of milligrams of total lipids per gram of the sample.

ANNEXURE-VIII

Estimation of crude fibre

Principle

During the acid and subsequent alkali treatment, oxidative hydrolytic degradation of the native cellulose and considerable degradation of lignin occurs. The residue obtained after final filtration is weighed, incinerated, cooled and weighed again. The loss in weight is the crude fiber content.

Reagents

0.255 ± 0.005 N standard H2SO4

0.313 ± 0.005 N standard NaOH

Method

Weighed amount of (2.5-5g) of moisture and fat free sample was transferred to a fibre bag which was pre heated and weighed. These bags were inserted into tubes and placed in a beaker provided in the instrument. The sample was boiled with 300ml 0.255 ± 0.005N H_2SO_4 for 30 minutes. Then the residue was washed with boiling water until acid free. Then residue was boiled with 300ml of 0.313 ± 0.005 N NaOH for 30 minutes. Again the residue was washed with boiling water followed by alcohol wash. The residue was transferred to pre weighed crucibles(W1) and it was dried to 2 hours at 130± 2 °C, cooled in a desiccator then weighed (W2). The dried dessicators containing samples were then ignited for 30 minutes at 600 ± 15°C. Finally the sample was cooled and weighed again.

$$\text{Per cent crude fiber} = \frac{\text{Loss in weight on ignition}}{\text{Weight of sample used (g)}}$$

$$= \frac{(W2\text{-}W1\ g) - (W3\text{-}W1\ g)}{\text{Weight of sample used (g)}} \times 100$$

ANNEXURE-IX

Estimation of Ascorbic acid by 2,6-dichlorophenol indophenols visual titration method.

The fruits and vegetables are important sources of ascorbic acid. The most satisfactory chemical methods of estimation are based on the reduction of 2,6-dichlorophenol indophenols by ascorbic acid with 2,4-dinitro phenyl hydrazine.

The dye is blue in alkaline solution and red in acid solution is reduced by ascorbic acid to a colorless form. The reaction is quantitative and practically specific for ascorbic acid in solution in the range 1 to 3.5.

Reagents:

1. **3% metaphosphoric acid –** prepared by dissolving sticks or pellets of metaphosphoric acid in glass distilled water.

2. **Ascorbic acid standard –** accurately 100mg of L-ascorbic acid was weighed and made up to 100ml with 3% metaphosphoric acid.10 ml of this solution was diluted to 100ml with 3% metaphosphoric (1ml=0.1 mg of vitamin C)

3. **Dye solution –** 50 mg of sodium salt of 2.6-dichloro indophenols was diluted in approximately 50 ml of hot glass distilled water containing 42mg of sodium bicarbonate. The mixture was cooled and diluted to 200ml with glass distilled water.

Procedure:

Standardization of dye: The mixture of 5 ml of standard ascorbic acid solution and 5 ml of metaphosphoric acid was taken in a conical flask. The micro burette was filled with dye solution and titrated to a pink color

which persisted for 15 seconds. Dye factor was determined i.e. mg of ascorbic acid /ml of the dye

$$\text{Dye factor} = \frac{0.5}{\text{Titre value}}$$

Preparation of the sample:

2-10g of sample was blended with 3% metaphosphoric acid and made up to 100ml with metaphosphoric acid. The solution was filtered and centrifuged.

An aliquot of 2-10ml of sample extract was titrated against standard dye to an pink end which was persisted for 15 seconds. Titration was carried out rapidly and accurately.

Calculation:

$$\text{Mg of ascorbic acid/100g} = \frac{\text{titre X dye factor X volume made upX100}}{\text{Aliquot of extract Taken for estimation} \times \text{weight of the sample taken for estimation}}$$

ANNEXURE-X

Estimation of total sugars

The total soluble sugar content in the sample was estimated by the phenol – sulphuric acid method.

Reagents:

1. 5% phenol
2. 96% sulphuric acid
3. Standard glucose solution: 100mg of glucose was dissolved in 100ml of distilled water. 1 ml of this solution was diluted to 10ml with water to obtain working standard solution containing 100µg/ml.

Extraction of sample: The sample of 0.5 g was accurately weighed and dissolved in 10 ml of distilled water. The mixture was shaken well.

Estimation: 0.1 ml extract was diluted to 1.0 ml with water. 1.0 ml of 5 % phenol and 5 ml of 96% sulphuric acid was added and mixed well for 10 minutes. The mixture was incubated in a water bath at 25-30°C for 10 minutes. After cooling to room temperature, the absorbance was read at 496nm against the reagent blank.

A standard graph was constructed with glucose as a standard in the range of 20-100µg. The results were expressed as percen

ANNEXURE-XI

Preparation of mineral solution

To the ash that was obtained was added 5 ml of a 1:1 solution of distilled water and fuming HCl. This mixture was then heated over a water bath to dryness before another 5 ml of the solution was added. It was heated further over the water bath until it started fuming and at this point, the crucible was retrieved and its contents filtered into a 100 ml volumetric flask using Whatman No.4 filter paper. After thorough rinsing of the crucible and the filter paper, the volume was made up to the mark with distilled water. Aliquots of this mineral solution were taken for the estimation of all the minerals in this study.

ANNEXURE-XII

Estimation of calcium

Procedure

To an aliquot (2ml) of mineral solution was diluted to about 150ml with distilled water. Few drops of methyl red indicator added and neutralized with ammonia till the pink colour changes to yellow. The solution was heated to boiling followed by the addition of 10ml ammonium oxalate. Again the solution was allowed to boil for a few minutes and glacial acetic acid added till the colour was distinctinctly pink and kept aside in a warm place till the precipitate settles down. The precipitate was then filtered through Whatman No.40 filter paper and washed with warm water till free of oxalate. The precipitate along with filter paper was transferred to a beaker and 10ml, 2N sulphuric acid poured over it. Finally, the solution was hot titrated against N/100 potassium permanganate solution.

1 ml of N/100 $KMnO_4$ = 0.2004 mg of Ca.

$$\text{Per cent calcium (mg)} = \frac{\text{N/100 } KMnO_4 \text{ X } 0.2004 \text{ X Vol. of } H_2SO_4}{\text{Weight of sample used for ashing x aliquot taken}} \text{ X } 100$$

ANNEXURE-XIII

Estimation of phosphorus

Determation of phosphorus was carried out by measuring colorimetrically the blue color formed when the ash solution is treated with ammonium molybdate and thus phosphomolybdate formed is reduced.

To an aliquot, 0.1 ml of mineral solution was added with 1 ml of ammonium molybdate, 1ml of hydroquinone and 1ml of sodium thiosulphate solutions in this order, mixing well after each addition. The volume was then made up to 15 ml with water and the solution mixed thoroughly. After 30 minutes the optical density of this solution is measured in a photoelectric calorimeter against a reagent blank prepared in the same way as the test, except that the test solution is omitted, at 660 nm. The phosphorus content of the sample was obtained from a standard curve prepared with standard phosphate solution (range 0.01 to 0.1 mg phosphorus).

ANNEXURE-XIV

Estimation of iron

Iron is determined calorimetrically making use of the fact that form iron gives a blood red colour with potassium thiocyanate.

Reagents

1. 30 % sulphuric acid: 30 ml concentrated H_2SO_4 diluted to 100m
2. Saturated potassium per sulphate solution: 7 gm of potassium per sulphate is dissolved in glass distilled water and the solution made up to 100 ml.
3. Potassium thiocyanate 40 % solution: 4 g KCNS dissolved in 90 ml glass distilled water, 4 ml acetone added and the volume made up to 100ml.
4. Standard iron solution : 0.722 g ferrous ammonium sulphate is dissolved in 100 ml glass distilled water and after addition of 5 ml of 1.1 HCl, the solution is made up to 1 litre and mixed thoroughly (1 ml 0.1 mg Fe) the standard solution is prepared once in six months. Working standard solution (0.01 mg Fe/ml) is prepared by diluting the above solution tenfold.

Procedure

To an aliquot (6.5 ml or less) of the mineral solution enough water is added (if necessary) to make up to volume of 6.5 ml followed by 1.0 of 30 per cent H_2SO_4, 1.0 ml of potassium per sulphate and 1.5 ml 40 per cent KCNS solution. The red colour that develops is measured within 20 minutes at 540 nm.

Note

For iron estimation all the reagents used should be free from iron. Use of glass distilled water preferred. If use of reagents containing traces of iron cannot be avoided it should be seen that the final solution of standard and test contain identical quantities of those reagents contain identical quantities of those reagents containing iron as impurity.

Calculation – e.g.

Standard OD = 0.322 contains 0.03 mg

Sample OD = 0.209 contains o.o2 mg

$$\frac{0.209 \text{X} 0.03}{0.322} = 0.019472$$

5 ml ⟶ 0.019472

100 ml ⟶ 0.389

Sample wt. / 4.322 5 g ⟶ 0.0389

100 g ⟶ $\frac{100 \text{ X } 0.0389}{4.311} = 9.03$

:. G contains 9.03 mg

ANNEXURE-XV

Estimation of ß-carotene (Ranganna, 2002)

The carotene present in sample is first extracted with acetone, and then the carotene is brought to petroleum ether phase. The concentration of ß-carotene in the solution was determined by measuring the optical density of the solution using a colorimeter @ 452 nm.

Preparation of standard curve

25mg of ß-carotene was accurately weighed and dissolved in 2.5 ml of chloroform and volume was made up to 250ml using petroleum ether in a volumetric flask (1ml=100µg). 10 ml of this solution was diluted to 100ml with petroleum ether in 100 ml volumetric flask to serve as a working standard (1ml=10µg). After words 5, 10, 15,20,25 and 30 ml of working ß-carotene standards were transferred to a series of 100ml volumetric flask containing 3ml acetone and diluted up to the mark. Thus the ß-carotene contents will be 0.5, 1.0, 1.5, 2.0, 2.5 and 3.0 µg/ml in these volumetric flasks. Absorbance of these solutions will be measured using a colorimeter @ 452 nm. Absorbance was plotted against concentration and the standard ß-carotene curve is obtained.

Extraction of ß-carotene from sample

10 g of sample is taken to which 25ml of acetone is added. It si transferred to a beaker and allowed to stand for 15 minutes and filtered. The residue is decanted and again subjected to acetone extraction. This procedure is repeated for 3-4 times till the residue becomes colorless. The filtrate from each extraction is pooled and is transferred to separating funnel.15 ml of petroleum ether and 100 ml of 5 % sodium sulphate solution is added to the extract and funnel is thoroughly shaken before allowing it to stand. The carotene get transferred to

petroleum ether layer from acetone solution and is repeated until the acetone layer becomes colorless. Petroleum ether extracts are pooled and volume is made up to 50 ml and is determined by measuring absorbance @ 452nm.

$$\text{ß-carotene } \mu g/g = \frac{\text{concentration of carotene read from standard curve} \times \text{volume made up}}{\text{weight of the sample}} \times 100$$

ANNEXURE -XVI

Estimation of oxalic acid

Principle: The oxalic acid was extracted from food stuffs and precipitated as calcium oxalate which is then titrated against standard potassium permanganate.

Reagents:

1. 2N HCl
2. Phosphoric – tungstate reagent: To 24 gm of sodium tungstate, dissolved in water was added to 40 ml of phosphoric acid and solution was diluted to liter with water.
3. Calcium chloride buffer: To 25g calcium chloride was dissolved in 500ml of 50%glacial acetic acid, was added to 330g of sodium acetate dissolved in 500ml of water. The solutions were mixed well
4. 0.1 % of methyl red.
5. N/100 $KMnO_4$

Extraction of sample:

To 5 g of well grounded sample was added 100ml of 2N HCl and the mixture was shaken well for about 2 hr in a mechanical shaker. It is then centrifuged and filtered (in case of fresh sample 10 g of the sample was well grounded in a mortar along with 25ml of 2N HCl.)

The mixture was transferred to the same beaker and weighed. It was then boiled for about 15min and cooled. The mixture was adjusted to the previous weight with distilled water, made up to 100m with 2N HCl shaken well and filtered.

To 25 ml of filtrate was added 5 ml of phosphoric tungstate reagent. The stirred well and kept overnight. The next day it was

centrifuged and filtered. To 20 ml of the filtrate was added 2 0r 3 drops of methyl red, neutralized with ammonia. 5 ml of calcium chloride buffer was added and stirred well.

The mixture was allowed to stand overnight at the end of which it was filtered through whatman No 40 and 44filter paper and washed from chloride using distilled water. The precipitate along with the filter paper is transferred to the same beaker and some distilled water was added followed by 5ml of 2N H_2SO_4. The mixture was heated to 80°C over a burner and titrated against N/100 potassium permanganate.

1ml of N/100 $KMnO_4$ = 0.45 mg of Oxalic acid.

$$\text{mg of Oxalic acid /100 g sample} = \frac{\text{Titre value X N of } KMnO_4 \text{ X } 0.45 \text{ X dilution factor}}{0.01 \text{ X weight of the sample}} \text{ X } 100$$

ANNEXURE-XVII

Estimation of Tannins

Tannins were estimated calorimetrically based on the measurement of blue colour formed by the reduction of phosphotungstomolybdic acid in alkali solution.

Preparation of standard curve

Zero to 10 ml of aliquots of the standard tannic acid solution was taken into 100 ml volumetric flask containing 75 ml of water added, three ml of folin-denis reagent and 10 ml sodium carbonate solution into each of the volumetric flasks and volume was made up to 100ml with distilled water. Solution was mixed well and colour measured after 30 minutes at 760 nm against experimental black adjusted as "O" absorbency.

Preparation of sample

Five gram of sample was extracted with 85 ml of methanol containing one per cent of HCl for 30 minutes with occasional shaking. The content was filtered using Whatman No. 1 filter paper. The filtrate was used for tannin determination.

Procedure for tannin estimation.

One ml of extract was taken in 100 ml volumetric flask to which five ml of folin denis reagent and 10 ml of sodium carbonate solution was added. The contents were mixed and diluted to 100ml using distilled water and allowed to stand for 30 minutes and absorbance was measured at 760 nm. The tannin content of the samples was calculated as tannic acid equivalents from the standard graph.

$$\text{Tannins a tannic acid \%} = \frac{\text{mg of tannic acid x Dilution x100}}{\text{ml of sample taken for colour development x Wt.of sample x100}}$$

ANNEXURE-XVIII

Estimation of *in vitro* protein digestibility (AOAC, 1980)

In vitro protein digestibility was determined by calculating the difference between the amount of nitrogen in the sample before and after hydrolysis with pepsin. Two hundred milligram of whole seed or dehulled lour was incubated with 50 ml of 0.2 per cent pepsin in 0.075 N HCl for 24h at 37^0 C. Digestion was performed in duplicate. The digests were filtered through Whatman No.2 filter paper and the residues were washed with warm water on the filter. Nitrogen in the residues was estimated by the microkjeldhal method. IVPD was obtained by calculating the difference between the amount of total nitrogen in the sample before and after in vitro digestion with pepsin. Kjeldhal nitrogen was multiplied by the factor 6.25 to obtained crude protein.

ANNEXURE–XIX

In vitro- digestability of carbohydrates by α-amylolysis.

Principle : Amylase splits starch into maltodextrins and glucose. Maltose and glucose form major products of α-amylolysis.

Reagents:

1. **Pancreatic amylase.**
2. **0.02 M glycerophosphate –HCl buffer pH 6.9**
3. **Alkaline salicylate reagent:** 20 g of 3,5-dinitro salicylic acid was suspended in approximately 400 ml of water. A solution of 32g of NaOH in 300ml of water was added dropwise while stirring and gently heated on water bath (if necessary) until a clear solution was obtained. 600g of potassium sodium tartarate was added in small amount and water was added to final volume of 2 liter. The reagent was stored in dark at room temperature.
4. **Starch 1% suspension in glycerophosphate buffer**
5. **Maltose : 2mg/ml**

Procedure : One percent suspension (with reference to starch content) of test material in 0.02 M glycerophosphate-HCl buffer pH 6.9 was prepared after thorough homogenization. This material was used as substrate for α-amylolysis. The reaction mixture containing 0.01 mg of α-amylase suspended in 1ml of 0.02 M glycerophosphate buffer pH 6.9 and 1.0 ml of the substrate were taken in individual test tubes and incubated in a metabolic shaker at 37°C. The reactions in different test tubes are stopped at different intervals (i.e. 0, 3, 6 and 9 minutes) by adding 1.0 ml of alkaline salicylate reagent. The mixture was heated in a boiling water bath for 5 minutes, cooled and diluted to a total volume of 10 ml with water. The absorbance at 540nm is measured in Coleman

spectrophotometer. The *in vitro* α-amylolysis thus determined, gives an idea of the rate of starch digestion and can also be applied to cooked materials. One unit of α-amylase activity is expressed as the reducing sugar value equivalent to1 mg maltose released per mg of the enzyme in 3 minutes. The calibration curve with standard maltose solution with a range of 0 to 2 mg is established using the alkaline salicylate reagent.

ANNEXURE-XX

Subjects were studied on separate days in the morning after a 10 to 12 hour overnight fast. A 50 g carbohydrate portion of standard food (white bread) was given after that the fasting blood glucose level (G_0) was noted. Subsequent blood glucose levels were recorded at 30 minutes of interval up to 120 minutes (G_1, G_2, G_3, G_4, G_5). After one day wash out period, on the third day 50 g carbohydrate portion of the test food (mulberry leaf powder incorporated chapathi) was given and blood glucose levels were recorded as for standard food. The calculation of area under curve for standard and test food was calculated as per method given below.

CALCULATION OF GLYCEMIC INDEX

Assuming that at times t_0, t_1 .....t_n (here equalizing 0, 30 ...120 minutes respectively). The blood glucose concentrations are G_0, G_1.....G_n respectively.

$$AUC=\sum_{x=1}^{N} A_x$$

Where A x = the AUC for the X^{th} time interval and the X^{th} time interval is the interval between t_{x-1} and t_x.

For the first time interval (i.e.x=1):

If G1>G0 A1= (G1-G0) X $(t_1-t_0)/2$ otherwise A1=0

For the other time intervals,

1). If Gx >Go and $G_{(x-1)}$ > Go, Ax= $\{[(Gx-Go)/2]\}+(G_{x-1}-Go)/2\}X(tx-t_{(x-1)})$

2). if Gx >Go and $G_{(x-1)}$ < Go, Ax= $\{[(Gx-Go)^2/(Gx-G_{(x-1)})]X\ (t_x - t_{(x-1)})/2$

3). If Gx<Go and $G_{(x-1)}$ >Go, Ax= $[(G_{(x-1)}-Go)^2/G_{(x-1)}-G_x)]x\ (t_x - t_{(x-1)})/2$

4). If Gx<Go and $G_{(x-1)}$ < Go, Ax=0.

ANNEXURE-XXI

Profile of subjects taken for the Glycemic index study

Sl. No.	Height in cm	Weight in Kg	BMI	Age (yr)	Gender	Occupation
1	153	54	23.07	25	F	Student
2	148.5	44	20.09	22	F	Student
3	155	48	20.00	22	F	Student
4	147	52	24.06	22	F	Student
5	152	47	20.34	22	F	Student
6	147.8	44	20.14	22	F	Student
7	153	48	20.15	22	F	Student
8	167	56	20.14	24	F	Student
9	157	50	20.28	22	F	Student
10	158.4	51	20.33	22	F	Student

F-female

BMI=body mass index

ANNEXURE-XXII

TEST MEAL COMPOSITION

PRODUCT	Moisture g	Protein g	Carbohydrate g	Fat g	Fiber g	Energy Kcal
white bread(100g)	39.0	7.8	51.9	0.7	0.2	245
Spinach Chpathi mix (65g)	9.67	11.30	50.65	2.32	2.32	265.8
Mulberry Chapathi mix (65 g)	9.45	11.59	50.55	2.26	1.50	263.9