A Study on Production of Functional Paneer via Incorporation of Water Chestnut

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(2014-V-257-M)

Division of Livestock Products Technology

Faculty of Veterinary Sciences & Animal Husbandry

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Thesis

Submitted to

The Faculty of Veterinary Sciences & Animal Husbandry
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in partial fulfilment of requirement for the award of the degree of

Master of Veterinary Sciences
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2016

Dedicated
To
My Loving Parents and
My Major Advisor

Sher-e-Kashmir
University of Agricultural Sciences & Technology of Kashmir
Faculty of Veterinary Sciences and Animal Husbandry
Division of Livestock Products Technology

Certificate – I

This is to certify that the thesis entitled, **"A Study on Production of Functional Paneer via Incorporation of Water Chestnut"** submitted in partial fulfilment of the requirements for the award of the degree of **Master of Veterinary Sciences (Livestock Products Technology)**, to the **Faculty of Veterinary Sciences & Animal Husbandry, Sher-e-Kashmir University of Agricultural Sciences & Technology of Kashmir** is a record of bonafide research work carried out by **Dr. Tarique Ahmad Padder (Regd. No. 2014-V-257-M)** under my supervision and guidance. No part of the thesis has been submitted for any other degree or diploma.

It is further certified that information received during the course of investigation has duly been acknowledged.

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ABSTRACT

The present investigation was carried out to evaluate the effect of incorporation of water chestnut (*Trapa natans*) on the quality of paneer. In the first phase of our study, the effect of water chestnut on physico-chemical, sensory and functional characteristics of paneer made from cow milk was determined. Water chestnut was incorporated at the rate of 0% (control), 0.25%, 0.5%, 0.75% and 1% in the raw milk before heating for coagulation. The study revealed that 0.25% and 0.5% water chestnut fortified paneer samples were having more desirable sensory characteristics as compared to other treatments. The moisture content showed a significant increase from control to 1% water chestnut incorporated paneer. The protein, ash, fat in whey and yield % showed a significant increase when compared with the control while the pH revealed a significant reduction with increase in water chestnut level; however fat values of paneer were comparable for all the treatments. DPPH-RSA and ABTS-RSA percentage showed significant increase with increase in water chestnut per cent in

paneer, thereby imparting functional value to the product. Based on the sensory, physico-chemical and functional characteristics, 0.25% and 0.5% water chestnut levels were selected optimum for incorporation into paneer. In our next phase, the control and selected treatment (0.25% and 0.5%) samples were stored aerobically at refrigeration temperature (4±1^{o}C) for 28 days and the samples were analyzed at regular interval of 7 days. The sensory evaluation showed marked reduction in control from 14th day, while significant reduction in selected treatments was observed on 21st day. The microbiological assay revealed SPC count to be well below the prescribed limits of FSSR (2011) for all the treatments on 28th day. The Coliform count of fortified paneer samples was lower than the set limits of FSSR (2011) on day 21 whereas the count was beyond the limit for control on day 21st. The yeast and mould count was below the prescribed limits for all treatments on 28th day. The TBARS value was lower for *Trapa* incorporated paneer as compared to control sample on 7th day of storage, thereafter the values were comparable; however, the values were lower than the prescribed value of FSSR. The tyrosine values were comparable for all treatments on storage period. It was concluded that incorporation of paneer with 0.25% and 0.5% of water chestnut extended the shelf life of paneer up to 21 days at refrigeration temperature in comparison to control samples (7-12 days shelf life).

Key words: Fortification, FSSR, Functional, Paneer, Physico-Chemical, Water Chestnut

Signature of Student
Dated: _____

Signature of Major Advisor
Dated: _____

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Place : Shuhama, Srinagar — ***Dr. Tarique Ahmad Padder***

Dated:

CONTENTS

Chapter	Particulars	Page No.
1.	INTRODUCTION	1-3
2.	REVIEW OF LITERATURE	4-26
3.	MATERIALS AND METHODS	27-38
4.	EXPERIMENTAL FINDINGS	39-51
5.	DISCUSSION	52-61
6.	SUMMARY & CONCLUSIONS	62-64
	LITERATURE CITED	i-xii
	APPENDICES	1

LIST OF TABLES

Table No.	Particulars	Page No.
1	Physicochemical Characteristics of *Trapa bispinosa*	8
2	Mineral composition of *Trapa bispinosa*	8
3	Physico-chemical characteristics of paneer fortified with varying levels of Water chestnu	41
4	Sensory quality of paneer with varying evels of Water chestnut	43
5	Functional characteristics of paneer with water chestnut added at varying levels	44
6	Sensory evaluation of paneer with selected levels of water chestnut during storage at 4 ± 1^0C	46
7	Standard Plate Count of water chestnut fortified paneer during storage at 4 ± 1^0C (log cfu/g)	47
8	Coliform Count of fortified paneer during storage at 4 ± 1^0C (log cfu/g)	48
9	Yeast and Mould Count of water chestnut fortified paneer during storage at 4 ± 1^0C (log cfu/g)	49
10	TBARS Value of paneer fortified with water chestnut during storage at 4 ± 1^0C (mg of malonaldehyde per kg)	50
11	Tyrosine Value of water chestnut fortified Paneer during storage at 4 ± 1^0C (mg of tyrosine per gram)	51

LIST OF FIGURES

Fig. No.	Particulars	After page No.
1	Physico-chemical characteristics of different paneer samples	41
2	Effect of varying level of water chestnut on pH of paneer	41
3	Effect of various levels of water chestnut on yield of paneer	41
4	Sensory score of paneer with varying levels of Water chestnut	43
5	Radical scavenging percentage of different paneer samples	44
6	Sensory scores of samples stored at refrigerator temperature (4±1 °C)	46
7	SPC count of samples stored at refrigerator temperature (4±1 °C)	47
9	Coliform count of samples stored at refrigerator temperature (4±1 °C)	48
10	Yeast and Mould count of samples stored at refrigerator temperature (4±1 °C)	49
11	TBARS Value of paneer fortified with water chestnut during storage.at 4 ± 1^0C	50
12	Tyrosine Value of samples stored at refrigerator temperature (4±1 °C)	51

LIST OF PLATES

Fig. No.	Particulars	After page No.
1	Functional paneer product samples presented for sensory evaluation	**45**

ABBREVIATIONS USED

ABTS	2,2 Azino bis thiazoline 6- sulfonic acid
AOAC	Association of Official Analytical Chemists
APHC	American Public Health Association
BHT	Butylated Hydroxytoluene
BIS	Bereau of Indian standards
°C	Degree Celsius
Cfu	Colony Forming Units
CWC	Chinese water chestnut
DCM:Me OH	Dichloromethane: Methanol
DPPH	Diphenyl-1 Picrylhydrazyl
FAO	Food and Agricultural Organization
FRAP	Ferric Reducing Antioxidant Power
FSSA	Food Safety and Standards Act
FSSR	Food Safety and Standards Regulation Act
HPLC	High-Performance Liquid Chromatography
METN	Methanol Extract Of *Trapa natans*
MDA	Malonaldehyde
NDN	Not Detectable Numbers
SNF	Solids Not Fat
PAS	Passive Avoidance Response
ROS	Reactive Oxygen Species
RSA	Radical Scavenging Activity
SPC	Standard Plate Count
SRBC	Sheep Red Blood Cells
TBARS	Thio-barbituric acid reacting substances
TBAE	Aqueous Extract of *Trapa natans*
TCA	Trichloroacetic acid
TL	Transfer Latency

Chapter- 1

INTRODUCTION

India is the largest producer of milk in the world with annual production of 147 million tonnes (Economic survey, 2016). About 5% of this milk is utilized for paneer production. Annual production of paneer being 700 metric tonnes (FAO, 2016). Paneer is an important nutritious and wholesome indigenous dairy product which occupies a prominent place among traditional milk products and carries a significant market potential. Paneer is highly popular product throughout the country, having many uses starting from its consumption in raw form to preparation of sweet meats and several varieties of culinary dishes and snacks. In Kashmir, although a predominantly non-vegetarian society, paneer holds a remarkable position in the common dietary schedule. Good quality paneer is characterized by a marble white to yellowish white color, sweetish, mildly acidic taste, nutty flavour, compact body and closely knit, smooth texture. According to the FSSA (2006), paneer means "product obtained from cow or buffalo milk or combination thereof, by precipitation with sour milk, lactic acid, or citric acid". Paneer should contain not less than 50% fat on dry matter basis and not more than 70% moisture. However, BIS stipulates a maximum moisture content of 60% in paneer. In order to meet these legal standards the raw milk should contain about 5.8% fat and 9% SNF (Sachdeva and Singh, 1988). In the local Kashmir scenario, the cow milk is almost invariably available that obviously does not match these required standards. As a result of this inherent inadequacy, the paneer made available in the market exhibits a poor texture and considerably high drip rate due to less moisture retention. This situation is further aggravated owing to exploitation by some unscrupulous marketeers by way of making undue profits out of increasing weight of paneer due to presence of unnecessary and illegitimate levels of water. Thus, the quality of paneer is compromised. In

order to contain this situation there is a need to find some way out and in this direction it is surmised that extraneous incorporation of certain suitable ingredients in raw milk would help bind water in paneer matrix and reduce the surface drainage of moisture thus imparting a desirably smooth and closely knit texture in paneer. Exploitation of a locally available edible additive that in addition to possessing the water binding and body/texture improving properties has an extra-nutritional /functional benefit offers an evident advantage.

A functional food is a food which gives an additional function often one related to health-promotion or disease prevention by adding new ingredients or more of existing ingredients (Martirosyan *et al*., 2011). Due to changing lifestyle and introduction of fast foods in our daily life, we are quite prone to oxidative stress from free radicals generated by cellular metabolism. These reactive oxygen species (ROS) are responsible for a number of diseases like cancer, neurological disorders, atherosclerosis, ischemia/perfusion, asthma etc. Therefore consumers are interested in functional foods which not only provide basic nutrition but promote health as well. It is, in this context, plausible to add some functional ingredients in dairy products which not only improve its quality characteristics but also provides therapeutic benefits. One such important locally available source of functional ingredients is water chestnut (*Trapa natans*), the incorporation of which is surmised to mitigate the problems highlighted along with the conferring of the antioxidant effect.

Keeping the above background in view, the present study is proposed with following objectives:

Objectives:

- To explore the influence of incorporation of water chestnut at different levels on the quality characteristics of paneer.
- To ascertain the functional effect of incorporation of water chestnut in paneer.

- To establish the relationship of incorporation of water chestnut with shelf life of paneer.

Chapter – 2

REVIEW OF LITERATURE

A) Water Chestnut (*Trapa natans*)

2.1 Historical Perspective

Trapa bispinosa had been introduced from Europe as an ornamental plant. Dispersal is limited because of the large, sinking nuts, but water chestnut has persisted and spread in the Northeastern states. In the Chinese Zhou Dynasty, water caltrop was an important food for worship as prayer offerings. The rites of Zhou (2nd century BC) mentioned that a worshipper should use a bamboo basket containing dried water caltrops. In India it is known as singhara or paniphal (eastern India) and is widely cultivated in fresh water lakes. The fruits are eaten raw or boiled. When the fruit has been dried, it is ground to a flour called singhare ka atta which is used in many religious rituals and can be consumed as a Phalahar diet on the Hindu fasting days, in Indian traditional festival "Navratri" (Chandana *et al.* 2013). The *Trapa bispinosa* is native to Eurasia. It was first introduced to North America in the 1870s, where it is known to have been grown in a botanical garden at Harvard University in 1877.The plant had escaped cultivation and was found growing in the Charles River by 1879.

2.2 Habitat

Trapa bispinosa Roxb. (Family: Trapaceae) is native to India. The fruit is commonly known as "Paniphal." It grows abundantly in the lakes of Kashmir, India. The plant is commercially cultivated in tropical parts of the world such as Pakistan, Sri Lanka, Indonesia and Africa. The plant is also abundant in Indonesia, southeast Asia and the Southern part of China and in the eutrophic waters of Japan, Italy and tropical America. It has become naturalized in a few places in the Eastern United States (Karmakar *et al*. 2011). It is commercially

cultivated across different parts of India for its consumable seasonal fruit commonly known as singhara which is a good source of nutrition having considerable amount of carbohydrate, protein and vitamins. *Trapa bispinosa* Roxb plant floats just beneath the water surface and thus forms a thick mat in the water column. Only its upper leaves float over water surface in an artistic radial pattern with swollen, air-filled petioles that keep the upper part of the plant afloat (Karmakar *et al*. 2011). *Trapa bispinosa* Roxb was first observed in North America, growing "luxuriantly" in Sanders Lake, Schenectady, New York, in 1884.The plant subsequently spread to many other areas in the Northeastern United States including Connecticut, Delaware, Maryland, Massachusetts, New Hampshire, Pennsylvania, Vermont, Virginia and Washington D.C. The plant is now present in the Great Lakes Basin and recently has been found in Quebec, Canada.

2.3 Cultivation and Collection

Trapa bispinosa seedlings are transplanted in May/June in a perennial pond. These plants make use of the available organic matter for their growth. Stock of 800 (50 g) common carp fingerlings is maintained in September-October. *Trapa bispinosa* fruits ripen in winter and are harvested from November to January (Singh *et al*. 2011).

2.4 Biogeographical Description

Common names

English: water chestnut

Hindi: singhara; singhada

Marathi: shingade

Sanskrit: smgtakah; jalphala

Biogeography and ecology

Kingdom: Plantae

Subkingdom: Tracheobionta

Family: *Trapaceae*

Genus: *Trapa*

Species: *Trapa natans L*

2.5 Botanical Description

It is an annual aquatic floating herb found in lakes and ponds. Floating leaves are rhomboid in shape, 2–6.5 cm in diameter, dark green above and reddish purple beneath, broader than long, denticulate, dentate, serrate or incised with entire base, apex acute red, and densely pubescent or villous beneath. The reddish green leaves are villous on the dorsal side, are about 5 to 8 cm long, and have hairy petioles from10 to 15cm in length. The submerged leaves are laterally dissected into capillary segments.

2.5.1 Flowers

Flowers are axillary, white in color, and with a solitary peduncle. They open above the surface of the water towards the afternoon. After pollination, the flowers submerge to facilitate fruit formation.

2.5.2 Fruit

It is obovoid, triangular with two horns and is about 2 cm in diameter. One seeded nut, has very unequal cotyledons and a top-shaped drupe. The fleshy pericarp covers a large 2–4 horned, stony endocarp (Karmakar et al. 2011). It is green in fresh condition, but after drying it becomes blackish; pulp of the fruit is whitish, sweet in taste.

2.5.3 Stem

The stem anchors into the mud by numerous branched roots and extends upward to the surface of the water. Cord like stems are spongy and buoyant and can reach lengths

2.6 Pharmacognostic Characters

Trapa bispinosa contains a great quantity of non-nutritional antioxidants, such as flavonoids, flavones and total phenol contents. Flavonoids are present in plant tissues, such as fruits, vegetables, nuts, seeds and leaves, in relatively high concentrations. Flavonoids act as natural antioxidants. Phytochemical screening of seed extract of *Trapa bispinosa* fruits reveals the presence of carbohydrates, saponins, phytosterols, fixed oils and fat, while the pericarp extract of the fruits of *Trapa bispinosa* revealed the presence of tannins, flavonoids and glycosides alkaloids, saponins, steroids and phenolic compound. The literature reveals the presence of saponins, tannins, flavonoids and glycosides in the pericarp extract of fruit (Bhatiwal *et al*. 2012). The kernel is delicious and contains carbohydrates, proteins, and essential minerals. It also contains plentiful B vitamins (including B1, B2, B5 and B6), E, A, and C vitamins. Seeds also contain thiamine, B6, E, A and C vitamins. Seeds also contain thiamine (Singh *et al*. 2011).

2.7 Phytochemistry

Trapa bispinosa (singhara) contains many organic and inorganic constituents which are mentioned below.

2.7.1 Inorganic Constituents

Acids, minerals, calcium, phosphorus, iron, copper, manganese, magnesium, sodium and potassium (Khare et al. 2007) and the physico-chemical characteristics of *Trapa bispinosa* are shown in **Table 1**. Biochemical analyses of fruits of *Trapa bispinosa* in 100 g showed 22.30 and 71.55% carbohydrate in fresh and dry fruits, respectively. The protein contents were 4.40% and 10.80% in fresh and dry fruits, respectively. The percentage of moisture, fiber, ash and fat contents was 70.35 and 7.30, 2.05 and 6.35, 2.30 and 8.50 and 0.65 and 1.85, in fresh and dry fruits, respectively. The mineral contents of the seeds were 32mg and 102.85mg calcium, 1.4 and 3.8mg iron and 121 and 325mg phosphorus in 100 g, in fresh and dry fruits, respectively (Table 1). In 100 g fresh and dried seeds of

Trapa bispinosa produced 115.52 and 354.85 Kcal of energy, respectively (Khare *et al.* 2007).

Table 1: Physicochemical Characteristics of *Trapa bispinosa*

Constituent	Percentage (wet basis)
Moisture	81.12 ± 0.5
Total soluble solids (Brix)	7.2 ± 0.2
Total acidity	0.142 ± 0.03
Crude lipids	0.36 ± 0.02
Total ash	1.33 ± 0.04
Crude fiber	0.72 ± 0.02
Total proteins	1.87 ± 0.03

Table 2: Mineral composition of *Trapa bispinosa*

Minerals	Content in *Trapa bispinosa*
Ca	365 ± 0.23
K	98.2 ± 1.23
Na	37.24 ± 0.36
Zn	6.926 ± 0.12
Ba	0.482 ± 0.32
Cr	0.106 ± 0.02

2.7.2 Chemical Composition of Water Chestnut Kernel.

Chemical analysis of the fruit showed that the moisture content of *Trapa bispinosa* kernel was 81.12% (wet basis). Fresh nuts having considerable water content are taken at breakfasts and are believed to suppress stomach and heart burning. The total soluble solids content of the fruit was 7.2%. The total acid in terms of citric acid present was 0.142%. Negligible amount of fat content was noticed in the fruit as 0.36% which substantiates its importance as dietary food. Also reported low crude lipid content in Chinese water chestnut was 0.06%. Total ash content obtained in fruit was 1.33% confirming good amount of minerals contained in the fruit. The potassium content of 0.41% has been reported as the major mineral present with iron and manganese contents which were 0.21 and 0.08%, respectively, being the minor minerals present (Khare *et al.* 2007). Crude fiber content of the water chestnut kernel was found to be 0.72% slightly higher than reported in Chinese *Trapa bispinosa* (Khare *et al.* 2007) as 0.60%. The total protein content calculated in the fruit was 1.87%. Low protein content has been earlier reported in *Trapa bispinosa.* Previously isolated classes of constituents, ascorbic acid, amylase, and amylopectin were isolated from the fruits of *Trapa bispinosa* (Rahman *et al*. 2000).

2.7.3 Organic Constituents

It contains carbohydrates and vitamins, namely, Vitamin B-complex (thiamine, riboflavin, pantothenic acid, pyridoxine, nicotinic acid), vitamin-C, vitamin-A, D-amylase, amylase and considerable amount of phosphorylase (Khare *et al*. 2007) Cycloeucalenol, ursolic acid, and 2β,3α,23-trihydroxyurs-12-en-28-oic acid (Song *et al.* 2007). The phytochemical content of *Trapa bispinosa* showed high quantity of saponins (36.92 ± 0.67%). Alkaloids present in the plants function as spasmolytic, anticholinergic, and anesthetic agents. The alkaloid content in *Trapa bispinosa* was found to be 0.775 ± 0.33%. Reports suggest that phenols antioxidant activity is due to their redox properties, H-donation, prevention of chain initiation by donating electrons or by binding transition metal

ion catalysts, and singlet oxygen quenchers. Flavonoids are important for their pharmacological activities as scavengers. Flavonoids prevent platelet stickiness and hence platelet aggregation. Colorimetric study of the two extracts of *Trapa bispinosa* showed that acetone solvent system was able to extract more phytochemicals in comparison to DCM:Me OH.

2.8 Ethnopharmacology

Pharmacological actions of water chestnut include aphrodisiac, astringent, appetizer, anti-pyretic, diuretic, haemostatic, nutritive, anti-diarrheal and tonic (Chatterjee and Prakash, 1995). Indications for use include are dyspepsia, diarrhoea, dysentery, intermittent fevers, leprosy, pharyngitis, lumbago, bronchitis, sore throat, hemorrhage, generalized debility, leucorrhea, threatened abortion, dysuria and inflammation (Khare *et al*., 2007). The fruits are used as intestinal astringent, aphrodisiac, anti-inflammatory and in leprosy, urinary discharges, fractures, sore throat and anemia (Rahman *et al*. 2000).

In Kashmir the water nuts form a staple farinaceous food. Fruit or nut or seed contains manganese and starch. It is nutritive, sweet, tonic and has freshening effect. Fresh fruits are edible, raw and cooked; dried ones are baked and eaten. They are also grated into flour and made into cakes. The nutritive value of the kernels is shown by analysis to be equal to that of rice. Fruits are refrigerant and useful in diarrhea and bilious affections with diarrhea. The upper portion of the stem was used in poultices as a discutient and the expressed juice in eye diseases (Nadkarni *et al.* 2007).

2.8.1 Uses in Unani medicine

It is used in cases of sexual debility, spermatorrhea, general debility, fatigue, tuberculosis, intermittent fevers, dysentery, dry cough bilious affections, bleeding disorders, anal fissure, lumbago, dental caries and sore throat (Ghani *et al*. 2010).

2.8.2 Uses during Pregnancy, Sexual Transmitted Disease and Fertility

Water chestnut fruits with milk are used in nervous and general debility, seminal weakness and leucorrhoea, menorrhagia etc. (Nadkarni *et al*. 2007).

2.9 Nutritional Aspects

Biochemical composition of fruits of *Trapa bispinosa* was studied and concluded that *Trapa bispinosa* could be important sources of carbohydrate, protein, and minerals, which is suitable for incorporation in human diet (Alfasane *et al* 2011). Nutrient composition of water chestnuts revealed moisture 62.5, ash 1.04, crude fiber 2.13%, total soluble sugar 0.92%, reducing sugar 0.33%, non-reducing sugar 0.59%, starch 8.7%, lipid 0.84%. One hundred gram of green variety contained water soluble protein 0.275mg, beta-carotene 60 microgram, vitamin-C 1.1mg, and total phenol 0.5mg. The minerals contents of green variety were potassium 5.22%, sodium 0.64%, calcium 0.25%, phosphorus 6.77%, sulphur 0.38% and iron, copper, manganese and zinc 200, 430, 90 and 600 ppm, respectively. The red variety contained moisture 62.7%, ash 1.30%, crude fiber 2.27%, total soluble sugar 0.90%, reducing sugar 0.30%, non-reducing sugar 0.60%, starch 8.2% and lipid 0.83%.The red variety contained water soluble protein 0.251mg, beta-carotene 92 microg, vitamin- C 0.9mg and total phenol 0.60mg per 100g.The red variety contained potassium 5.32%, sodium 0.59%, calcium 0.26% phosphorus 6.77%, sulphur 0.32%, iron 200 ppm, copper 450 ppm, manganese 110 ppm and zinc 650 ppm. The free amino acids, glutamic acid, tryptophan, tyrosine, alanine, lysine and leucine were commonly found in both varieties. In addition, green and red varieties contained cysteine, arginine and proline and glutamine and asparagines, respectively. Thus, the water chestnuts may play a crucial role in human nutrition (Faruk *et al*. 2012).

2.10 Antifungal and antimicrobial activity

In recent years, attempts have been made to investigate indigenous drugs against infectious disease. *Trapa bispinosa* can be used as antimicrobial agent

(Razvy *et al*. 2011) which has evaluated antifungal activity of fruit extracts of different water chestnut varieties. A strong antifungal activity of ethanol and petroleum extract was found against the treated fungi resulting in remarkable inhibition zone in comparison to both dithane-M fungicide and control. It was also evident that wild variety of water chestnut was comparatively more efficient in respect to antifungal activity compared to the red and green varieties of the same plant (Rahman *et al* 2001). It was mentioned that the extracts of *Trapa bispinosa* showed interesting antimicrobial activity against Gram-positive and Gram-negative test organisms and significant cytotoxic activity (Razvy *et al*. 2011).

2.11 Antibacterial Activity

Antibacterial activities of the fruit extract of two varieties (green and red) of water chestnut by the disc diffusion method from methanol extract were studied. The extract of red variety of water chestnut showed high antibacterial potential (31 mm) against *Bacillus subtilis* with the concentration of 600 micron. On the other hand, green variety showed highest antibacterial activities (12 mm) against both *Staphylococcus aureus* and *Shigella sonnei* with the concentration of 600 microgram Kanamycin used as standard. In this disc diffusion assay, the methanol extract of red variety was found to have a significant antibacterial efficiency compared to the extract of green variety of water chestnut. These findings pinpoint the efficiency of these extracts to inhibit microbial growth (Razvy *et al*. 2011). Rahman *et al*. (2001) investigated the in vitro antibacterial activity of compound from *Trapa bispinosa.* Anti-bacterial activity was carried out against fifteen pathogenic micro-organisms, both gram positive and gram negative bacteria, by Disc diffusion method. Compound MTC- 4 isolated from choloroform extract showed significant anti-bacterial activity against all test micro-organisms but maximum against *Bacillus subtilis* and *Shigella dysentrie* and produced zone of inhibition between 12 to 15 mm.

2.12 Radical Scavenging Activity

ABTS scavenging assay is applicable for screening both lipophilic and hydrophilic antioxidants which shows the percentage inhibition of ABTS radical by *Trapa bispinosa* extracts and standard trolox. Acetone extract (IC_{50}= 5 ± 0.24mg/mL) and DCM: Me OH (IC_{50} = 7 ± 0.76mg/mL) showed less scavenging than that of standard trolox (IC_{50} = 1 ± 0.01mg/mL).There was significant difference ($P < 0.05$) in ABTS scavenging activity of both the extracts (Gupta *et al.*, 2012). Malivya *et al*. (2010) investigated the *in vitro* antioxidant potential of aqueous extract of *Trapa natans* L. fruits rind. The extract was found to contain a large amount of polyphenols and also exhibited an immense reducing ability. The total content of phenolic, flavonoid and tannin compounds was estimated as 63.81 mg of gallic acid equivalents/g of dry material, 21.34 mg of rutin equivalents/g of dry material and 17.11 mg of total tannin equivalent /g of dry material, respectively. IC_{50} values for different antioxidant model were calculated as 128.86 µg/mL for DPPH radicals, 97.65 µg/mL for $O2^-$, 148.32 µg/mL for H_20_2 and 123.01 µg/mL for NO, respectively. Reducing power and inhibition of OH radical-induced BSA oxidation were also determined. Their study revealed that the aqueous extract of *Trapa natans* L. fruit rind had significant antioxidant activity against free radicals. You *et al*. (2007) conducted the experiment to test the antioxidant activity and then determine the major phenolic compound components present in Chinese water chestnut (CWC). CWC phenolic extract strongly inhibited linoleic acid oxidation and exhibited a dose-dependent free-radical scavenging activity against α,α-diphenyl-β-picrylhydrazyl (DPPH) radicals, superoxide anions and hydroxyl radicals, which was superior to ascorbic acid and butylated hydroxytoluene (BHT), the two commercially used antioxidants. Furthermore, the CWC extract was found to have a relatively higher reducing power, compared with BHT. The major phenolic compounds present in CWC tissues were extracted, purified and identified by high-performance liquid chromatography (HPLC) as (–)-gallocatechin gallate, (–)-epicatechin gallate and

(+)-catechin gallate. This study suggested that CWC tissues exhibit great potential for antioxidant activity and may be useful for their nutritional and medicinal functions. Barreiraa *et al*. (2007) evaluated the antioxidant properties of chestnut (flowers, leaves, skins and fruits) extracts through several biochemical assays: DPPH (2,2-diphenyl-1-picrylhydrazyl) radical-scavenging activity, reducing power, inhibition of b-carotene bleaching, inhibition of oxidative hemolysis in erythrocytes, induced by 2,20-azobis(2-midinopropane)dihydrochloride (AAPH), and inhibition of lipid peroxidation in pig brain tissue through the formation of thiobarbituric acid-reactive substances (TBARS). These assays have been extensively studied as models for the peroxidative damage in biomembranes. The EC_{50} values were calculated for all the methods in order to evaluate the antioxidant efficiency of each chestnut extract. The phenol and flavonoid contents were also obtained. Chestnut skins revealed the best antioxidant properties, presenting much lower EC_{50} values, particularly for lipid peroxidation inhibition in the TBARS assay. Furthermore, the highest antioxidant contents (polyphenols and flavonoids) were found for these extracts. Zhao *et al*. (2011) studied the extraction and isolation procedure for tannins from chestnut burs, and to assess their potential antioxidant activity. Aqueous ethanol solution was used as extraction solvent, and HPD 100 macroporous resin column was applied for isolation. The influence of solvent concentration in the extraction and elution process on extraction yield, tannins and polyphenols content, as well as antioxidant potential, including DPPH and ABTS radical scavenging ability, reducing power ability and cellular antioxidant ability were assessed. In both the extraction and isolation process, 50% aqueous ethanol led to superior total tannins and polyphenols content as well as significantly higher antioxidant activity. In addition, the antioxidant activity and the total tannins content in extracts and fractions had a positive linear correlation, and the predominant components responsible for antioxidant activities were characterized as hydrolysable tannins

2.13 Pharmaceutical uses

2.13.1 Excipients

Starch obtained from *Trapa bispinosa* has comparable physicochemical and binding activities compared to official starches. Physicochemical property of water chestnut starch (WCS) was comparatively evaluated with official potato and maize starch. The granule shape is round to oval with the particle size diameter 18–130 μm. The powder characteristics are nearby similar to the official starches. Hydration and swelling capacity of WCS is approximately similar which make this potential excipient in pharmaceutical formulation development. Thus, it has potential to be used as binder industrial (Singh *et al.* 2011).

2.13.2 Metal Chelation Activity

Lipid peroxidation by the Fenton reactions is initiated by ferrous iron. Thus, minimizing Fe^{+2} concentrations in Fenton reactions by metal chelation affords protection against oxidative damage. The chelating of Fe^{+2} ions by the extracts was estimated by the method of Dinis . In this assay, both extracts interfered with the formation of ferrous and ferrozine complex in an almost similar manner, suggesting that they have chelating activity and capture Fe^{+2} ion before ferrozine.

2.13.3 Freeze Thaw Stabilization

Among the four gums tested, Guar gum (GG) was effective in increasing freeze thaw stability, when 0.2% gum was added; while at 0.3%, gum acacia was more effective than GG. It was noted that the addition of salts increases the stability of the gel towards low temperature. The addition of NaCl at 0.5%, 1%, and 2% showed maximum stability compared to other salts due to the hydrophilic nature of the sodium chloride enhanced water-holding ability of the starch pastes thereby limiting amount of water exuded but the reduced stability was observed in the presence of $CaCl_2$. The addition of salts increased the stability of the mixtures against the freeze thawing at varying concentrations (Lutfi *et al*. 2009).

2.13.4 Starch as Additive in Pharmaceuticals.

The surfaces of the granules of all samples are smooth with no evidence of cracks. Some granules appeared to be either round or oval in shape with “horn(s)” protruding from the surface (Ghani et al. 2010). The physicochemical properties of the starch extracted from Krajub (Thailand known water chestnut) *Trapa bispinosa* were investigated. Scanning electron microscopy of the starch granules showed that they were either oval or round in shape with small horn(s) protruding from the surface. Amylose content of the Krajub starch was 29.62% (dry weight basis). The pasting temperatures of 6–8% starch suspension were 81– 83^0C. Bra bender amylogram showed no peak viscosity and very low breakdown, indicating high heat and shear stability of the starch suspension.The starch pastes highly retrograded and formed an opaque gel. The X-ray diffraction patterns of the starch revealed a C-type crystallite. The starch granules were more resistant to acid hydrolysis (2.2 NHCl at ambient temperature) than mung bean starch (C-type crystallite) (Tulyathan *et al.* 2005).

2.14 Analytical Evaluation

2.14.1 Yoghurt Stabilizer

Enriched yoghurt with *Trapa bispinosa* starch at different levels was studied with physicochemical and sensory analysis. Yoghurt prepared by incorporation of *Trapa bispinosa* starch at concentration of 0.5%, 0.75%, 1%, and 1.25% was compared for these characteristics to the yoghurt containing stabilizer gelatin 0.5% w/w. Physiochemical parameters (fat, pH, acidity, synergies, water holding capacity, viscosity, protein, etc.) , sensory evaluation and microbial analysis (total viable count and coli form test) were studied. Use of *Trapa bispinosa* starch produced better results in terms of lowering synergies and increasing water holding capacity, viscosity and overall acceptability for all sensory attributes. Addition of *Trapa bispinosa* starch did not influence the taste and overall acceptability. *Trapa bispinosa* starch 1.25% gave most excellent

results for water holding capacity, synergies and viscosity and *Trapa bispinosa* starch 0.75% gave most excellent results for all sensory attributes. Yoghurt shelf life was increased up to 25 days (Malik *et al.* 2012).

(B) Paneer

2.1 Historical Perespective

Paneer is a South Asian variety of soft cheese obtained by acid and heat coagulation of milk. It is non-fermentative, non-rennet, non-melting and unripened type of a cheese. It is believed that the nomads of south west Asia were the first, to develop several distinctive heat and acid coagulated varieties of cheese (Mathur et al.19860), as there is documental evidence suggesting that the people of Kusana and Saka Satavahana periods (AD 75-300) used to consume solid mass prepared from mixture of warm milk and curd, which seems to be earliest version of present day of acid and heat coagulated milk products like paneer (Mathur, 1991). Paneer khaki is one of unique Iranian nomadic cheese, developed by bakhtiari tribe of Iran (Roa et al., 1992), when salted it is known as paneer-e-shour. The literal meaning of word 'paneer' is container and that of khiki is skin. Paneer is also the Hindi name of *Withania coagulans*, a vegetable rennet that yields bitter curd. The nomads of Afghanistan developed white paneer which served as a staple food for them, they developed two distinct varieties of paneer, when made from raw milk, it is known as paneer-e-kham, and from boiled milk, paneer-e-pokhta. It has been surmised that sour milk, pieces of creeper called putika, bark of palasa tree or Kuyala (jujuka), etc. might have been used for coagulation of milk by nomads. Paneer was probably first introduced into india by Persian and Afhgan invaders which is most likely reason for its wide popularity in the North Western parts of india and southern regions of jammu and Kashmir due to influence of foreign settlers in these regions. However, it was only during the last five decades that paneer has spread to other parts of india probably due to wide spread migration of people from one region to another.

Cheese varieties that are made without any starter culture using high heat, acid precipitation is practiced in many countries of south asia and central, south and latin America. White cheese, which is present throughout south and central America, mexico and carribean islands, is a product that is quite similar to paneer. Some varieties of fresh unripened cheese found throughout the world which are quite similar to paneer are, Kareish in Egypt, Armavir in western Caucasus, Zsirpi in Himalyas, Feta in Balkans and Queso Criollo, Queso Llanero and Queso Blanco etc. in Latin America, Anari in Cyprus, Farm cheese in western countries and BEyaz paneer in Turkey (Torres and Chandan, 1981 and Wikipedia 2009) and Krandi cheese which is semisoft dried cheese found in Jammu and Kashmir, India (Punoo et al., 2007).

2.2 Diversification

Paneer is highly popular product throughout the Indian sub-continent and its popularity is growing. Now-a-days the production of paneer is not restricted to South Asia only but it has spread throughout the world. The most important features which has helped it to become popular worldwide is its ability to be deep fried and makes great snakes, pakoras or fried paneer chunks which are great success all over the world. Paneer is used in variety of forms, it forms a base for a variety of culinary dishes, stuffing material for various vegetable dishes (especially matar paneer and palak paneer), snacks and sweatmeats for prepration of rasgolla, rasamalai and Sandesh etc. Due to ever growing demand for paneer, researchers were encouraged to develop new types and varieties of paneer. The different types of paneer manufactured in recent times are low fat paneer, reduced fat paneer, dietry fibre enriched low fat paneer, low fat paneer enriched with whey protein concentrate, low protein paneer with soy protein isolates, skim milk paneer, soy paneer, filled paneer, protein enriched filled paneer, microfiltered paneer , ultrafiltered paneer, vegetable impregnated paneer, long life paneer, paneer curry, paneer spreads, paneer pickles, spiced paneer, masala paneer, fruit paneer and processed paneer (Nakazawa *et al*., 1989; Sanyal and Yadav, 2000; Pal

and Kapoor, 2000; Kanawjia and Rizvi, 2000 and 2003; venkateshwarlu *et al.*, 2003; Sreedhara and Balasubramanyam, 2003; Shukla and Vaid, 2004; Bajwa *et al.*, 2005; Kanawjia and Khurana, 2006; Yellamanda *et al.*,2006;Pal and Londhe, 2006; Roa and Patel, 2006; Yadav *et al.*, 2007; Gupta *et al.*, 2007; Chatli et al., 2007; Pal and Kapoor, 2007 and Prince *et al.*,2007).

2.3 Raw Materials

Types of milk

Various types of milk have been usedfor manufacture of paneer. The quality of paneer is determined by quality of milk from which it is produced (Nayak and Bector, 1998).

2.3.1 Buffalo milk

For making good quality paneer with desirable characteristic, buffalo milk is considered more suitable than cow milk (Bhattacharya *et al.*, 1971; Sachdeva *et al.*, 1985; Singh and Kanawjia, 1988). Research work of Bhattacharya *et al*. (1971), Shukla *et al.* (1984) and Chawla *et al.* (1985) held bigger fat globule size in buffalo milk responsible for spongy characteristics in buffalo milk paneer compared to cow milk. Ghodekar (1989) reported higher amount of casein and minerals (phosphorus and calcium) are responsible for imparting firm and rubbery body to paneer from buffalo milk. However Sindhu (1996) reported that not only bigger size of fat globules but also higher amount of casein, fat, minerals (calcium and phosphorus) and casein micelles, in buffalo milk compared to cow milk, makes the former better suited to paneer manufacture. Ramasamya *et al*. (1999) advocated use of buffalo milk having 6 per cent fat for prepration of good quality paneer. Masud (2002) also used buffalo milk with 6 per cent fat for making paneer. Other workers (Bhattacharya et al., 1971; Arora and Gupta, 1980; Roa *et al.*, 1984; Chawla *et al.* 1987; Sachdeva and Singh, 1988b; Singh and Kanawjia, 1990 and Kumar *et al*., 2008) also recommended buffalo milk with 5-6 per cent fat for paneer manufacture.

2.3.2 Cow milk

It is well established fact now that the good quality paneer can be obtained from cow milk using certain modifications in the manufacturing along with use of certain additives (Visheshwaraiah and Anantakrishnan 1986; Singh and Kanawjia, 1988; Sachdeva et al., 1991; Arya and Bhaik, 1992). Visheshwaraiah and Anantakrishnan (1986) reported that paneer from cow milk standardized to 4.5% fat also conforms to PFA standard. However, Pruthi and Koul, (1989) observed that the milk from cross bred cows (HF × Sahiwal) having 3.7% fat and SNF (8.25-8.42% or more, yields product which conforms to PFA standards. Jadhavar *et al*. (2009) prepared good quality paneer from cow milk.

Low fat paneer of acceptable quality can be produced from cow milk containing as low as 3.5% fat (Visheshwaraiah and Anantakrishnan, 1986). However, the use of cow milk with fat levels lower than 3.5% did not meet desired success as product lacked softness and typical flavour(Arya and Bhaik, 1992). Chandan (2007) reported that acceptable quality paneer with only 24% of FDM is available in western countries.

2.4 Fortification of Paneer and Other Dairy Products

Bandyopadhyay *et al.* (2007) assessed the antioxidant activities of beet (*Beta vulgaris*), mint (*Mentha spicata L*.) and ginger (*Zingiber officinale L*.) alone or in combination after their fortification in sandesh (a heat desiccated product of coagulated milk protein mass) using Randox's total antioxidant level determining chemicals and ultimately it was compared with the synthetic antioxidants like TBHQ (tert-butylhydroquinone), BHA and BHT. Addition of beet or mint alone in sandesh showed lower antioxidant level than the addition of ginger alone. However, combination of beet with ginger showed highest antioxidant level among the natural sources and value was almost equal to TBHQ (200 mg kg^{-1}). Besides, the suitable stage and form of addition of these herbs in sandesh were also investigated using the Randox's antioxidant level evaluating chemical.

Among the four forms of herbs such as paste, tray-dried powder, freeze-dried powder and solvent extracted form, addition of solvent extracted form in sandesh showed highest antioxidant level than any other form. Similarly, addition of all these herbs at final stage of sandesh preparation showed highest antioxidant level than their addition at the initial stage of sandesh preparation. Comparative evaluations of the proximate composition of herbal sandesh with the control sandesh showed that herbal sandesh was more or less similar with control sandesh except in fat and moisture content. But according to sensory characteristics, sandesh containing beet, ginger or combination of beet with ginger or mint was more acceptable to panelist than control sandesh. Results of the study indicate that herbal sandesh is more value added health food than control sandesh. Bandyopadhyay *et al*. (2007) incorporated herbs such as turmeric (*Curcuma longa L*.). coriander (*Coriandrum satiaurn L*.), curry leaf *(Murraya koenigi;* L.), spinach (*Spinacia oleracea*) and aonla (*Emblica fficinalis*), separately as a paste at 10% level into Sandesh to induce the antioxidant properties into the product. The antioxidative level of these herbs was compared with the synthetic antioxidants TBHQ (tert-butylhydroquinone) and BHA: BHT (1:1') at 100 and 200 mg/kg levels. The authors have reported that the total antioxidative status of herbal Sandesh was lower than the samples with TBHQ but similar to those with 200 mg/kg BHA: BHT (1: 1). The authors have also reported that the use of coriander herb with its antimicrobial and antioxidant properties increased the shelt life of herbal sandesh upto 8 days and 30 days when stored at 30+ 1^0C and 7 + 1^0C, respectively when compared with the remaining samples.

Nalkar *et al*. (2009) conducted a study to find the effect of *bhendi* gum as stabilizers on the quality and composition of the paneer. Due to the use of stabilizers, moisture retention, fat, FDM and acidity were increased. Within the particular stabilizer group, in general, positive correlation was observed between yield and stabilizer level. The differences in sensory score for these quality attributes due to different treatments were highly significant. Use of *Bhendi* gum

at 0.45 per cent level, yields the paneer of desirable quality at comparatively lower cost.

Sharma *et al*. (2009) studied the effect of different levels of carboxymethyl cellulose (CMC) on the uptake of vegetable oil during the deep-fat frying of paneer by way of : (i) direct addition of CMC to the milk and (ii) dipping the paneer cubes into solutions of CMC for different time intervals. The fried paneer samples were evaluated for chemical composition, hardness and sensory characteristics. Method (i) proved to be beneficial in all respects as compared to (ii), as the absorption of water from the suspensions of CMC destroyed the texture of the paneer

Landge *et al.* (2011) successfully prepared shrikhand using *ashwagandha* herb powder as an additive. *Ashwagandha* herb powder @ 0.3%, 0.5% and 0.7% with 40% cane sugar by weight of chakka was mixed for manufacture of shrikhand. The samples were stored at 7^0 C and sensory and microbial qualities were evaluated at regular intervals. It was found that addition of O.5%(T_2) ashwagandha powder to srikhand was superior in organoleptic parameters followed by 0.7%(T_3), 0.3%(T_1),and control (T_0),respectively. The treated product remained acceptable up to 52 days at refrigerated temperature.

El-Aziz *et al.* (2012) evaluated Soft cheese fortified with ginger extract as a functional dairy food. Buffalo's milk retentate was divided into three equal portions. One batch had no ginger extract that served as control. The latter batches were fortified with extract at the rate of 1.5 or 3.0 g/kg. All batches were salted with @ 4% NaCl/water phase. The resultant cheese samples were divided into 2 parts; one was separately pickled in salted permeate (4%), while the other was stored without pickling at 5±2°C for 6 weeks. The results revealed that cheese pickling increased the cheese proteolysis and lipolysis, and decreased pH and TVFAs. Fortification with ginger extract enhanced cheese proteolysis and TFVA and reduced pH value and oxidative rancidity of cheese. Physically, un-pickled soft cheese was more springy, harder, darker and more yellowish compared with

pickled cheese samples.Ginger extract caused an increase in cohesiveness, whiteness and yellowish colour degree, and decrease in hardness of both pickled and un-pickled soft cheese. Ginger extract fortifi ed cheese enhanced the growth of *L. lactis ssp. lactis and L. lactis ssp. cremoris* compared with control cheese. However, ginger extract exhibited the highest growth for *Lactococcus strains* in pickled cheese. Yeasts and moulds were detected only in cheese control sample after 2 weeks. Ginger extract fortified cheese gained the highest scores for flavour, texture and overall acceptability in both pickled and un-pickled cheese, which became more acceptable to panelists than control cheese over storage

Cimo *et al*. (2013) observed that the bioactive components of *ginseng* can decrease oxidative stress, which is a mechanism associated with prevention of chronic disease development. Since the efficacy of ginseng is dependent on gut microbiota, combining ginseng with a probiotic yogurt may improve microflora health and enhance the health benefits associated with consumption of this herb. To identify if yogurt is a suitable medium for North American Ginseng and probiotic bacteria *Lactobacillus rhamnosus* GR-1, ginseng fortified probiotic yogurt was developed by inoculating milk with probiotic mother culture, starter cultures, and various concentrations of aqueous ginseng extract. *L. rhamnosus* GR-1 viability and ginsenoside stability were measured at 1, 14, and 28 days of refrigerated storage through microbial analysis and high performance liquid chromatography, respectively. Throughout storage, viability of L. rhamnosus GR-1 increased in the presence of ginseng. While ginsenosides were observed to be present, stability in the yogurt medium is inconclusive. This study demonstrated that yogurt combined with aqueous ginseng extract is suitable for *L. rhamnosus* GR-1, and there is a synbiotic relationship between the prebiotic components of ginseng and *L. rhamnosus* GR-1.

Singh *et al*. (2014) conducted a study to evaluate the properties of turmeric incorporated paneer prepared from different types of milk, i.e. cow milk, buffalo milk and mixed milk. Turmeric was incorporated in the products at the rate of 0.0

(control) and 0.6 % by weight of expected yield of paneer after heat treatment of milk but before addition of coagulant. The paneer was packed into almunium foil and divided into two groups. One was stored at room temperature and the other at refrigerated temperature.The prepared samples of paneer were subjected to sensory evaluation when fresh and after the interval and during storage for 3 days at room temperature(27±1 °C) and for 15 days ats refrigerated temperature(below 5^0C). During this period of storage different tests, such as sensory evaluation, texture profile analysis and safety study (chemical analysis and microbial analysis) were conducted. The samples of paneer with 0.6 % turmeric by weight of expected yield of paneer remained acceptable up to 15 days on storage at refrigerated temperature. The present study entailed to conclude that addition of turmeric in paneer prepared from either cow milk, buffalo milk or mix milk at the rate greater than 0.6 % by weight of expected yield of paneer results into sharp decline in sensory score and texture of paneer but it is still acceptable and safe for usage. Addition of turmeric at the rate of 0.6 % by weight of expected yield of paneer and packed in aluminium foil extends the shelf life of paneer up to 15 days on storage at refrigerated temperature(below 5^0C).

Palthur *et al*. (2014) prepared the milk products by partial substitution of milk with *Ocimum sanctum* and investigated the proximate quality, textural characteristic, keeping quality and sensory attributes of the developed product. The physico- chemical and organoleptic studies were performed for the Herbal Milk analysis. Laboratory analysis was carried out to study the variation in moisture, protein, fat and ash content. PH, Acidity and Specific Gravity were slightly changed when compared to normal milk. The organoleptic studies appearance, colour, flavor, taste and overall acceptability were studied and overall acceptability was good for herbal milk. Microbial studies like total plate count (TPC), yeast and mould count, coliform and E.coli count were carried out to evaluate the safety and keeping quality of the products. Antioxidant and iron

chelating activity of the Herbal Milk was determined. The Herbal Milk product was the most preferred and recommended for market exploration.

Perna *et al*. (2014) evaluated the antioxidant activity of yogurt made from milk characterized by different casein (CN) haplotypes (αs1-, β-, κ-CN) and fortified with chestnut and sulla honeys. The CN haplotype was determined by isoelectric focusing, whereas antioxidant activity of yogurt was measured using 2,2'-azino-bis-3-ethylbenzothiazoline-6-sulfonic acid and ferric-reducing antioxidant power. The statistical analysis showed a significant effect of the studied factors. The results showed that chestnut honey presented the highest phenolic acid and flavonoid contents, which are closely associated with its high antioxidant activity. The antioxidant activity of fortified yogurt samples was affected both by different CN haplotypes and by type of honey added. Yogurts fortified with chestnut honey showed higher antioxidant activity than those fortified with sulla honey. The different behavior observed among the fortified yogurts suggested that the effects of protein-polyphenol complex on antioxidant activity are interactive. The results suggest that milk proteins polymorphism and polyphenols play different roles in affecting the bioavailability and the antioxidant activity of yogurt.

Ali *et al*. (2014) carried out a study to find the quantitative antioxidant activity of herbal powder inclusion (*Asparagus racemosus, Asparagus adscendens R, Punica granatum L and Dactylorhiza hatagirea*) in the ice cream. Ice cream prepared by this method was subjected to sensory analysis. The best result of DPPH and FRAP activity in herbal ice cream were found with the inclusion @ 4% herbal powder of selected ice cream.

Sohail *et al*. (2014) conducted a study to introduce *Lallemantia royleana* seeds as yogurt stabilizer instead of gelatin. The yogurt was prepared by the standard method. The *tukhm milanga* in the powder form, was added to the milk after pasteurization @ 0.15%, 0.2% and 0.25% concentration. The yogurt was stored at 4±2 ^{0}C for 20 days and analyzed for different physiochemical (pH,

titratable acidity, synersis, water holding capacity, total solids, viscosity, hardness, fat, protein and ash), microbiological and sensory attributes at specified day intervals. The utilization of stabilizer and its rate of incorporation affected the given attributes. Among different concentrations of *L. royleana*, Y3 (with 0.25% *L. royleana*) gave best results for physical and chemical parameters but Y2 (with 0.20% *L. royleana*) attained highest score for overall sensory acceptability throughout the storage period. It was also committed from data incorporation of *L. royleana* into yogurt promoted overall effective outcomes for sensory evaluation although physicochemical and microbiological results were almost comparable with yogurt containing gelatin

Srivastava *et al*. (2015) used different levels of *Zingiber officinale* and *beta vulgaris* to produce the herbal yoghurt from cow, buffalo and goat milks. The aim of the study was to find the antioxidant activities of the herbal yoghurt samples. The samples were analysed by using 2,2 diphenyl-1 picrylhydrazyl (DPPH) scavenging activity and ferric reducing antioxidant power (FRAP). The results indicated that the highest antioxidant activities with DPPH & FRAP methods was found in goat milk yoghurt with 2% level of ginger rhizome and 2% level of beet root extracts followed by cow milk yoghurt with 2% level of ginger extract. The lowest antioxidant activity was found in buffalo milk herbal yoghurt. The yoghurt prepared by this method was subjected to sensory evaluation. It was concluded that yoghurt prepared by this method had high antioxidant properties which are beneficial for human health.

Chapter-3

MATERIALS AND METHODS

3.1 Raw materials

a) Milk: Milk was procured from local known source. The composition of raw milk was determined in terms of fat, SNF and total solids. Milk was subjected to parameters like p^H, titratable acidity, specific gravity and electrical conductivity

b) Water chestnut: water chestnut fruits were procured from local market. Fruits of water chestnut (*Trapa natans*) were sun dried and then ground by using laboratory scale grinder, into fine powder of 20 mesh size. It was analysed for proximate composition. The powder was stored in edible food grade plastic containers at a cool and dry place for further use.

3.2 Incorporation of water chestnut at various levels

Paneer was prepared by standard procedure of Sachdeva *et al.* (1985). Water chestnut of 20 mesh size was added @ 0% (control), 0.25%, 0.50%, 0.75% and 1% levels to milk samples . Now, these samples were heated to 90^0 C without holding and then cooled to 70^0C. Citric acid (2%) was added as a coagulating agent at 70^0C with continous and gentle stirring. After coagulation the contents were allowed to stand for 5 minutes. Then the whey was drained out by filtering contents through a fine muslin cloth. The coagulum was collected in clean muslin cloth and filled in hoops with cloth linings followed by pressing of freshly prepared product with the weight of 230kg/$m^2$for 15-20 minutes. Finally the freshly prepared paneer samples were chilled in cold water at 4^0 C for 30 minutes. The paneer was taken out of water and drained well and weighed again to obtain final paneer yield and per cent moisture absorption. The product was then subjected to sensory, antioxidant, physico-chemical and shelf-life estimation.

$$\text{Moisture absorption (\% green weight)} = \frac{\text{final paneer wt. after immersion} - \text{green paneer wt.}}{\text{Green paneer weight}} \times 100$$

Experimental Protocol

Ingredients	**T_1 (control)**	**T_2**	**T_3**	**T_4**	**T_5**
Milk (ml)	1000	1000	1000	1000	1000
Water chestnut (g/l)	0	2.5	5.0	7.5	10

3.3 Laboratory analysis

All the analytical procedures required for the analysis of milk, whey and paneer produced during the experimentation were carried out in duplicate in the laboratory of Divison of Livestock products technology, Faculty of Veterinary sciences and Animal husbandry, SKUAST-K, Shuhama Alusteng, Ganderbal. For physico-chemical analysis about 100gm of paneer and 60ml of each of milk and whey were used for determination of various parameters.

3.3.1 pH

pH of milk and whey

The pH of milk and whey samples were taken by directly dipping the combined electrode of digital pH meter after proper calibration of the instrument, into the samples. These readings were taken for each sample and average pH recorded.

pH of paneer

For determination of pH of paneer samples, about 10g of paneer was blended with 50ml distilled water in a beaker to form slurry. The pH of resultant slurry was recorded using the same procedure

3.3.2 Titratable acidity

A 10ml quantity of thoroughly mixed milk samples was taken in a conical flask with the help of a dry pipette. To this few drops of phenolphthalein indicator were added. Then the milk was carefully titrated against 0.1N sodium hydroxide till faint pink colour appeared and persisted for 15 seconds. The volume of 0.1 N sodium hydroxide used was recorded and titratable acidity was calculated as per the formula given below:

$$\text{Titratable acidity (\%)} = \frac{\text{No. of ml of 0.1N NaOH used} \times 0.009}{\text{weight of sample in g}} \times 100$$

3.3.3 Electrical conductivity (EC)

Electrical conductivity of the sample was taken by dipping the electrode of electrical conductivity meter into the sample after proper calibration of instrument. Two or three readings were taken for each sample and average electrical conductivity was calculated.

3.4 Proximate composition

All the milk, paneer and whey samples were analysed for determination of various physico-chemical parameters using the standard procedures of Association of Analytical Chemists (AOAC, 2000). Brief description of methods is outlined below:

3.4.1 Moisture

For the determination of moisture about 3gm of paneer sample was weighed accurately on electronic balance, corrected up to 0.1mg, in a dry, preweighed, flat bottomed moisture cups and kept in hot air oven at 102 ± 1^0C

for 4 hours. Then moisture cups were transferred immediately to a dessicator to cool to room temperature (at least 30 minutes). The process of drying, cooling and weighing was repeated at 30 minutes interval until the difference between two consecutive weighing was less than one milligram. Weight loss of cup after drying was recorded and expressed in terms of moisture per cent.

Calculation

Dry matter per cent = $[(W_1 - W/(W_2 - W)] \times 100$

Moisture per cent = (100 − Dry matter per cent)

Where ,

W = weight of empty dried cup (g)

W_1 = weight of cup + sample after drying (g)

W_2 = weight of cup + sample (g)

3.4.2 Ash

About 3-5 g of paneer samples in duplicate were weighed accurately on electronic balance, corrected up to 0.1mg, in a dried and pre-weighed crucibles and kept in hot air oven at 102 ± 1^0C for 4 hours. The sample in crucible was subjected to carbonization and then the crucible was placed in muffled furnace to incinerate the sample at 550 -600^0C for 2 hours. After incineration the sample in crucible was allowed to cool and weighed again. Ash percentage was calculated as given below

Calculation

Ash per cent = $[(W_1 - W/(W_2 - W)] \times 100$

Where,

W = weighed of empty dried crucible (g)

W_1 = weight of crucible + sample after ashing (g)

W_2 = weight of crucible + sample (g)

3.4.3 Crude Protein

Microkjeldhal's method was followed for estimation of total nitrogen present in paneer products. About 0.2-0.3g of well mixed homogenized paneer samples in duplicate were digested with 10 ml of concentrated sulphuric acid. A pinch of catalyst (mixture of sodium sulphate and copper sulphate in the ratio of 95:5) was added to hasten the digestion. 30 ml of distilled water was added to the digested sample present in digestion tube large (DTL). 25 ml of 4% boric acid solution along with few drops of indicator was taken in a 250ml volumetric flask and loaded in the receiver end of distillation apperatus of CALCULOUS. The sample was neutralized with 40% NaoH and then distillation was done. The ammonia collected in boric acid solution was filtrated against 0.1 N Hcl

Calculation:

$$(\%)\text{Nitrogen} = \frac{14 \text{ x normality of acid x } (\text{BRT} - \text{BRB})}{\text{Sample weight x } 1000} \times 100$$

Where,

BRT= burette reading for taken sample

BRD= burette reading of blank

% CP = 6.38 x % N

3.4.4 Ether Extract (Paneer) Estimation

Crude fat content of paneer samples was determined using fat extraction tubes of soxlet apparatus. About 4 grams of the well mixed and homogenized paneer sample in duplicate was taken into a thimble made of Whatman filter paper #2. It was then plugged with small amount of cotton to prevent sample from coming out of thimble. The sample was kept in a beaker for drying in the

oven at 100- 102^{0}C for 4-6 hours. Thimbles were then transferred to soxlet apparatus for extraction of fat. Petroleum ether having boiling point of 40-60^{0}C was taken in a clean dried pre-weighed round bottom flask and allowed to run for 6-8 hours or 21cycles. The flask was finally removed from apparatus and last traces of solvent evaporated in hot air oven at 60^{0}C. Flask was then transferred to a dessicator and weighed. The flask was then again put in oven at 100-102^{0}C for an hour and cooled in dessicator and weighed again. This was continued till two consecutive weights did not differ more than 0.001g. The per cent ether extract was calculated as follows:

Calculation

$$\text{Ether extract per cent} = \frac{W2 - W1}{W0} \times 100$$

Where,

W_0 = weight of sample

W_1 = weight of empty dried round flask after extraction

W_2 = weight of dried round flask after extraction

W_2-W_1 = amount of ether extract

3.4.5 Fat

Fat of milk and whey was estimated by Gerber's sulphuric acid (90 ml of concentrated sulfuric acid added to 10ml of distilled water) was taken carefully in a clean dry butyrometer (ISI marked) with the help of automatic dispenser (tilt measure) without wetting the neck. To this 10.75ml of thoroughly mixed milk/whey sample was added with the help of milk pipette on the side walls of the butyrometer. Then 1ml of amyl alcohol was added to the butyrometer on the sides. Dry rubber lock stopper was used to close the butyrometer. These were then shaken and inverted 2-3times till complete dissolution of the acid and milk or whey contents. Then tubes were placed in water bath for 5 minutes at 65 ±

2^0C to ensure that all casein particles were dissolved. The butyrometer tubes were then placed in a centrifuge in a radial symmetry and as evenly spaced as possible. Centrifugation was done for 4 minutes at 1100 rpm. Butyrometer tubes were then removed from centrifuge and placed again in water bath for 5 minutes at 65±2^0C. With the help of stopper and key, the fat level was adjusted in such a way that scale reading corresponds to the lowest point of the fat meniscus and the surface of separation of the fat and acid. The observed fat level was recorded as per cent fat of test sample.

3.4.6 SNF

SNF of the milk was calculated by indirect method. The difference between total solids (%) and fat (%) gave the SNF content in milk.

SNF (%) = TS % − Fat%

3.5 Sensory Evaluation

The paneer samples were presented to a panel of 10 to 15 trained and semi-trained judges comprising of scientists of LPT Divison and the PG students of F.V.Sc and A.H. The panelists evaluated the coded samples of paneer for appearance, body and texture, flavour and overall acceptability as per 9 point Hedonic scale (Periyam and Pilgrim, 1957).

3 .6 Determination of Antioxidant Activity

3.6.1 ABTS Method (2,2 Azino bis thiazoline 6- sulfonic acid)

For ABTS assay, the procedure followed the method of Arnao *et al.* (2001) with some modifications. The stock solutions included 7.4mM $ABTS^{\bullet+}$ solution and 2.6mM potassium persulfate solution. The working solution was then prepared by mixing the two stock solutions in equal quantities and allowing them to react for 12 h at room temperature in the dark. The solution was then diluted by mixing 1mL $ABTS^{\bullet+}$ solution with 60mL methanol to obtain an absorbance of 1.1±.02 units at 734 nm using the spectrophotometer.

Fresh ABTS$^{\bullet+}$ solution was prepared for each assay. 150µl of paneer extract were allowed to react with 2850µL of the ABTS$^{\bullet+}$ solution for 2 h in a dark condition. Then the absorbance was taken at 734nm using the spectrophotometer (UV-VIS). Radical scavenging percentage was obtained using the formula:

$$\text{Radical scavenging } (\%) = \frac{\text{Absorbance of blank} - \text{Absorbance of sample}}{\text{Absorbance of blank}} \times 100$$

3.8.2 DPPH (2,2–diphenyl –1 –picryl – hydrazyl – hydrate) Method

The DPPH assay was done according to the method of Brand-Williams *et al*. (1995) with some modifications. The stock solution was prepared by dissolving 24 mg DPPH with 100mL methanol and then stored at -20^{0}C until needed. The working solution was obtained by mixing 10mL stock solution with 45mL methanol to obtain an absorbance of 1.1±.02 units at 515 nm using the spectrophotometer (UV-VIS).. 150µl of paneer extract was allowed to react with 2850µl of the DPPH solution for 24 h in the dark. Then the absorbance was taken at 515 nm. The radical scavenging activity was measured using the formula:

$$\text{Radical scavenging } (\%) = \frac{\text{Absorbance of blank} - \text{Absorbance of sample}}{\text{Absorbance of blank}} \times 100$$

3.7 Microbiological Analysis

The paneer which was prepared in the hygienic condition of the laboratory of Divison of LPT, F.V.Sc. and A.H., SKUAST-K, was subjected to microbiological analysis for Standard Plate Count, Coliform Count and Yeast and Mould Count as per the method described by APHA (1993)

Sample prepration and serial dilution

10g of paneer sample was aseptically weighed and transferred to pre-sterilized mortar. A 90 ml volume of sterile 0.9 per cent sodium chloride was added to it and samples were homogenized for 2 minutes using a sterile pestle for uniform dispension getting 10^{-1} dilution. For obtaining 10^{-2} dilution, 1 ml of this diluted solution was transferred to another tube containing 9 ml of sterile 0.9 per cent sodium cholride. This procedure was repeated to obtain 10^{-3} dilution and so on, until appropriate dilution was achieved which yielded plates with 25 to 250 colony forming units (cfu)..These procedures were performed in the sterilized environmental conditions of laminar flow.

3.7.1 Standard Plate Count

For determination of SPC, plate count agar were used. About 23.5 g of it was dissolved in 1000ml of distilled water followed by sterilization in an autoclave at 15 lb pressure (121^0C) for 15 minutes. With the help of sterile pipette about 1ml of inoculum from $10^{-1,}$ 10^{-2} and 10^{-3} dilution was taken and inoculated into a double set of presterlised petridish. Then the inoculum and media in petridish were mixed thoroughly and uniformly by rotating the plates alternatively in clockwise and anticlockwise directions followed by back and forth motion on level surface. When media in plates solidified, they were inverted and incubated aerobically at 32 ± 1^0C for 48±3 hours. The number of micro-organisms per ml/g of sample was calculated by selecting plates containing 25 to 250 cfu/ml or g or selecting plates with count closest to this range. The cfu/g was calculated by using the formula

$$\mathbf{N = \sum C/[(1\times n_1) + (0.1\times n_2)]d}$$

Where,

N = number of colonies per milliliter or gram of product

$\sum C$ = sum of all colonies on all plates counted

n_1 = no of plates in lower dilution counted

sn_2 = no of plates in next higher dilution counted

d = dilution from which the first counts were obtained

Finally, the cfu/g was expressed as log cfu/g of sample

3.7.2 Coliform count

41.53 grams of Violet Red Bile Agar procured from Himedia laboratories Pvt. Ltd. Mumbai was dissolved in 1000 ml of distilled water followed by sterilizing in an autoclave at 15 lb pressure (121^{o}C) for 15 minutes. The 1ml inoculum from 10^{-1}, 10^{-2} and 10^{-3} dilution was inoculated into already sterilized double set of Petri dishes. About 15-20 ml of sterilized media maintained at about 45 ± 1^{o}C was poured in Petri dishes. Sample dilution and media was mixed thoroughly and uniformly by alternate rotation and back and forth motion of plate on level surface. The plates were incubated at 37°C for 24-48 hours. Colonies were counted from the plates. The cfu/g was calculated as above formula and expressed as log cfu/g of sample.

3.7.3 Yeast And Mould Count

39 g of Potato Dextrose Agar (obtained from Hi-media Laboratories Pvt. Limited. Mumbai) was suspended in 1000ml of distilled water, boiled to dissolve the medium completely and sterilized by autoclaving at 15 lbs pressure (121°C) for 15 minutes. The pH of sterilized medium was set to a value of 3.5 by acidifying with 10 ml of 10 per cent tartaric acid. Precaution was taken not to heat the medium after addition of the acid. Pour plate technique was followed for inoculation of suitable sample dilution and plates were incubated at 25°C for 5 days. Colonies appeared on the plates were counted and expressed as log cfu/g.

3.8 Tyrosine Value Determination

Procedure put forward by Strange et al (1977) with slight modifications. Trichloroacetic acid (TCA) extract was prepared by blending 20 g minced paneer with 50ml of precooled 20% TCA solution for 2 minute. After homogenization, the contents were transferred to a beaker by rinsing with 50ml cold distilled water, mixed and filtered through a Whatman's filter paper no. 42. To estimate the tyrosine value, 2.5ml TCA extract was mixed with equal amount of distilled water. The mixture was then blended with 10ml 0f 0.5N sodium hydroxide. Then 3ml of diluted folin and ciocalteau's reagent (1volume concentrated F and C reagent + 2volumes distilled water) added to it and again shaken. The mixture was allowed to stand in dark place at room temperature for 15minute for proper color development. The optical density was measured at 700nm by a spectrophotometer. Tyrosine value was calculated as mg tyrosine per g of paneer by referring to a standard graph, which was prepared as per the procedure described by pearson (1968).

3.9 Thiobarbituric Acid (TBA) Value Determination

Procedure followed was Strange et al 1977 with slight modifications. 20g paneer sample was blended with TCA .Temperature was maintained at 4^0 C. The slurry made was transferred to 100ml volumetric flask with 40 ml distilled water. Volume was made 100ml and shaking done. Half of this was transferred through filter paper whatman paper no.1. 5ml of this filterate transferred to test tube to which 5ml of 2-TBA was added. Tube was stoppered and inversion was done . then the test tube was kept at dark for 15 hours. Absorbance was measured at 530nm (UV-spectrophotometer). Value was obtained by multiplying the factor 5.2.

3.10 Statistical Analysis

The data from duplicate samples were averaged and the data so generated were analyzed statistically following the method of Snedecor and Cochran (1980) and Gomez and Gomez (1984). The data was processed in a computer using

SPSS version 20 software package. The analysis of variance of group mean was computed and significance of means tested by using least significance difference test of 5 per cent level of significance One way and two way analysis of variance with all possible interactions was carried out. The nested means were compared when the interaction was found to be significant. In the absence of such significance the overall means were compared.

Chapter – 4

EXPERIMENTAL FINDINGS

For the purpose of preparation of functional paneer, nine trials were conducted. In order to ascertain the physico-chemical, sensory and antioxidant characteristics six trails were conducted. Control sample and samples selected on the basis of physico-chemical, sensory and functional characteristics were subjected to shelf life determination on weekly intervals for one month. For estimation of shelf life, that included sensory evaluation, microbiological examination, TBARS and tyrosine value determination, three trials were conducted. The results which were obtained from the experiments are presented below. The tabular and graphical representation of the data has been made with the aim of making the text easily comprehensible.

4.1 Milk Characteristics

The milk used for paneer manufacture in all trails was cow milk with an average fat content of 4.02±0.06 and SNF content of 8.42±0.03.. The levels of total solids, protein, pH, titratable acidity and electrical conductivity were found with the mean value of 12.56±0.04, 3.67±0.08, 6.59±0.37, 0.13±0.25 and 0.005S. These values are within the prescribed limits of FSSA, 2006.

4.2 Phsico-Chemical Characteristics of Water Chestnut FortifiedPaneer

The data pertinent to the study related to the effect of water chestnut on physico-chemical characteristics of paneer is presented in table 3 and graphically depicted in Figs 1, 2 and 3. As is evident from the table, the moisture content showed a significant ($p \leq 0.05$) increase from control through T_5. The moisture percentage for T_5 appeared highest whereas control sample had the lowest value .The overall mean was of the order of 60.64±0.86. The fat content of all the treatments was comparable with the control and did not reveal any significant

difference. The overall mean was to the tune of 19.68±0.19.As inferred from data in the table under discussion, the protein content in control samples had significantly lower values compared to all test samples, the latter in turn did not differ significantly from one another ($p > 0.05$). The overall mean was found to be 18.95±0.21. The ash content of control samples was lowest among all samples and was comparable with T_2.however, there was significant increase in ash content of T_3, T_4 and T_5 as compared to control. The overall mean was found to be 2.32±0.062. The pH value revealed a significant decrease in value of other test samples as compared to control. But the values for T_3, T_4 and T_5 showed no significant difference. The "fat loss in whey" of control varied significantly ($p \leq 0.05$) from other treatments, however the latter did not vary significantly among themselves. The overall mean value obtained was 1.28±0.008. From the table 3 and Fig.3, it is clear that yield of water chestnut fortified samples(T_2, T_3, T_4 and T_5) showed a significant ($p \leq 0.05$) increase as compared to control. But within the treatments the values were comparable. The overall mean was of the order of 21.95±0.26.

Table 3: Physico-chemical characteristics of paneer fortified with varying levels of Water chestnut

Parameters	Treatments (Levels of Water chestnut in paneer)					Overall mean
	Control (T_1)	0.25% (T_2)	0.5%(T_3)	0.75%(T_4)	1.0% (T_5)	
Moisture (%)	53.74±0.58^{a}	57.57±0.32^{b}	61.68±0.18^{c}	63.68±0.40^{d}	66.53±0.67^{e}	**60.64±0.86**
Fat (%)	20.10±0.45^{a}	19.99±0.12^{a}	19.77±0.29^{a}	19.65±0.47^{a}	18.88±0.56^{a}	**19.68±0.19**
Protein (%)	17.21±0.19^{a}	18.91±0.25^{b}	19.23±0.33^{b}	19.60±0.37^{b}	19.70±0.39^{b}	**18.95±0.21**
Ash (%)	1.98±0.04^{a}	2.18±0.08^{ab}	2.33±0.13^{bc}	2.49±0.13^{bc}	2.62±0.122^{c}	**2.32±0.062**
pH	5.36±0.08^{c}	4.82±0.04^{b}	4.60±0.05^{a}	4.55±0.01^{a}	4.39±0.07^{a}	**4.74±0.06**
Fat in whey (%)	1.21±0.016^{a}	1.28±0.016^{b}	1.3±0.01^{b}	1.3±0.01^{b}	1.31±0.016^{b}	**1.28±0.008**
Yield (%)	20.06±0.22^{a}	21.20±0.40^{b}	22.28±0.25^{c}	22.58±0.39^{c}	23.65±0.32^{d}	**21.95±0.26**

Means± SE, row wise with common superscripts, do not differ significantly ($p > 0.05$).

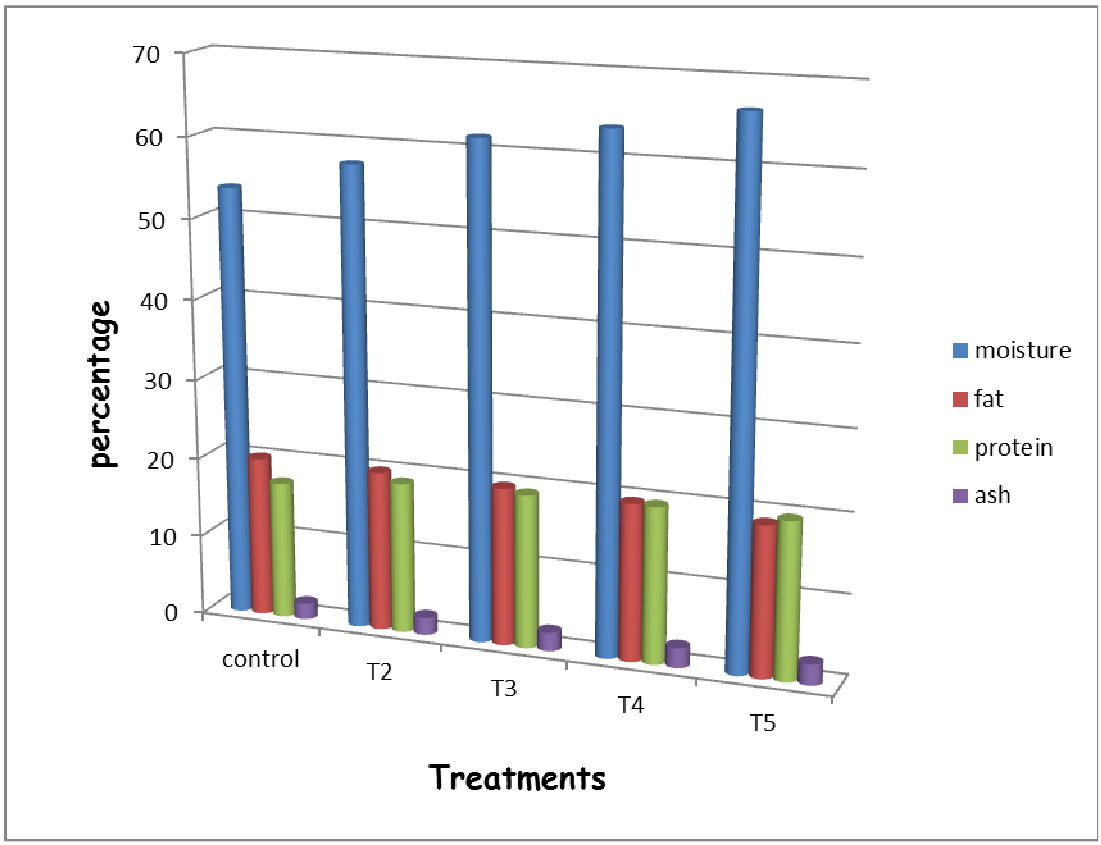


Fig No. 1: Phsico-Chemical Characteristics of Water Chestnut Fortified Paneer

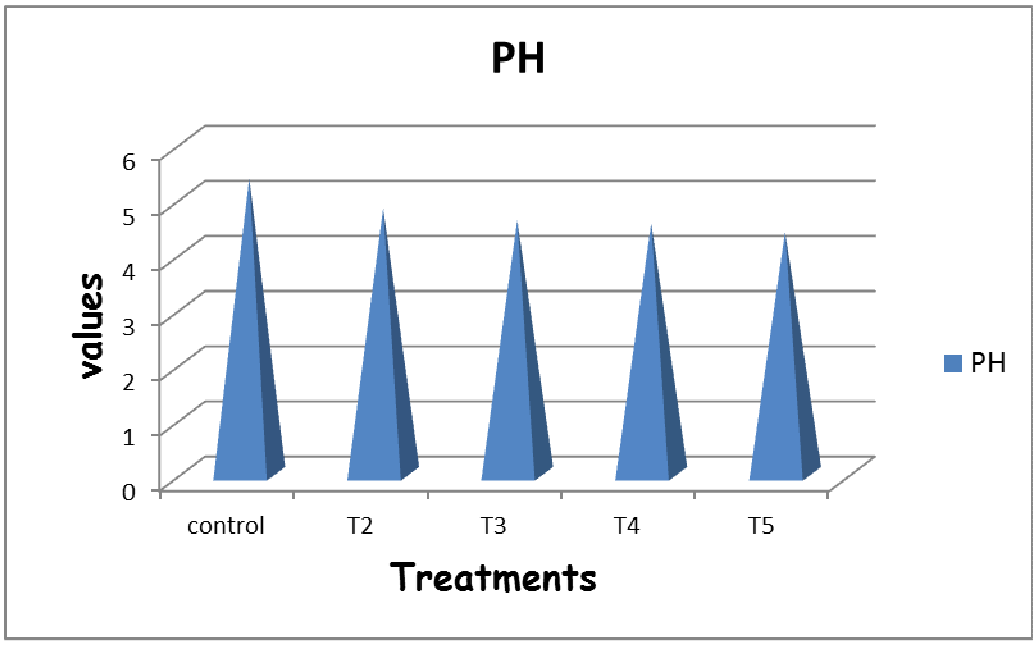


Fig.No. 2: Effect of varying level of water chestnut on pH of paneer

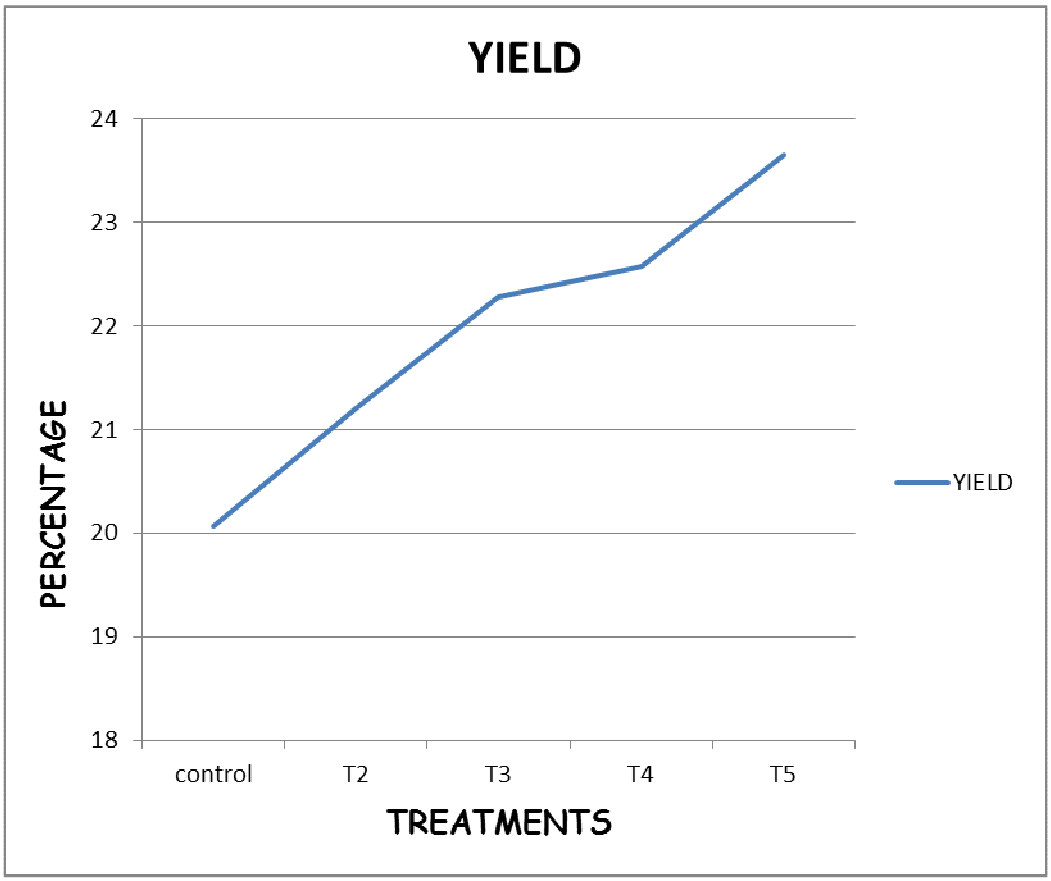


Fig.No. 3: Effect of various levels of water chestnut on yield of paneer

4.3 Sensory Characteristics of FunctionalPaneer

The data related to the effect of varying levels of water chestnut on sensory quality of paneer is displayed in table 4 and graphically presented in Fig 4. The scores with regard to the sensory attributes like appearance, flavour, body and texture and overall acceptability showed increasing trend from control to 0.25% water chestnut level. Thereafter, a decreasing trend was followed from T_3 to T_5. As can be judged from the table and the graph, the T_2 showed the remarkable increase of scores for appearance as compared to control while there was marked reduction in scores for T_4 and T_5. The overall mean was to the tune of 7.47±0.15. The flavour scores of T_2 and T_3 were comparable with thecontrol. But the scores of T_4 and T_5showed significant reduction compared to other test samples. The overall mean was of the order of 7.22±0.14. The body and texture score showed a comparable value for control, T_3 and T_4. The T_2illustrated eminent increase in score and T_5 showed conversely the marked decrease, but both differed significantly from each other and also from the control. The overall mean was 7.25±0.13. The overall acceptability of control samples was comparable with T_3 and varied significantly with the T_2, T_4 and T_5. The latter also differed significantly when compared with one another. The overall mean was of the order of 7.5±0.13.

Table 4: Sensory quality of paneer with varying levels of Water chestnut

Treatments (Levels of Water chestnut in paneer)						
Parameters*	**Control (T_1)**	**0.25% (T_2)**	**0.50% (T_3)**	**0.75% (T_4)**	**1% (T_5)**	**Overall mean**
Appearance	7.85 ± 0.177^{c}	8.57 ± 0.17^{d}	8.21 ± 0.18^{cd}	6.92 ± 0.24^{b}	5.78 ± 0.26^{a}	**7.47±0.15**
Flavour	7.57 ± 0.13^{c}	8.14 ± 0.17^{c}	8.00 ± 0.20^{c}	6.71 ± 0.16^{b}	5.71 ± 0.30^{a}	**7.22±0.14**
Body and texture	7.5 ± 0.13^{bc}	8.14 ± 0.20^{d}	7.92 ± 0.19^{cd}	7.04 ± 0.17^{b}	5.57 ± 0.20^{a}	**7.25±0.13**
Overall acceptability	7.85 ± 0.14^{c}	8.5 ± 0.20^{d}	8.14 ± 0.17^{cd}	7.07 ± 0.07^{b}	5.92 ± 0.22^{a}	**7.5±0.13**

Means± SE, row wise with common superscripts, do not differ significantly ($p > 0.05$).

*9- point Hedonic scale (9= like extremely, 1= dislike extremely)

4.4 Characteristics of Functional Paneer

The characteristics of functional paneer are shown in table 5 by means of DPPH and ABTS methods. In both the methods the ability of a product to scavenge the free radical is determined and expressed in terms of percentage. From the table 5, it is evident that incorporation of water chestnut in paneer resulted in the increase in scavenging activity. The control showed an inherent scavenging activity with average values of 16.27±1.36 (ABTS) and 13.07±0.58 (DPPH). The various treatments showed significant increase in scavenging activity as compared to the control. The overall mean was to the tune of 42.62±3.72 (ABTS) and 39.60±3.92 (DPPH).

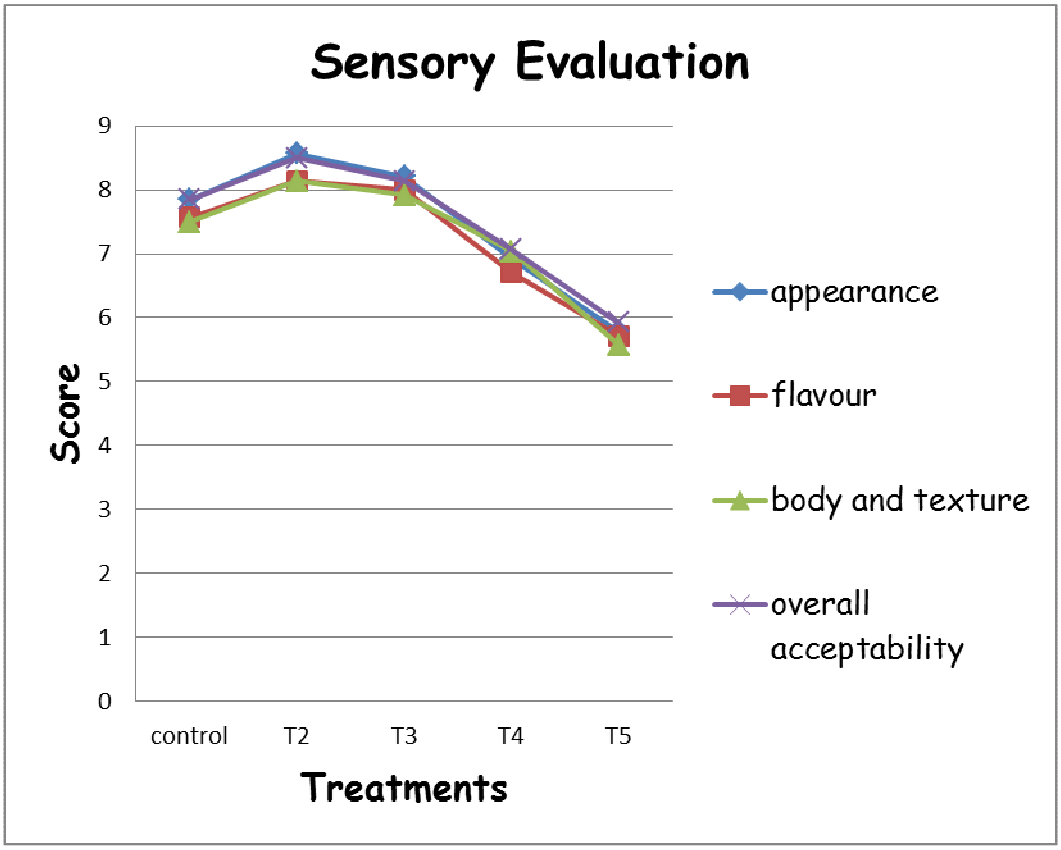


Fig.No. 4 Sensory score of paneer with varying levels of Water chestnut

Table 5: Functional characteristics of paneer with water chestnut added at varying levels.

Parameter	(Treatments) Water chestnut levels in paneer					Overall mean
	Control (T_1)	**0.25% (T_2)**	**0.50% (T_3)**	**0.75% (T_4)**	**1% (T_5)**	
ABTS	16.27±1.36[a]	44.39±2.39[b]	45.84±2.18[b]	51.65±1.14[c]	54.95±0.69[c]	**42.62±3.72**
DPPH (%)	13.07±0.58[a]	36.39±1.46[b]	43.74±1.20[c]	51.66±2.02[d]	53.17±0.71[d]	**39.60±3.92**

Means± SE, row wise with common superscripts, do not differ significantly ($p > 0.05$).

4.5 Sensory Characteristics of Functional Paneer duringStorage at 4±1^{0}C

Based on the sensory, physio-chemical and functional characteristics two treatments T_2 and T_3 with 0.25% and 0.5% water chestnut levels repectively, were selected and the latter along with the control were analysed for sensory evaluation and other shelf life characteristics i.e. Microbiological assay, TBARS, Tyrosine value at 4±1^{0}C on weekly intervals up to 28 days storage period.

On day 0, appearance score of T_3 appeared comparable with control while there was a remarkable increase ($p \leq 0.05$) in appearance score of T_2 in comparison to control sample. The same trend was observed on day 7th. But on 14th and 21st day, there occurred a significant decrease in scores of control as compared toT_2 and T_3. Sensory evaluation was not conducted on 28th day owing

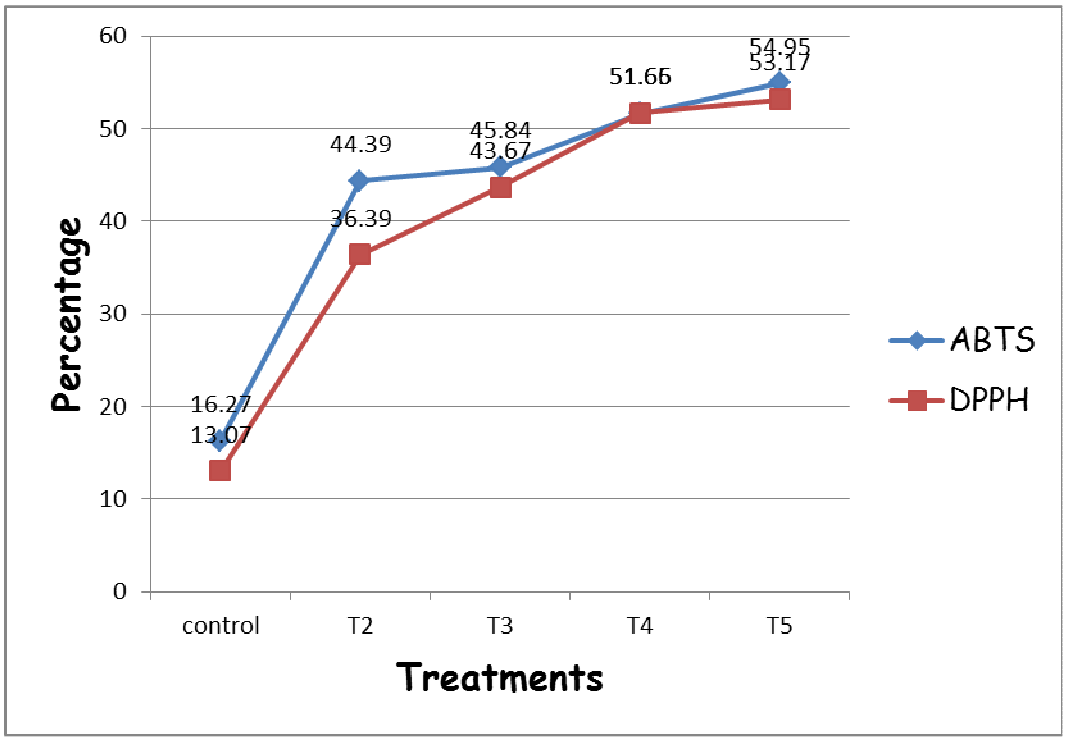


Fig No. 5: Radical scavenging percentage of different paneer samples

to frank deteriorative changes. There was a progressive reduction in all samples from day 0 to 21st day.

The flavour scores on day 0 were more or less similar in all test samples. However, onday 7, 14, 21 the control sample revealed drastic reduction in flavour scores as compared to other treatments. On 21st day, the control sample showed a score of4.04±0.18 that signified “Dislike slightly” on hedonic scale. While the flavour score of both T_2 and T_3 was6.07±0.17 that amounts to “Like slightly” on hedonic scales. In all the treatments there was an observable significant decrease in score from day 0 to 21st day.

In case of body and texture, remarkable increase in score was obtained in T_2 and T_3 on day 0,as compared to control. Similar trend was observed on day 7, 14 and 21 with control sample revealing marked reduction in the score as compared to other treatments. The body and texture scores in all treatments were found to show significant decrease from day 0 to 21st day. However, the control sample depicted “Dislike moderately” score on hedonic scale whereas it was “Like slightly” for other test samples on 21st day.

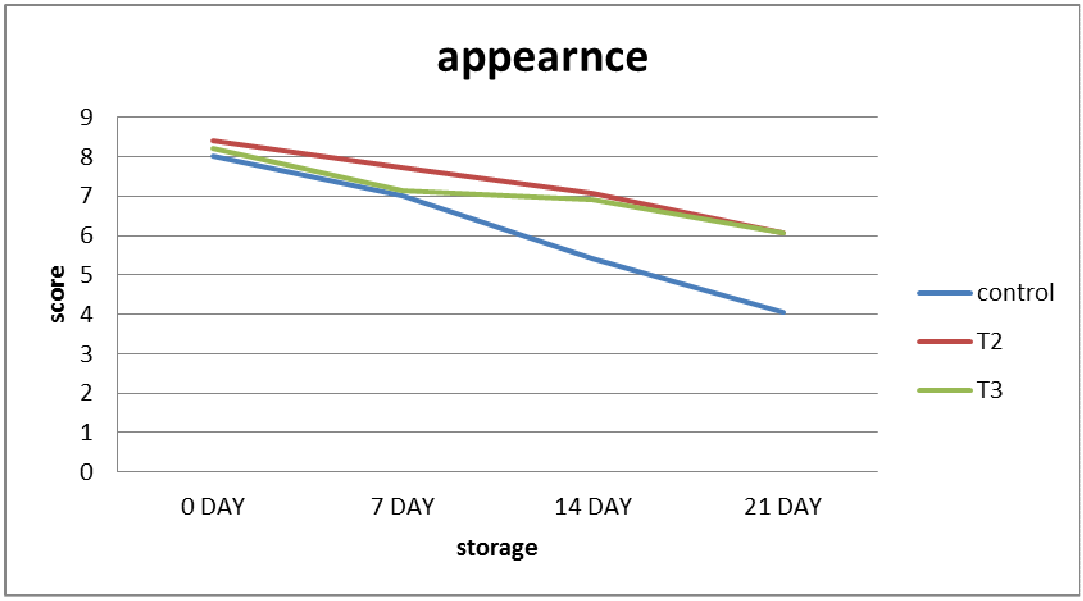
The overall acceptability scores of control,T_2 and T_3, on day 0 were comparable and did not differ significantly. On 7th day, control sample had significantly lower score as compared to other treatments. Similar was the trend followed on 14th and 21st days, with control showing marked deterioration than T_2 and T_3 samples. There was significant decrease in overall acceptability values of control,T_2 and T_3 samples from day 0 to 21st day. But the control showed extreme reduction in score as compared to other treatments.

Plate 1: Functional paneer product samples presented for sensory evaluation

Table 6: Sensory evaluation of paneer with selected levels of water chestnut during storage at 4±1^{0}C

Treatment	STORAGE PEROID (DAYS) 0	7TH	14TH	21st	28TH	Overall Mean
APPEARANCE						
Control	7.78±0.18^a	7.07±0.19^a	5.42±0.17^a	3.94±0.18^a	NC	**4.86±0.19**
0.25% (T_2)	8.42±0.17^b	7.71±0.19^b	7.07±0.16^b	6.04±0.17^b	NC	**5.85±0.18**
0.5% (T_3)	8.14±0.17^{ab}	7.42±0.17^{ab}	7.00±0.14^b	5.92±0.17^b	NC	**5.72±0.16**
Overall mean	**8.11±0.10**	**7.40±0.11**	**6.5±0.14**	**5.38±0.10**		
FLAVOUR						
Control	8.00±0.15^a	7.01±0.23^a	5.42±0.17^a	4.04±0.18^a	NC	**4.89±0.19**
0.25% (T_2)	8.42±0.17^a	7.71±0.19^b	7.07±0.16^b	6.07±0.17^b	NC	**5.85±0.18**
0.5% (T_3)	8.21±0.18^a	7.14±0.14^b	6.92±0.12^b	6.07±0.17^b	NC	**5.66±0.18**
Overall mean	**8.21±0.18**	**7.27±0.10**	**6.47±0.14**	**5.38±0.10**		
BODY AND TEXTURE						
Control	7.78±0.21^a	7.01±0.23^a	5.22±0.17^a	3.94±0.18^a	NC	**4.81±0.19**
0.25% (T_2)	8.5±0.17^b	7.78±0.19^b	7.14±0.16^b	6.14±0.17^b	NC	**5.91±0.18**
0.5% (T_3)	8.35±0.19^b	7.57±0.14^b	7.07±0.12^b	6.07±0.17^b	NC	**5.81±0.18**
Overall mean	**8.21±0.12**	**7.43±0.10**	**6.54±0.14**	**5.45±0.10**		
OVERALL ACCEPTABILITY						
Control	8.00±0.13^a	7.07±0.21^a	5.64±0.21^a	4.04±0.18^a	NC	**4.95±0.18**
0.25% (T_2)	8.50±0.17^a	7.78±0.17^b	7.14±0.17^b	6.14±0.17^b	NC	**5.91±0.18**
0.5% (T_3)	8.21±0.18^a	7.50±0.19^b	7.07±0.19^b	6.07±0.17^b	NC	**5.77±0.18**
Overall mean	**8.23±0.08**	**7.43±0.12**	**6.61±0.12**	**5.45±0.10**		

Means± SE, row wise (small letters) and column wise (capital letters) with common superscripts, do not differ significantly ($p > 0.05$).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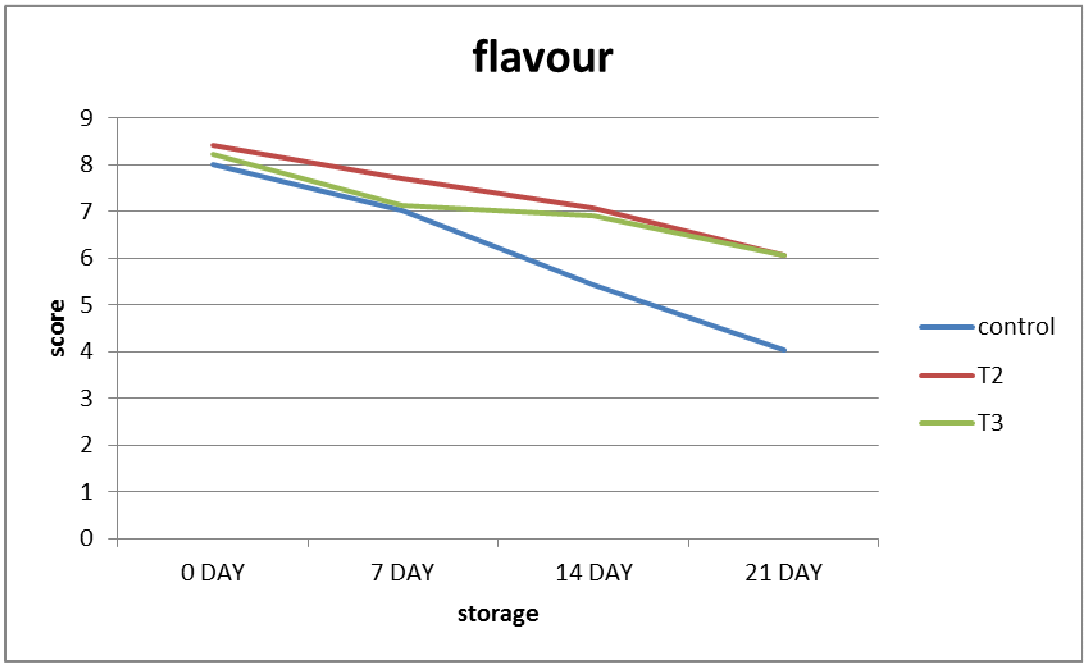
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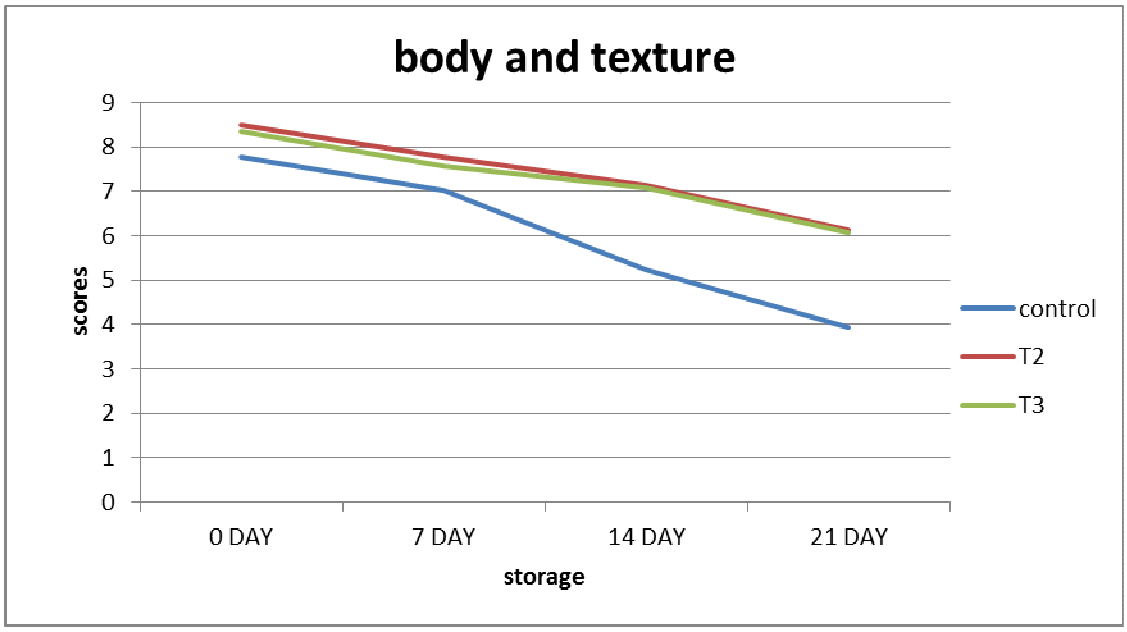

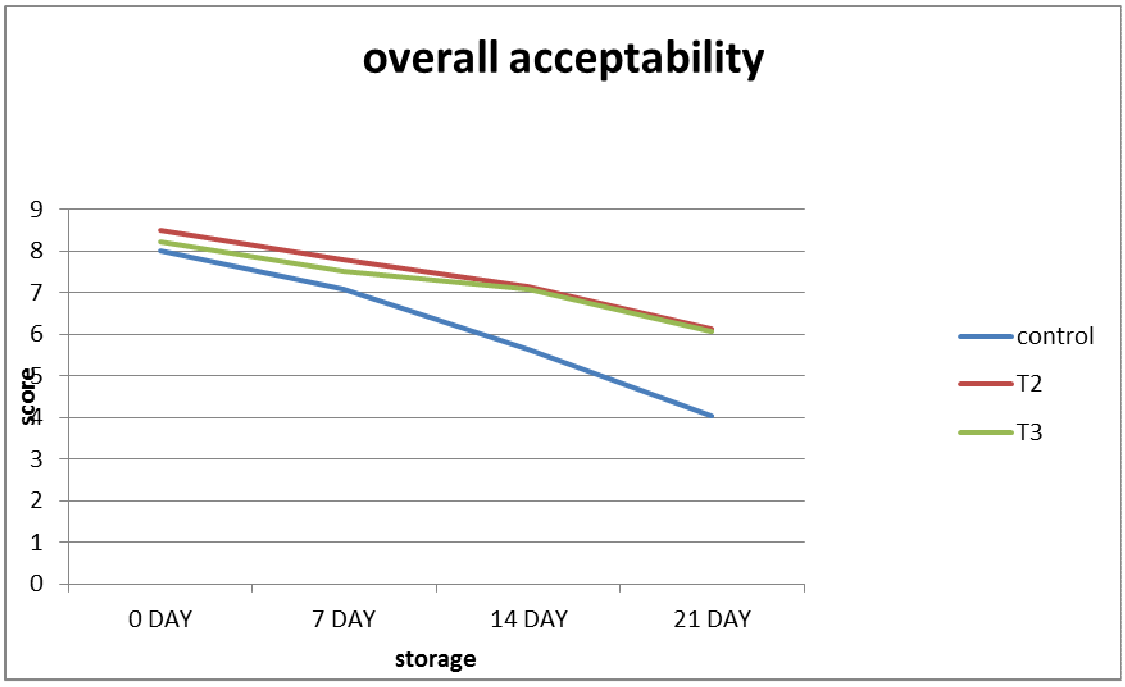

Fig. No.6: Sensory scores of samples stored at refrigerator temperature (4±1 °C)

4.5 Microbiological Analysis

Microbiological analysis for selected samples T_1 and T_2 along with the control was carried out on 0, 7^{th}, $14^{th,}$ 21^{st} and 28^{th}day. The count obtained was compared with prescribed limits to assess quality and effect of water chestnut onshelf life of functional paneer. The SPC count, Yeast and Mould count and Coliform count of test samples was determined.

Standard plate count of functional paneer is shown in Table 7 and Fig 7. On day 0, SPC count of various treatments was comparable and did not show any significant difference. The overall mean was of the order 1.81±0.04logcfu/g. On day 7^{th}, there was a significant decrease in SPC count of T_2 and T_3 as compared to control. On day 14, 21 and 28 the SPC count values among all treatments were comparable. The overall mean of 3.29±0.07, 4.16±0.15 and4.72±0.07 logcfu/g SPC count was observed on 14^{th}, 21^{st} and 28^{th} day respectively. Within all the treatment groups, there was a significant increase in SPC count from day 0 to $28^{th.}$ The overall mean of SPC count for control, T_2 and T_3 was3.32±0.24, 3.26±0.273.26±0.28logcfu/grespectively.

Table 7: Standard Plate Count of water chestnut fortified paneer during storage at 4±1^{0}C (log cfu/g).

	Storage Period					
Treatment	**0 day**	**7 day**	**14 day**	**21 day**	**28 day**	**Overall mean**
Control (T_1)	$1.82±.05^{Aa}$	$2.59±0.13^{Bb}$	$3.33±0.09^{Ac}$	$4.18±0.09^{Ad}$	$4.72±0.04^{Ae}$	**3.32±0.24**
0.25% (T_2)	$1.80±0.06^{Aa}$	$2.37±0.14^{Ab}$	$3.28±0.04^{Ac}$	$4.15±0.10^{Ad}$	$4.72±0.06^{Ae}$	**3.26±0.27**
0.5% (T_3)	$1.83±0.04^{Aa}$	$2.35±0.09^{Ab}$	$3.28±0.06^{Ac}$	$4.16±0.06^{Ad}$	$4.71±0.05^{Ae}$	**3.26±0.28**
	$1.81±0.04^{A}$	**2.44±0.13**	**$3.29±0.07^{A}$**	**4.16±0.15**	**4.72±0.07**	

Means± SE, row wise (small letters) and column wise (capital letters) with common superscripts, do not differ significantly ($p > 0.05$).

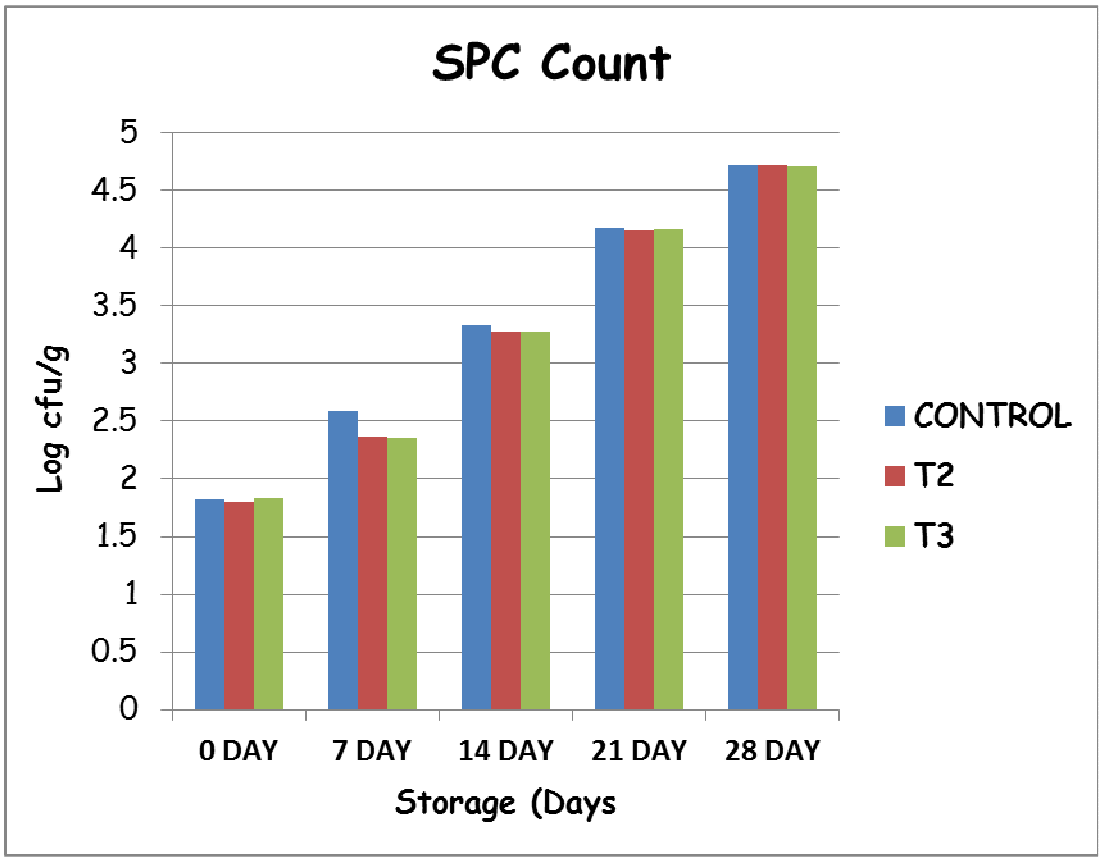

Fig. No. 7: SPC count of samples stored at refrigerator temperature (4±1 °C)

Coliform count of control and selected experimental samples is shown in Table 8 and Fig 8. On day 0, Coliform count was not detected in any treatment. While on 7thday, the count for control was 1.20±0.09 log cfu/g. There was significant decrease in counts of T_2 and T_3as compared to control. Similar was the trend on day 14, 21and 28, the selected treatments showing significantly lower count than control. Within all the treatment groups there was a significant increase from day 7th to 28th day.

Table 8: Coliform Count of fortified paneer during storage at 4±1^{0}C (log cfu/g).

Treatment	Storage Period					Overall mean
	0 day	7 day	14 day	21 day	28 day	
Control (T_1)	NDN*	1.20±0.09^{Bb}	1.88±0.09^{Bc}	2.56±0.01^{Bd}	3.40±0.03^{Be}	**1.80±0.32**
0.25% (T_2)	NDN*	0.96±0.02^{Ab}	1.28±0.03^{Ac}	1.89±0.05^{Ad}	3.11±0.01^{Ae}	**1.44±0.30**
0.5% (T_3)	NDN*	0.92±0.01^{Ab}	1.24±0.05^{Ac}	1.90±0.03^{Ad}	3.03±0.02^{Ae}	**1.39±0.29**
Overall mean	NDN*	**1.02±0.05**	**1.89±0.07**	**2.63±0.04**	**3.18±0.05**	

*NDN = Not detectable numbers

Means± SE, row wise (small letters) and column wise (capital letters) with common superscripts, do not differ significantly ($p > 0.05$).

The Yeast and Mould count was not detected in any of the test samples upto 14th day. On 21st day, the count was comparable among different treatments. Similar was the trend with no significant difference in Yeast and Mould counts of control,T_2 and T_3 on day 28. However, in allthe treatment groups there was a significant increase in count from day 21 to 28thday.

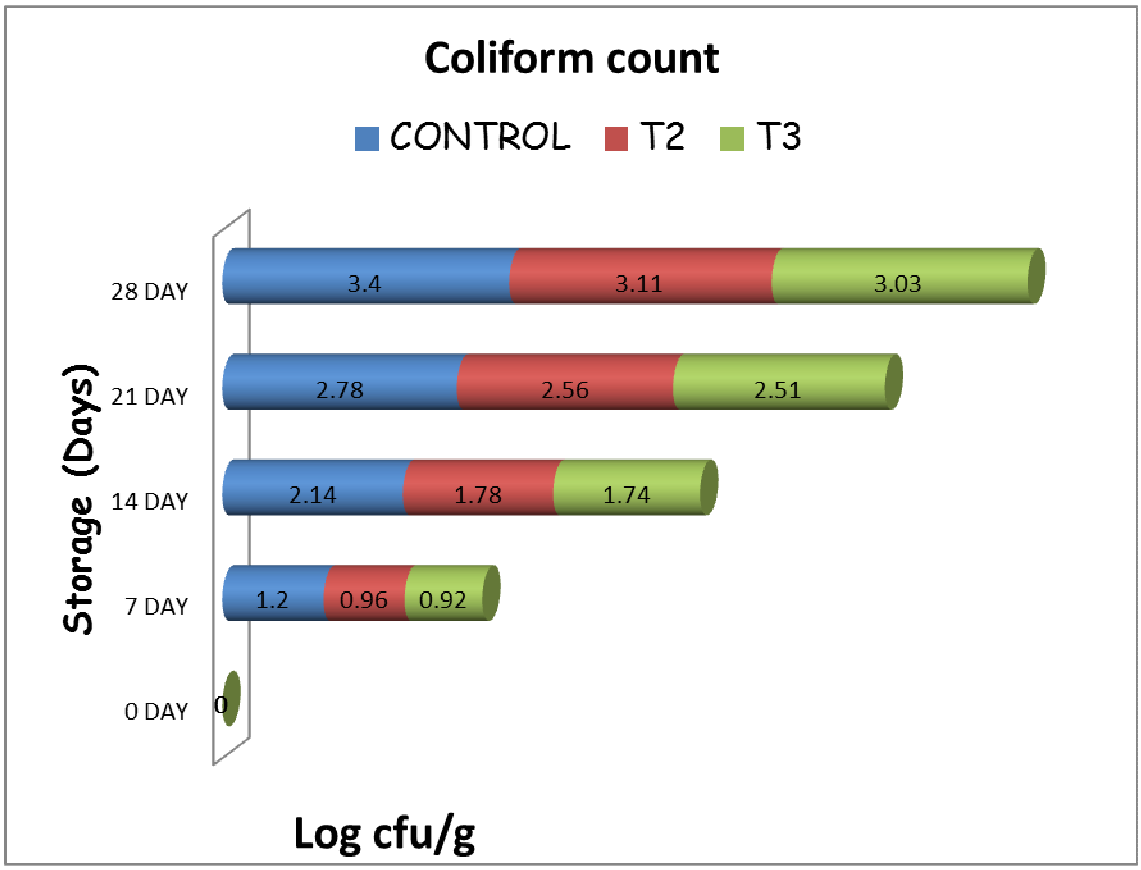

Fig. No. 8: Coliform count of samples stored at refrigerator temperature (4±1 °C)

Table 9: Yeast and Mould Count of water chestnut fortifiedpaneer during storage at 4±1^{0}C (log cfu/g)

Treatment	Storage Period					Overall mean
	0 day	7 day	14 day	21 day	28 day	
Control (T_1)	NDN*	NDN*	NDN*	1.87±0.02^{Ab}	2.26.07^{Ac}	**0.82±0.27**
0.25% (T_2)	NDN*	NDN*	NDN*	1.83±0.03^{Ab}	2.24±0.06^{Ac}	**0.82±0.27**
0.5% (T_3)	NDN*	NDN*	NDN*	1.85±0.04^{Ab}	2.25±0.07^{Ac}	**0.82±0.27**
Overall mean	NDN*	NDN*	NDN*	**1.85±0.01**	**2.25±0.03**	

*NDN = Not detectable numbers

Means± SE, row wise (small letters) and column wise (capital letters) with common superscripts, do not differ significantly ($p > 0.05$).

4.6 TBARS Value

TBARS values obtained for control and fortified samples is shown in the Table 10 and Fig 10 . The degree of lipid peroxidation produces primary products as hydroperoxides which get converted to some secondary products mostly carbonyl compounds e,gmalonaldehyde. These carbonyl compounds react with thiobarbituric acid forming conjugate which is detected by a spectrophotometer. The values obtained are expressed inmg malonaldehyde per kg of the product. On day 0, the values obtained were comparable among the all test samples with overall mean of 0.30±0.05 mg MDA/Kg. On day 7, there was significant decrease in TBARS value of T_2 and T_3 as compared to control. On 14th, 21st and 28thday,

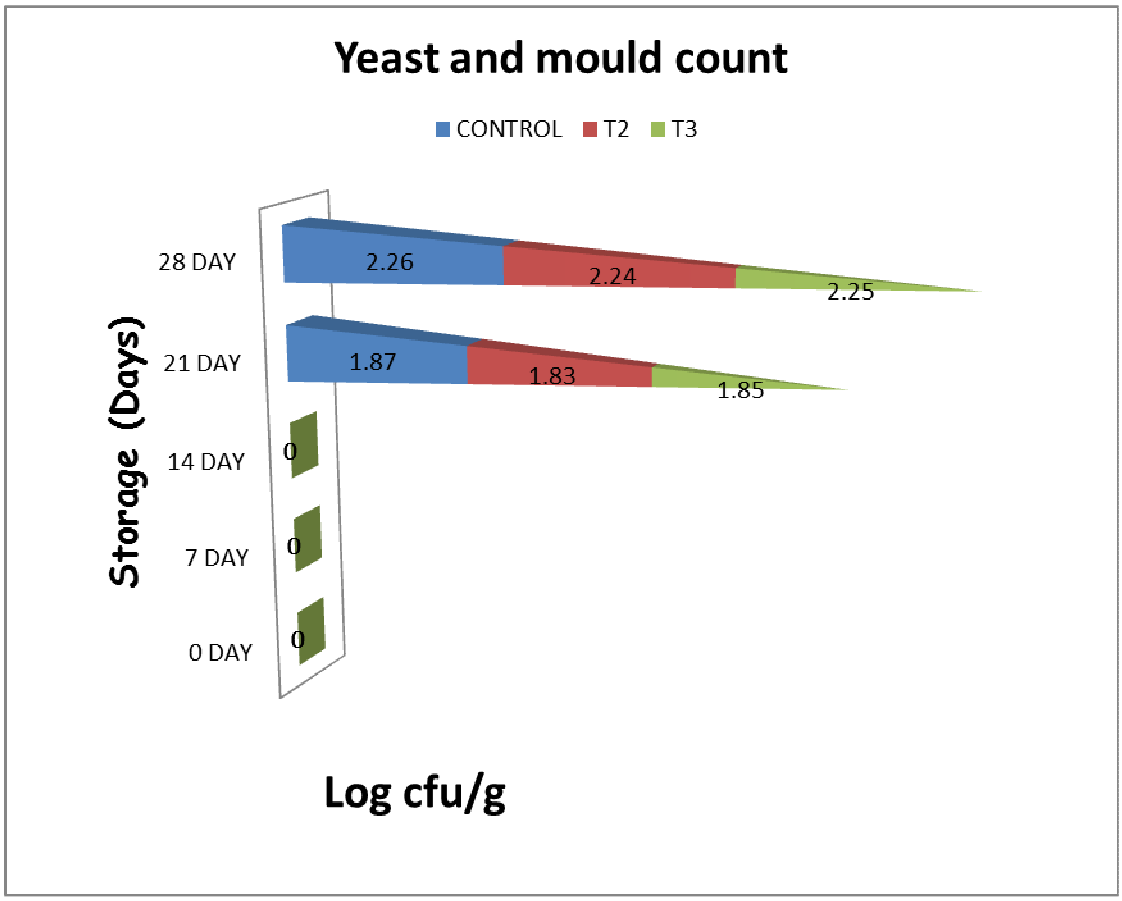

Fig. No. 9: Yeast and Mould count of samples stored at refrigerator temperature (4±1 °C)

the values for all the treatments were comparable. The overall mean of control, T_2 and T_3were 1.00±0.04, 1.39±0.02 and 1.61±0.02mg MDA/Kg repectively. Within the test samples the TBARS value showed a significant increase from day 0 to 28th day.

Table 10: TBARS Value of paneerfortified with water chestnut during storage at 4±1^{0}C. (mg of malonaldehyde per kg)

Treatment	Storage Period					Overall mean
	0 day	7 day	14 day	21 day	28 day	
Control (T_1)	0.47±0.05^{a}	0.86±0.02^{Ab}	1.12±0.02^{Ac}	1.43±0.05^{Ad}	1.79±0.05^{Ae}	**1.14±0.12**
0.25% (T_2)	0.45±0.04^{a}	0.68±0.12^{Ab}	0.98±0.06^{Ac}	1.25±0.04^{Ad}	1.54±0.02^{Ae}	**0.93±0.13**
0.5% (T_3)	0.47±0.02^{a}	0.65±0.10^{Ab}	0.92±0.08^{Ac}	1.25±0.02^{Ad}	1.51±0.01^{Ae}	**0.90±0.13**
Overall mean	0.46±0.05^{A}	**0.73±0.05**	**1.00±0.04**	**1.39±0.02**	**1.61±0.02**	

Means± SE, row wise (small letters) and column wise (capital letters) with common superscripts, do not differ significantly (p > 0.05).

4.7 Tyrosine Value

Tyrosine value is an estimation of hydrolysis of proteins. There is either bacterial proteolysis or proteolysis due to inherent tissue enzymes in the product. Therefore tyrosine value is an indicator for spoilage. However with advanced spoilage it becomes less sensitive. Tyrosine value is shown in the Table 11. From the table it is quite evident that the control sample was comparable with other treatments (T_2 and T_3) and did not show any significant variation on 0, 7th, 14th, 21st and 28thday . The value of control showed a marked increase from 0.12±0.005 mg of tyrosine per gram of product on day 0 to 0.62±0.01 on day 28th. Similar inclination was obtained for other treatments with a progressive increase in tyrosine value from day 0 to 28th day.

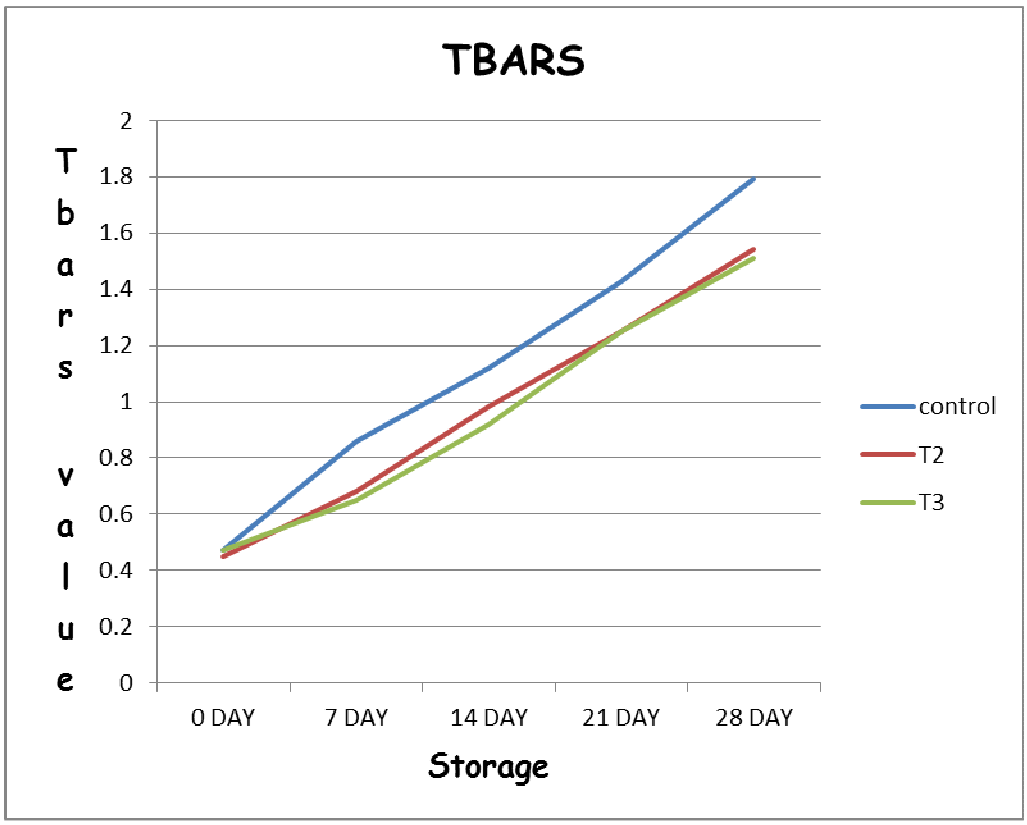

Fig.No.10; TBARS Value of paneer fortified with water chestnut during storage at 4±1^{0}C

Table 11:Tyrosine Value of water chestnut fortified Paneer during storage at 4±1^{0}C(mg of tyrosine per gram)

	Storage Period					
Treatment	**0 day**	**7 day**	**14 day**	**21 day**	**28 day**	**Overall mean**
Control (T_1)	0.12±0.005^{Aa}	0.20±0.008^{Ab}	0.35±0.018^{Ac}	0.47±0.017^{Ad}	0.62±0.01^{Ae}	**0.35±0.04**
0.25% (T_2)	0.10±0.008^{Aa}	0.17±0.015^{Ab}	0.33±0.012^{Ac}	0.46±0.020^{Ad}	0.62±0.03^{Ae}	**0.34±0.03**
0.5% (T_3)	0.10±0.011^{Aa}	0.16±0.01^{Ab}	0.30±0.008^{Ac}	0.44±0.027^{Ad}	0.60±0.006^{Ae}	**0.30±0.03**
Overall mean	**0.10±0.005**	**0.18±0.009**	**0.27±0.020**	**0.39±0.023**	**0.53±0. 027**	

Means± SE, row wise (small letters) and column wise (capital letters) with common superscripts, do not differ significantly ($p > 0.05$).

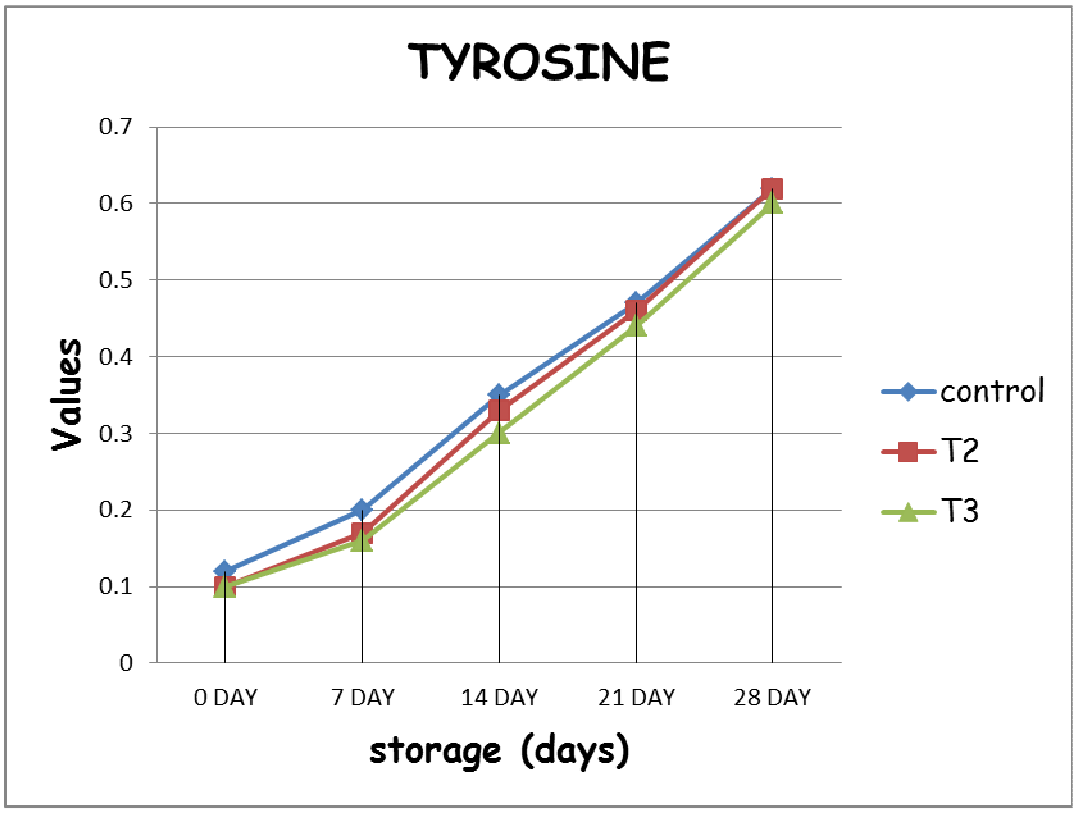

Fig. No.11: Tyrosine Value of samples stored at refrigerator temperature (4±1°C)

Chapter - 5

DISCUSSION

5.1 Milk Characteristics

The cow milk with an average fat content of 4.02±0.06 and SNF content of 8.42±0.03 was used for manufacture of paneer. The levels of total solids and protein were found to be with the mean values of 12.56±0.04 and 3.67±0.08 respectively. These values are well within the prescribed limits of FSSA, 2006. The average TA of milk samples was 0.13±0.25 which falls within the normal limits for cow milk (Sherbon,1999; Jay *et al*.,2005 and Walstra *et al*., 2006). The pH of milk samples was recorded with an overall mean of 6.59±0.37 which is within the range found normally in cow milk (Sherbon,1999; Jay *et al*.,2005 and Walstra *et al*., 2006). The electrical conductivity obtained was on an average 0.005S. A similar phenomenon was noted by Henningson *et al*., (2005), who observed that the EC is proportional to the milk concentration. The probable increase could be proportionate increase in the concentration of minerals and activity of ions in the milk with the increase in the TS content of milk which are responsible for conducting electric current in liquid medium.

5.2 Phsico-Chemical Characteristics Of Water Chestnut Fortified Paneer

In this phase different levels of water chestnut were used in raw milk in order to obtain the desired quality characteristics in paneer manufactured. The moisture content of different samples showed a significant change. When moisture content of experimental paneer was compared with that of control (T_1), all the treated paneer samples showed significantly ($p< 0.05$) higher moisture retention. Further, the moisture retention was found to be increasing concommitantly with the increase in the level of water chestnut. The higher moisture content with increase in latter could be attributed to higher water

binding capacity of water chestnut. The values obtained in all test samples except control (comparable) were higher than obtained by Rani *et al.* (2014) during the study of effect of oil based pickling on shelf life of paneer but closer to the values obtained by (Jadhavar *et al*., 2009; Pal *et al*., 1996 and Sachdeva *et al*., 1991). Sachdeva and Singh (1988) reported increase in the moisture retention and yield of paneer by using hydrocolloids such as CMC (0.1 to 0.2%), sodium alginate, carrageenan and starch with different levels. Roy and Singh (1994) also reported increase in moisture retention and yield of paneer where hydrocolloids were used. Differences in fat content of paneer due to different treatments were statistically non-significant. The value of control was comparable with the all other test samples. Numerically, slight reduction in fat content was observed from T_1 to T_5. The fat content on dry matter basis helps to compare the paneer of different groups more accurately as it eliminates the moisture variation. The values for fat content in control samples and other test samples observed in our study are in agreement with those reported by Sachdeva *et al*. (1991) in cow milk paneer. The values obtained were lesser than observed by Rani *et al.* (2014) for whole milk with 6% fat and 8.5% SNF. The moisture and fat content of the paneer is influenced by several factors such as composition of milk (Bhattacharya *et al.*, 1971; Sachdeva *et al*., 1985; Vishweshwaraiah and Anantakrishna, 1986; Singh and Kanawjia, 1988;, temperature of coagulation (Bhattacharya *et al.*, 1971; Sachdeva *et al.*, 1985; Singh and Kanawjia, 1988; Sachdeva *et al.*, 1988; Sachdeva and Singh, 1988; Singh and Kanawjia, 1991; Roy and Singh, 1994). The protein content varied from 17.21±0.19 to 19.70±0.39. The control sample showed significantly lower value as compared to others. The results for the control sample agree favourably with those of Jadhavar *et al*. (2009); Pal *et al*. (1996) and Sachdeva *et al*. (1991) for cow milk paneer. However, the protein content values of all other samples were comparable and were close to that reported by Singh and Kanawjia (1992) who reported no specific trend in protein content of paneer in response to added calcium chloride as additive at different

levels. Sachdeva and Singh (1988) found increased protein content in paneer with the decrease in milk fat content. Further the slight increase in protein content of water chestnut fortified samples can also be attributed to the incorporation of water chestnut which might have enhanced the protein content of fortified samples, albeit, marginally. It was found that control samples had significantly lower ash content compared to all other samples and was lower than the values reported by Jadhavar *et al*. (2009); Pal *et al*. (1996) and Sachdeva *et al*. (1991). Higher value in other samples could be attributed to diffusion of high inorganic salts from water chestnut to paneer as also reported by Rao and Patil, (1999) and Rani *et al.* (2014). The pH range of control samples was within the range obtained in the literature (Sachdeva *et al*., 1991; Pal and Yadav, 1991). The lower pH values for fortified samples can be attributed to inherent acidity of water chestnut being of the order of 0.142±0.03 (Majee *et al.*, 2013). The fat in whey value obtained in control was significantly lower than other samples. However, the values are in range of the reported values in the literature (Sachdeva *et al*., 1991). The yield of paneer showed a significant increase compared to control. The increase in the yield of water chestnut incorporated samples could possibly due to the increase in moisture content, due to maximum protein coagulation in such treated milk and greater entrapment of fat in coagulum as is evident from the analysis of paneer on dry matter basis. The yield of paneer is significantly influenced by composition of milk (Bhattacharya *et al.*, 1971; Sachdeva *et al.*, 1985; Vishwehwaraiah and Anantakrishnan, 1986; Singh and Kanwjia, 1988); temperature of coagulation (Bhattacharya *et al* ., 1971; Rao *et al*., 1984; Sachdeva *et al*., 1985; Singh and Kanwjia, 1988; Roy and Singh, 1994) and type of coagulant and additives etc. The hydrocolloids are well known for their moisture binding ability. In the present investigation, yield of paneer showed proportionate increase with moisture retention of different types of paneer prepared with various levels of water chestnut. Similar reports were reported by Sachdeva and Singh (1988), who stated that the binding capacity and yield of paneer can be

improved by the sodium alginate (0.1%), Carrageenan (0.15 per cent), pre-gelatinized potato starch (0.15 per cent) and CMC (0.1%). Roy and Singh (1994) also reported increase in the moisture retention and yield of paneer where hydrocolloids were used. Sachdeva and Singh (1995) observed higher paneer yield by coagulation at 90^0 C with addition of stabilizers. Rao *et al.* (1984) observed paneer yield of 22.35 per cent from buffalo milk heated at 85 ^{0}C and coagulated at 70 ^{0}C with 0.3 per cent citric acid.

5.3 Sensory characteristics of functional paneer

All the sensory parameters like appearance, flavour, body and texture and overall acceptability were significantly affected by the level of incorporation of water chestnut. There was found a remarkable increase in appearance score of T_2 but marked reduction in T_4 and T_5 as compared to control. The lower scores observed in latter might be due to slight change in colour of paneer owing to increase in water chestnut incorporation. It indicates that up to 0.5 per cent levels, water chestnut did not have any adverse effect on the finished product as far as colour and appearance is concerned. Similar results were obtained by Bhadekar *et al.* (2008) in paneer made by incorporation of Sago powder in buffalo milk. However, Sachdeva and Singh (1988) had recorded the score for appearance in between 7.4 to 7.6 for the paneer with addition of pre-gelatinized potato starch. The flavour scores for control, T_2 and T_3 were comparable and were significantly higher than the T_4 and T_5 samples. The latter were criticized for acidic harshness (low pH) and unnatural flavours. The results corroborate the findings of Singh and Kanawjia (1992), who also observed that with the increase in total solids in reconstituted milk there was a decrease in the flavour scores of paneer. Body and texture scores of T_2 were significantly higher than control. The latter had comparable values with T_3, T_4 and T_5. T_5 samples had lowest value as compared to all treatments. The lower scores in former may be due to excess increase in moisture, as a result of which paneer body became loose and soft and crumbly in nature. The comparatively lower body and texture scores were also observed by

Singh and Kanawjia (1992) in paneer from higher solid reconstituted milk. Sachdeva and Singh (1988) studied the incorporation of hyrocolloids to improve the yield, TS recovery and quality of paneer and reported the scores between 7.50 to 8.10 for the body and texture of the paneer. Roy and Singh (1994) while studying the effect of coagulation temperature and hydrocolloids on production and sensory quality of filled paneer recorded a score range of 6.50 to 8.00 for body and texture of paneer. The results recorded in present investigation with respect to body and texture was found to be comparable with the above findings.

The overall acceptability scores of control, T_2 and T_3 again were significantly higher than the T_4 and T_5. The values obtained were 7.85, 8.5, 8.14 for control, T_2 and T_3 respectively. Gadhve (2000) prepared the paneer from safflower milk blended with buffalo milk and reported that decrease in acceptability due to the increase in proportion of safflower milk. John (2002) recorded the overall acceptability as 7.66 for Shrikhand, which was prepared from buffalo milk blended with sago powder. Kachare (2002) studied the preparation of lassi from buffalo milk blended with sago powder and reported that the acceptability score of the finished product was in the range of 7.36 to 8.26 for 0.5% sago powder incorporated lassi sample. In the present investigation, score values for overall acceptability was found to be comparable with the research findings recorded above. Conclusively, the incorporation of non-milk solids to paneer beyond a certain limit inflict some negative influence on the product. It is therefore, immensely important to calibrate the incorporation of such ingredients scrupulously in order to attain the most desirable results.

5.4 Characteristics Of Functional Paneer

In order to ascertain the antioxidant potential of the functional paneer integrated with water chestnut, ABTS and DPPH methods were used as the basis of determination of such capability. In both the methods the mechanism lies in

scavenging or neutralizing the free radicals. The results obtained were expressed in terms of radical scavenging percentage. The radical scavenging activity values were significantly higher in fortified samples both in ABTS and DPPH methods. The values obtained in control sample were lower (13% by DPPH and 16% by ABTS) and varied significantly from other fortified samples. The difference in the ABTS and DPPH can be attributed to a comparatively more acidic sensitivity of the former. The values increased with the increase in level of concentration of water chestnut, reached to near about 55% radical scavenging in both methods.. These results are in agreement with findings of Palthur *et al.* (2014) who also obtained the values of 45-55% radical scavenging (DPPH) for *tulasi* flavored herbal milk containing 10 gm of *Ocimum sanctum* powder. Shori *et al.* (2011) reported that the presence of *Allium sativum* during yogurt formation increased the antioxidant activities in both cow-milk yogurt (37.9 ± 0.8%; $p < 0.05$) and camel-milk yogurt (26.1 ± 0.8%; $p < 0.05$). The values were comparable to that obtained in 0.25% water chestnut fortified paneer during current investigation but lower as compared to other treatments.

Sensory Characteristics On Storage

As signified by the result on day 0, control and T_3 showed comparable scores for appearance while a remarkable increase ($p \leq 0.05$) was observed in appearance score of T_2 in comparison to control sample. The same trend was observed on day 7^{th}. The appearance scores were within the range as obtained by the Bhadekar *et al* (2008). But on 14 and 21^{st} day, there appeared significant reduction in scores of control compared to T_2 and T_3. Sensory evaluation was not conducted on 28^{th} day owing to frank deteriorative changes. Flavour is the main parameter for accepting the product. In the present study, flavour scores on day 0, revealed insignificant change among the treatments. The results corroborate the findings of Singh and Kanawjia (1992). However, on 7^{th}, 14^{th}, 21^{st} day the control sample revealed progressive reduction in flavour scores as compared to other treatments.

On 21st day, the control sample showed the score 4.04±0.18 that signified "Dislike slightly" on hedonic scale. While the flavour score of both T_2 and T_3 was 6.07±0.17 that amounts to "Like slightly" on hedonic scales. The body and texture is an important parameter as far as the consumers acceptability is concerned. The body and texture score showed significant decrease in control compared to T_2 and T_3. Similar trend was observed on day 7th, 14th and 21st with control sample revealed marked decrease in score as compared to other treatments.

The overall acceptability scores of control, T_2 and T_3, on day 0 were comparable and did not differ significantly. On 7th day, control sample had significantly lower score as compared to other treatments. Similar was the trend followed on 14th and 21th days, with control showing marked deterioration than T_2 and T_3 samples. There was significant decrease in overall acceptability values of control, T_2 and T_3 samples from day 0 to 21th day. But the control showed very much reduction in score as compared to other treatments.

5.5 Microbiological Analysis

As indicated by the result, irrespective of the level of water chestnut in paneer, on day 0 all the test samples had comparable values for SPC count with overall mean of 1.81±0.04. On day 7th, there appeared significant decrease in SPC in T_2 and T_3 as compared to control that can be attributed to antibacterial activity of water chestnut (Razvy *et al*., 2011). The counts on 14th and 21st day among different treatments were comparable and well below the prescribed limits of Food Safety and Standard Authority Of India Regulations (FSSR, 2011) for paneer that is 4.69 log cfu/g (50,000/g). However, on day 28th, the SPC of test samples was above the prescribed limits of FSSR (2011). There was a significant increase in SPC from day 0 through 28th day of storage in all test samples. The values for all the selected samples at 4^0C storage temperature was far below compared to those obtained by Rani *et al*. (2014) for spiced paneer pickles at

10^0C storage temperature. Pal (1998) also reported an increase in bacterial count in fresh paneer from 3.03 log cfu/g to 4.5 log cfu/g during storage on day 5^{th} at 10^0C. The Coliform Count was not obtained on day 0 for any treatment. This can be assigned to antibacterial activity against gram negative (-ve) bacteria as suggested by Rahman *et al*., 2001. On day 7 and 14, there was significant reduction in coliform count of T_2 and T_3 samples in contrast with control. On day 21^{st} Coliform number for control was 2.56±0.01 log cfu/g well beyond the FSSR limit that is 1.95 log cfu/g whereas the values for T_2 and T_3 were below the FSSR limits. On day 28^{th}, coliforms were above the FSSR approved limits for all the test samples. From day 7^{th} to 28^{th} day, the count showed significant increase in all the treatments. The above findings differ to some extent with that of Rani *et al*. (2014), who obtained 2.38±0.18, 2.44±0.22 log cfu/g values on day 15^{th} and 2.91±0.35, 2.93±0.06 log cfu/g values on day 30^{th} for spiced paneer pickles at 10^0C. The Yeast and Moulds Counts were not detectable till 14^{th} day of storage at 4^0C for the entire range of treatments. This can be probably due to observing sanitary conditions during manufacture and inhibitory effect of refrigeration on fungal growth. On day 21^{st} and 28^{th} there appeared an insignificant reduction in count of T_2 and T_3 as compared to control. Within the treatments, the control,T_2 and T_3 showed increase in Yeast and Mould count from day 21^{st} to 28^{th} day. However, their counts remained below the prescribed limits of FSSR (2011) that is 250/g (2.39 log cfu/g). The count obtained by Pal (1998) at 10^0C on day 5^{th} for fresh paneer was 2.19 log cfu/g comparable to that obtained in our study on 28^{th} day at 4 ± 1^0C. Our results differed from the Rani *et al*. (2014), who observed the Yeast and Mould Count results of 1.69±0.13 and 1.90±0.25 log cfu/g for spiced paneer pickles on day 30^{th}. Vashita (2000) observed similar results on the microbiology of paneer pickles. The lower number in the latter cases can be due to essential oils from spices which cause their inhibition.

5.6 Thiobarbituric acid reacting substances value

As evidenced by the result, the TBARS value showed a comparable picture on day 0 among various treatments. But on day 7th lower values were obtained for T_2 and T_3 compared to the control that may be due to inhibition of lipid peroxidation by water chestnut (Ambikar *et al*., 2010). On day 14th, 21st and 28th, the values obtained were comparable. The values of 21st day were below the set limits of FSSR that is 1.50 mg MDA/kg, while on day 28th extensive lipid peroxidation had occurred as suggested by the higher values. From day 0 to 28th day, there was a significant increase in TBARS values among various treatments. Singh *et al*. (2014) obtained the similar values of 0.496 and 0.486 mg malonaldehyde per kg of product for control and *turmeric* incorporated paneer samples respectively, on zero day at room temperature. However, these values on 3rd day showed abrupt increase with 2.38 and 0.801 mg malonaldehyde per kg of product for control and turmeric incorporated paneer at room temperature. Therefore, the oxidative deterioration of paneer samples is to some extent limited by both radical scavenging behavior of water chestnut fortified samples and by refrigeration temperature. However, to ascertain the individual effect of these two hurdles to check oxidative deterioration a separate comparative study is needed to be undertaken.

5.7 Tyrosine Value

As evidenced from the results the values for various treatments on 0,7th, 14th, 21st and 28th days, were comparable and did not show any significant difference. The values rose significantly in all samples from 0 to 28th day. However, the values were well below the prescribed limit of FSSR that is 0.92 mg tyrosine per gram of the product. Rai *et al*. (2008) observed the similar phenomenon of 0.12 mg tyrosine per gram of product for paneer samples packed under atm2 (25 inches Hg vacuum). However, on storage for 45 days initial mean tyrosine content value increased from 0.12 (mg/ g) to 0.34, 0.29 and 0.33

respectively, for the samples packaged under atm2 (vacuum), atm 3(100% CO_2) and atm 4 (100% N2), suggesting that the minimum proteolysis had been in the samples packaged under 100 % CO_2 and maximum in samples packed under vacuum, establishing a very significant influence of MAP on the proteolysis of paneer during storage. Thus in our study tyrosine value increases with advanced storage but was not very sensitive to changes during early stage of spoilage.

Chapter – 6

SUMMARY AND CONCLUSION

The current investigation was undertaken with the aim of manufacturing functional paneer by incorporation of water chestnut which in addition to nutritional benefits had functional characteristics and increased shelf life. For this purpose the study was conducted in two phases. In the first phase, different concentrations of water chestnut as 0%, 0.25%, 0.5%, 0.75%, 1% were incorporated in raw cow milk before heating to coagulation. The control and fortified paneer samples prepared were analysed for physico-chemical, sensory evaluation, antioxidant activity. The physico-chemical analysis of paneer samples revealed that the moisture content showed increasing trend with the rise in level of water chestnut with T_5 having the highest mean value and control showing the lowest value. The fat values were comparable among various treatments and did not show any significant difference. In case of protein, there was significant increase in various treatments in contrast with the control whereas within the treatments the values were comparable. The ash content showed significant increase in all treatments compared to control except T_2. The pH value revealed a significant decrease in all treatments as compared to control. While within the treatments T_3 ,T_4 and T_5 the values were insignificant. The fat in whey depicted a significant increase in value in contrast to control whereas other treatments revealed insignificant values. The yield of paneer showed a significant increase from control value of 20.06±0.22 to T_5 value of 23.65±0.32 with T_3 and T_4 had comparable values.

While studying the effect of water chestnut on sensory characteristics of paneer, it was found that the scores with regard to the sensory attributes like appearance, flavour, body and texture and overall acceptability showed highest mean squares to the 0.25% of water chestnut incorporated paneer(T_2) product

followed by T_3 . While as the lowest mean squares were obtained by the product with 1% water chestnut.

With regards to the effect of water chestnut on functional characteristics, it was observed that the radical scavenging percentage values as determined both by ABTS-RSA and DPPH-RSA methods showed highest increase in T_5 and lowest in contol product. In T_2 and T_3 the values obtained were significantly higher as compared to control paneer product.

Giving more preference to the sensory evaluation, keeping in view the other parameters also, we preferred 0.25% and 0.5% water chestnut levels for incorporation into paneer. Now, these three samples T_2 and T_3 along with the control were further subjected to shelf life estimation parameters at 4 ± 1^0C up to the storage period of 30days on weekly intervals.

Sensory analysis of selected treatments on weekly intervals revealed that appearance, flavour, and overall acceptability for T_2 showed significant rise on day 0^{th} and 7^{th} day as compared to control while the latter showed comparable values with the T_3. On day 14^{th} significant reduction was observed in control product as compared to T_2 and T_3. Lowest mean squares were observed on day 21^{st} for all treatments. The sensory was not performed on day 28^{th}. The body and texture score was significantly higher in T_2 and T_3 as compared to control on day 0 and 7^{th} day. On day 21^{st} marked reduction in control occured compared to other treatments.

With regards to microbiological analysis, the SPC of various treatments was insignificant on day 0^{th} while on day 7^{th} there was observed significant reduction in count of T_2 and T_3 as compared to control. However, the values appeared comparable on further storage days. On 28^{th} day the values in terms of log cfu/g appeared well beyond the prescribed limit of FSSR (2011), for all treatments. The coliform count was not observed on day 0, for any product sample. On day 7^{th}, 14^{th}, 21^{st} and 28^{th}, the count showed significant reduction in

T_2 and T_3 as compared to control. Within the treatment groups, there was observed significant increase in count for all treatments from 0 to 28th day. On 21st day the count for control sample was well beyond the standard 1.95 log cfu/g set by FSSR (2011). While the count of T_2 and T_3 was below the level upto 21st day. The yeast and mould count was not observed on days 0,7th and 14th for any treatment. The count was comparable on day 21st and 28th for all products and was well below the level of 2.39 log cfu/g.

The TBARS value of various selected treatments was comparable on day 0th while significant reduction was seen in the T_2 and T_3 as compared to control on day 7th. Thereafter the values were comparable on 14th, 21st and 28th day among all the treatments. The value obtained on day 28 was above the prescribed value of FSSR (2011) that is 1.5 mg MDA/kg.

The tyrosine value appeared comparable among all treatments irrespective of water chestnut incorporation on 0,7th, 14th, 21st and 28th day. However the value on 28th day was well below the FSSR set limits.

On the basis of the results obtained from the experimentation on different aspects during the current study the following conclusion can be drawn

- The functional paneer made from the cow milk incorporated with water chestnut was to a greater extent free from the defects like syneresis, poor body and texture and high drip rate.
- The paneer obtained by fortification of levels of water chestnut resulted in increase in yield of paneer (commercial importance)
- Paneer fortification with 0.25% and 0.5% water chestnut levels resulted in more desirable sensory characteristics
- The antioxidant potential in terms of RSA was improved to a very great extent in functional paneer integrated with water chestnut.

- The shelf life was extended from 7 to 12 days of fresh paneer to 21 days for functional paneer enriched with water chestnut at 4 ± 1^{0}C.

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APPENDIX- 1

Score Card for Sensory Evaluation of Milk Products

Faculty of PG studies

Division of Livestock Products Technology, Shuhama, Srinagar, Kashmir.

SCORE CARD FOR SENSORY EVALUATION OF MILK PRODUCTS

Name of Judge: …….. **Experiment No: ……**

Name of Product: ……

Scoring Guide for Hedonic Scale

9 = Like extremely

8 = Like very much

7 = Like moderately

6 = Like slightly

5 = Neither like nor dislike

4 = Dislike slightly

3 = Dislike moderately

2 = Dislike very much

1 = Dislike extremely

Sample No.	Appearance	Flavour	Body & Texture	Overall Acceptability	Comments, if any
1					
2					
3					
4					
5					

{Source: Peryam and Pilgrim, 1957}

Signature

Sher-e-Kashmir
University of Agricultural Sciences and Technology of Kashmir
Division of livestock Products Technology
Shuhama campus, srinagar-190006

CERTIFICATE

Certify that all the corrections/modifications suggested by the External Examiner Dr. Zuhaib Fayaz Bhat Assistant Professor, Division of livestock Products Technology, SKUAST-Jammu **have been incor**porated in the manuscript entitled **"A Study on Production of Functional Paneer via Incorporation of Water Chestnut"** submitted by of Tarique Ahmad Padder (Regd. No. 2014-V-257-M)

Dr. Mohammad Ashraf Pal
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