

A Thesis submitted to the
West Bengal University of Animal and
Fishery Sciences
in partial fulfillment of the requirement for
the degree
of Master of Technology
in
(Dairy Chemistry)
By
TRISHA ROY
B. Tech. (Dairy Technology)



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**Dedicated to my
Family and Well-Wishers**

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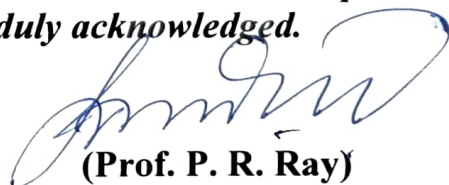
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This is to certify that the work recorded in the thesis entitled, "Evaluation of Physico-Chemical Characteristics and Antioxidant Potential of Lassi" submitted by Miss. Trisha Roy, in partial fulfillment of the requirements for the 'Degree of Master of Technology in Dairy Chemistry' in West Bengal University of Animal and Fishery Sciences (West Bengal), is the faithful and bonafide research work carried out under my personal supervision and guidance.

The research findings presented in the thesis have not so far been submitted for any other Degree or Diploma. The assistance and help received during the course of investigation have duly acknowledged.

Dated..... 12/02/23

Mohanpur, Nadia



(Prof. P. R. Ray)

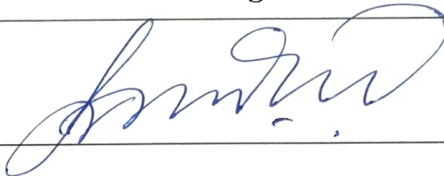
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**APPROVAL OF EXAMINERS FOR THE AWARD OF THE
DEGREE OF MASTERS OF TECHNOLOGY**

(DAIRY CHEMISTRY)

We, the under signed, having been satisfied with the performance of Miss. TRISHA ROY, in the viva-voce examination conducted today, the 13th. February 2023, recommended that the thesis be accepted for the award of the degree.

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(Trisha Roy)

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LIST OF ACRONYM

%	Percentage
&	And
@	At the rate of
<	Less than
>	More than
°C	Degrees centigrade
μ	Micro
α	alpha
β	Beta
μM	Micromole
cp	Centipoise
g	Gram
Kg	Kilogram
mEq	Milliequivalent
min	Minutes
nm	Nanometer
ABTS	2,2'-azinobis-(3-ethylbenzothiazoline-6-sulfonic acid).
ANOVA	Analysis of variance
AOAC	Association of official Agricultural Chemists
BCAA	Branched Chain Amino Acids
BIS	Bureau of Indian Standards
BOD	Biochemical oxygen demand
CHD	Coronary heart disease
COD	Chemical oxygen demand
DPPH	2,2,-diphenyl-1-picrylhydrazyl
ELU	Experiential Learning Unit.
<i>et al.</i>	Co worker
GRAS	Generally Recognized as Safe
i.e	That is
IBS	Irritable Bowel Syndrome
KDA	Kilodaltons
LA	Lactic acid
NDDB	National Dairy Development Board
No	Numbers
OD	Optical Density

PDCAAS	Protein digestibility-corrected amino acid score
PET	Polyethylene Terephthalate
POS	Point of sales
ROS	Reactive oxygen species
TA	Titrateable acidity
TCA	Trichloro acetic acid
TEAC	Trolox equivalent antioxidant capacity
TIDP	Traditional Indian Dairy Product
w/v	Weight/Volume
w/w	Weight/Weight
WPC	Whey protein concentrate

ABSTRACT

Lassi is a popular traditional fermented dairy product which possesses various functional properties and it is also known for its health beneficial effects and is established as dietary adjuncts. It is mainly dahi (yoghurt) based drink, consumed in South Asian region. Lassi has lot of variation in their preparation method. A study carried out to evaluate physico-chemical characteristics, chemical quality and antioxidative property of five types of different lassi samples were collected from the market of West Bengal. Lassi samples fortified with fermented, non-fermented whey along with control (without addition of whey) were collected from Experimental Learning Unit (ELU), Students Dairy, Mohanpur Nadia, West Bengal and analyzed for physico-chemical characteristics and antioxidant activity during storage at $7\pm 2^{\circ}\text{C}$. Physico-chemical analysis revealed a lot of variations in the constituents of lassi samples collected from West Bengal Market and whey fortified lassi sample collected from ELU (Experimental Learning Unit) Student Dairy, Mohanpur, Nadia. The average fat, protein, ash, total solids, sucrose, pH and titratable acidity, Tyrosine value of the market samples were found to be in the range between $1.21\pm 0.01\%$ to $2.12\pm 0.01\%$, $1.29\pm 0.01\%$ to $2.36\pm 0.02\%$, $0.43\pm 0.01\%$ to $0.67\pm 0.01\%$, $18.95\pm 0.05\%$ to $20.92\pm 0.01\%$, $10.43\pm 0.01\%$ to $11.91\pm 0.02\%$, 4.13 ± 0.05 to 4.44 ± 0.04 , $0.43\pm 0.01\%$ LA to $0.57\pm 0.02\%$, 3.80 ± 0.12 mg/100ml to 5.34 ± 0.04 mg/100ml, respectively. The average value of fat, protein, ash, total solid, sucrose, pH and titratable acidity content fermented whey fortified lassi was $1.85\pm 0.03\%$, $2.25\pm 0.01\%$, $0.35\pm 0.01\%$, $20.50\pm 0.02\%$, $11.28\pm 0.07\%$, 4.29 ± 0.04 and $0.59\pm 0.01\%$, respectively. The antioxidant activity of market lassi sample as determined by ABTS and DPPH were ranged between 73.98 ± 4.62 to 99.16 ± 7.11 μM ABTS absorbed in terms of TEAC and 9.12 ± 0.54 to 15.60 ± 0.09 % inhibition, respectively. The antioxidant activity of fermented and non-fermented whey fortified samples were found to be 67.01 ± 1.10 , 44.61 ± 1.49 μM ABTS absorbed in terms of TEAC, respectively at 7th day of storage. The antioxidant activity by DPPH method was found to be 15.90 ± 0.08 and 15.63 ± 0.03 % inhibition at 7th day of storage. The tyrosine value was increased to be 4.88 ± 0.04 , 4.29 ± 0.03 mg/100 mL for fermented and non-fermented whey fortified samples, respectively at 7th day of storage. The peroxide value was increased to 0.52 ± 0.02 , 0.48 ± 0.02 mEq/kg of fat for fermented and non-fermented whey fortified samples, respectively at 7th day of storage.

CHAPTER-1

Introduction.....

1. INTRODUCTION

Lassi is a traditional fermented milk beverage. Fermented milk products are the source of both energy and nutrition. Good quality lassi should have creamy consistency, smooth texture, glossy sheen and white colour with yellowish tinge. Mild acidic flavour and sweetish taste of lassi make it a refreshing soft drink. It is flavoured with either salt or sugar and other condiments or spices like ginger, coriander and mint depending on regional preferences. Lassi is obtained from pasteurized whole milk or partly skim milk, cultured with lactic and aroma/flavour producing organism. In many parts of the country products like butter milk, chhach, mattha obtained after churning of sweet cream or whole milk dahi and removal of butter are termed as lassi. It is usually consumed in salted or spiced form also a product prepared from cultured butter milk is classified as lassi.

Lassi is an excellent source of bio-functional peptides that may impart improved health benefits when ingested. These bio-functional peptides are shown to have antihypertensive, antimicrobial, immunomodulatory, antioxidative and mineral binding properties. The antioxidant defense system has the ability to remove the reactive oxygen species through enzymatic antioxidant such as peptides and proteins, Vitamins, trace elements co-enzyme and co-factors. Therefore, the present study has been carried out to determine the anti-oxidative property of lassi through different methods.

The different types of methods of preparation provide various sensory attributes and believed to have different physicochemical and microbiological characteristics. Average content of milk fat, milk total solids, sugar, low methoxy pectin (optional), sodium dihydrogen phosphate (optional) and acidity is 1.5 to 3.8%, 9%, 13 to 20%, 0.5%, 0.5% and 0.7% lactic acid, respectively (Aneja *et al.*, 2002).

1.1 Justification of work

Lassi is an excellent palatable product, full of nutrients, easy to digest, beneficial for gastrointestinal disorder (Padghan *et al.*, 2015). Bio-active peptides which are defined as specific protein fragments can exhibit several beneficial effects like preventing diseases or modulating the physiological systems once are absorbed in the human

body (Mora, 2019). Those peptides can be derived from milk and milk products. In case of lassi, it is observed that lassi is a potential source of various types of bio-active peptides.

Whey is a highly nutritious by product obtained from preparation like cheese, paneer, chhana, casein etc. It also contains lactose, protein, minerals and water soluble vitamins which enhance the nutritional and functional aspect.

Hence lassi with added whey can be more effective and very much helpful to increase functional and nutritional property of the product, and also most adequate component for human gut health who are suffering in constipation and other bowel diseases like bloating, lactose intolerance, IBS (Irritable Bowel Syndrome) etc.

So far, several researches were carried out on whey based food and dairy products. However, the production of these products is facing challenges because of their unwanted flavour, functional properties, textural problem due to sedimentation and improper solubility. To overcome these problems or to make whey useful it is better to use whey for fortification or enrichment. Addition of whey makes the product unique, provides the probiotic effect and gives authentic taste to the product. At the same time, it is very adequate in economical aspect due to proper utilization of whey. Hence, the present research work was undertaken to evaluate the physico-chemical quality and antioxidant properties of plain lassi along with lassi fortified with non-fermented as well as fermented whey.

1.2 Objective of Work

The present study was planned keeping in view the following objectives:

- 1) To evaluate the physico-chemical characteristics and antioxidant activity of lassi samples collected from market in West Bengal.
- 2) To determine the physico-chemical parameters and antioxidant activity of lassi samples collected from Experiential Learning Unit (ELU) of Milk Parlour (Point of Sales), Students' Dairy, Faculty of Dairy Technology, WBUAFS, Mohanpur Campus, Nadia, WB during storage at $7\pm 2^{\circ}\text{C}$.

1.3 Plan of work

The brief outlines of the research work are given below

PHASE I

- Analysis of physico-chemical characteristics of lassi samples available in market in West Bengal.
- Determination of Antioxidant activity of lassi samples by ABTS and DPPH method.

PHASE II

- Evaluation of physico-chemical characteristics and antioxidant activity of lassi samples which were fortified with
 1. Partially fermented whey
 2. Non-fermented whey
 3. Lassi without addition of whey referred as control sample
- Evaluation of physico-chemical characteristics and antioxidant activity of those samples during storage ($7\pm 2^{\circ}\text{C}$) at 2 days interval.

CHAPTER-2

Review of literature...

2. REVIEW OF LITERATURE

Among all the popular indigenous fermented milk beverage lassi is one of the most delicious and nutritious drink (Dhumal *et al.*, 2018). Lassi largely used as thirst quencher in summer. Fermented milk products possess greatest importance in human consumption as refreshing beverages and nutritious food in many parts of the world since earliest ages. Different flavours are used to enhance the flavor of the lassi.

2.1 Nutritional and Therapeutic Importance of Lassi

Different available preparation methods like traditional method and industrial method provide distinguishable characteristics to the lassi. Dixit *et al.* (2018) worked on development of lassi using whey and moringa powder at different concentration. Moringa powder has various nutritional and medicinal benefits as they possess flavonol and phenolic acid which have the ability to inhibit the prostate cancer and breast cancer.

Alovera is one of the undoubtedly eminent herb which possess emollient, anti-inflammatory, purgative, anti-microbial, aphrodisiac, antifungal and antioxidant properties. Alovera gel has excellent functional and nutritional value of food (Sonawane *et al.*, 2021). Monosaccharides (glucose and fructose) and Polysaccharides (glucomannose/ Polymannose), the components provided by alovera have antiallergic and anti-inflammatory properties. To enhance the excellent nutritional and therapeutic value, alovera juice is added in different concentration in lassi (Nichal *et al.*, 2022).

Swelling problem of human body is mainly due to overabundance of water in the body. Lassi consumption is an extraordinary way for preserving off stomach swells due to presence of beneficial microbes that help toward off horrible microbes. Cholesterol intake which is considered as the source of cardio-vascular diseases and coronary heart disease (CHD). The past 50 years, scientific literature about the cholesterol consumption is a great deal because it has a strong positive co-relation with heart diseases. Horrible dimensions of cholesterol in the human body are totally wiped out by lassi providing its probiotic attributes (Saha *et al.*, 2021).

To overcome oxidative stress use of antioxidants are the way of prevention and fruits are the excellent source of antioxidants (Kurutas, 2016). Phytochemicals like

phenolics, carotenoids, vitamin c and antioxidant potential in sapota was showed. Different names of sapota are chikoo or chiku in Hindi and Sapeta in Bengali. This fruit enriched in calories and sugars, and it consists a good amount of fructose and sucrose. Vitamin C and good amounts of proteins, minerals act as stimulant for enriching the blood, increasing muscle strength, present in sapota in good amount. Dairy products which are fortified with sapota juice improve the nutritional quality and taste (Gopalan *et al.*, 1977, Shambharkar *et al.*, 2011, Kasote *et al.*, 2015 and Kurutas, 2016). Sapota fruit pulp helps to reduce constipation and provide protection against colon cancer. This fruit pulp enriched with potassium, copper, iron and vitamins such as folic acid, niacin and pantothenic acid. Those components are very much essential for metabolic process in the body. It contains high amount of mucilage and used for manufacturing of chewing materials. These materials were used as substitute of stabilizer in ice cream (Gopalan *et al.*, 1977, Anon, 2007 and Padmavathi, 2018). Shaikh *et al.* (2016) worked on evaluation of the lassi prepared with different proportion of sapota pulp *i.e.* 5%, 10%, 15%, 20%. Lassi with 15% concentration has showed significantly superior due to its flavor, body, texture, colour and appearance. The author reported that blending lassi with 15% sapota pulp considered as good quality lassi.

There are two types of nutrients available in honey, almost 200 compounds in honey such as sugar, Protein, enzymes, minerals, vitamins, amino acids and wide range of polyphenols. Therapeutic activities, colour, taste, viscosity etc. depend on the ratio of those compounds present in honey. Inflammation acts as the main key in developing chronic diseases like cancer, cardio-vascular diseases, diabetes, arthritis and neurodegenerative disease which is untreatable in most of the time. Honey consists of distinctive phenolic and flavonoid compounds which fight against inflammation-mediated chronic diseases (Ranneh *et al.*, 2021). Shuwu *et al.* (2011) developed honey sweetened lassi by using 27.5, 30, 32.5, 35% honey syrup level. Among all this, lassi prepared with 30% honey syrup concentration secured the highest overall acceptability. The development of value added lassi using honey not only improves the sensory quality but also helps to enhance the nutritional properties of the product. Young grass of the common wheat plant is referred as wheat grass. It is power-house of nutrients such as chlorophyll, amino acids, minerals, vitamins and enzymes. The

grass considered as a humble weed, in the form of juice it contains higher concentration of chlorophyll, active enzymes, vitamins and other nutrients. Wheatgrass juice helps to reduce wastes which are responsible for creating problems in the body like clog cells, blood, tissues and organ related issues, it is also the richest source of vitamins A,B,C,E,K, calcium, potassium, iron , magnesium, sodium, sulphur and 17 forms of amino acids. Green Blood Therapy is a therapy in which wheat grass juice is used, known as green blood and considered as a complete for containing all the nutrients the body requires (Mujoriya and Bodla, 2011). Fortification of wheat grass extract in lassi is an innovative way to enhance the nutritional and therapeutic attributes of lassi. Kakde *et al.* (2019) used the different concentration of wheat grass extract for preparing lassi. They confirmed that wheat grass extract had no harmful effect in quality of the product and it can be used as flavoring and colouring agent in lassi. Ten parts of wheat grass extract are combined with 90 parts of curd for manufacturing wheat grass fortified lassi which was considered as better than other combination of concentration.

2.2 Chemical Composition of flavoured lassi

Composition of lassi depends upon the type of milk and other raw materials used in its preparation and the manufacturing conditions. Chemical specifications are given by several workers.

Table 2.1 Average chemical composition of Pudina flavoured lassi (Dhumal *et al.*, 2018)

Treatments	Acidity (% LA)	pH	Moisture (%)	Fat	Protein (%)	TS (%)	Ash (%)	Sugar (%)	Viscosity (cp)
T1	0.78	4.19	88.45	2.60	2.22	11.55	0.45	5.28	27.84
T2	0.76	4.24	88.68	2.53	2.53	11.33	0.45	5.18	26.51
T3	0.73	4.25	88.75	2.43	2.43	11.25	0.46	5.16	25.66
T4	0.71	4.27	88.84	2.40	2.40	11.16	0.47	5.16	2436

Treatment details: The pudina leaves extract was optimized for the preparation of lassi by taking its proportion 2.5, 5.0 and 7.5% as per following treatments combinations.

- T1 - 100 Parts of curd
- T2 - 97.5 Parts of curd + 2.5 Parts of Mentha arvensis extract

- T3 - 95.0 Parts of curd + 5.0 Parts of Mentha arvensis extract
- T4 - 92.5 Parts of curd + 7.5 Parts of Mentha arvensis extract

(Dhumal *et al.*, 2018)

Table 2.2 Chemical composition of Basil leaves Lassi (Sayyad *et al.*, 2022)

Treatments	Acidity (% LA)	pH	Fat (%)	Protein (%)	Total solid (%)	Ash (%)	Sugar (%)	Moisture (%)
T1	0.80	4.36	3.17	2.81	18.91	0.63	12.31	81.09
T2	0.86	4.04	2.92	2.72	17.39	0.64	11.11	82.62
T3	0.90	3.95	2.76	2.65	16.88	0.65	10.82	83.12
T4	0.93	3.92	2.58	2.58	15.35	0.66	09.57	84.67

For preparation of herbal *lassi* by using *Ocimum sanctum* extract, the treatment combinations were finalized in preliminary trials on weight basis as per follows:

- T1 - 100 Parts of curd
- T2 - 95 Parts of curd + 5 Parts of Basil leaf extract
- T3 - 90 Parts of curd + 7.5 Parts of Basil leaf extract
- T4 - 85 Parts of curd + 10 Parts of Basil leaf extract

(Sayyad *et al.*, 2022)

2.3 Antioxidant activity of lassi

Fermentation by lactic acid bacterium is the most efficient approach to enrich the bio-active peptides in fermented milk products. Those bio-active peptides are main precursor of antimicrobial, antioxidative, antithrombotic, anti-hypertensive and immunomodulatory activities. Oxidative stress which is the main precursor of many chronic diseases. Intake of food components that have improved antioxidant potential is an effective method to reduce the chance of oxidative stress. Food nutrients like vitamin C, E carotenoids are potential antioxidants. Vitamins K, Vitamin D, Niacin, and Pyridoxine are also antioxidant. Vitamin E and carotenoids presents in milk fat globule membrane of milk, responsible for prevention of autoxidation of milk fat. Vitamin C is an important which has a complex interaction with the iron and is an electron donor in the conversion of the tocopheroxyl radical back to antioxidant active vitamin E (Lindmark-Mansson and Akesson, 2000).

Ginger is a flowering plant whose rhizome is widely used as a spice, turmeric (*Curcuma longa*) used as a spice in Asian cuisine and as a medicinal herb. Carrot is an excellent source of potential antioxidant. It also contains vitamin K, Potassium and others nutritional elements. Maji *et al.* (2018) reported that lassi with turmeric extract showed best antioxidant activity and sensory scores and found acceptable upto 9 days when stored at $7\pm 2^{\circ}\text{C}$ in a glass bottle.

Beta vulgaris, popularly known as beet is a red root vegetable, it is used for sugar production due to containing high sucrose concentration. Betalains demonstrate antimicrobial, antiviral and free radical scavenging activities, as well as inhibiting lipid peroxidation and exhibiting hepatoprotective properties (Delgado-vargas *et al.*, 2000, Von Elbe *et al.*, 1974, Georgiev *et al.*, 2010, Jha and Gupta, 2016, Strack *et al.*, 2003 and Tesoriere *et al.*, 2003). Fortification of beta vulgaris can enhance the nutritional and functional attributes to the food. To increase the antioxidant activity, betalain powder is used because it contains higher polyphenol content, it is a natural bio-colour which can be used as a great substitute of chemical colourant which are harmful for health (Ganguly *et al.*, 2017).

Free radicals are mainly responsible for many carcinogenic diseases especially cancer. Antioxidant rich food products like yoghurt, kefir, acidophilus milk, Turkish yoghurt drink and the others fermented food products help to prevent the production of free radicals. In case of fermented dairy food products milk has its own antioxidative system added with antioxidant potential due to lactic acid bacteria. There are some compounds like casein, whey protein, peptides, amino acids, CLA, uric acid, vitamin C, A and carotenoids, coenzyme Q10, enzymatic systems (Superoxide dismutase, catalase, glutathione peroxidase) as well as from the antioxidant activity of the lactic acid bacteria or kefir culture. (Jimenez *et al.*, 2008, Liu *et al.*, 2005, Pihlanto, 2006 and Zulueta *et al.*, 2009).

Antioxidant's concentration in milk has a direct relation with the animal health and their food habits. Milk is an excellent source of potential antioxidant. Different antioxidant compounds have different type of reaction and the exact function of each antioxidant in milk cannot be determined. Each and every antioxidant compound of milk has different capacity to prevent oxidation of milk. The sum of the antioxidant

role of every compound can be determined by total antioxidant capacity of milk (Lindmark-Mansson and Akesson, 2000).

The main mechanism by which antioxidants act in food is radical scavenging. Single strains of lactic acid bacteria generated antioxidant activity in whey fractions. Proteolytic strain of lactic acid bacteria provides health promoting bio-active peptides (Fitzgerald and Murray, 2006).

Tinospora cordifolia (Giloy) is one of the protein rich herb and rich in calcium and phosphorus. Polysaccharides such as alkaloids, diterpinoid, lactones, glycosides, steroids, phenolic those are therapeutic component of Giloy. Development of herbal lassi using giloy stem juice increase anti-inflammatory, antineoplastic, anti-osteoporotic, antioxidant attributes of the specified products (Tripath and Rekha Rani, 2021).

Betalains are water soluble nitrogen-containing pigments. The most known edible sources of betalains in the caryophyllates are red beet roots. They are the potential source of antioxidant. As a natural colourant it is added in many dairy products like milk, ice cream, yoghurt and kefir. It helps to enhance the functional and nutritional property to the product. Betalains have been proven to eliminate oxidative and nutritive stress by scavenging DPPH, preventing DNA damage and reducing LDL cholesterol. Lassi fortified with beetroot juice is proved to be a highly nutritious fermented dairy product, rich in antioxidants, phytonutrients anti-inflammatory components (Sihag *et al.*, 2019).

Raspberries (*Rubus ellipticus*), plum (*Prunus domestica*), apricot (*Prunus armeniaca*) and jamun (*Syzygium cumini*) - these fruits are potential source of antioxidant. Kumar and Kumar (2016) worked on development of antioxidant rich fruit fortified probiotic buttermilk (lassi) using *Lactobacillus Rhamnosus* culture. Probiotic lassi was made with 5% probiotic *Lactobacillus Rhamnosus*, fortified with 10% (v/v) antioxidant rich fruit juice (i.e. *Rubus ellipticus*, *Prunus domestica*, *Prunus armeniaca* and *Syzygium cumini*). Apricot and raspberries fortified lassi were found to be the most stable product.

To maintain a healthy lifestyle antioxidants are very much essential. The function of spices and herbs as an antioxidant enhances the nutritional functional quality of the product when using at proper proportion. Artificial preservatives have a detrimental health effect on human health, whereas spices and herbs which are used in various way like as an extract, powder or as an essential oil to the food product exhibits no detrimental effects. Spices and herbs possess therapeutic, immuno-boosting characteristics with the minimal side effects (Sharma and Kumari, 2022). They are used as natural preservatives and contain a variety of phytochemicals, provide lot of health benefits. Herbs improve the quality of product, shelf life, mouth feel and overall acceptance through its qualitative attributes (Sharma and Kumari, 2022).

Shah *et al.* (2016) evaluated the antioxidative potential of two synbiotic product viz. synbiotic lassi with honey and whey based synbiotic drink with inulin and orange juice. Raw ingredients and probiotic cultures which are used for preparation of symbiotic products were evaluated for antioxidant activity. In this study, author reported that the storage of products had an adverse effect on antioxidant potential. Antioxidant activity was also affected by the composition of synbiotic products which was indicated by contrasting result of both products for DPPH and ABTS activity during storage.

β -lactoglobulin, α -lactalbumin, bovine serum albumin, immunoglobulin and lactoferrin components are whey proteins fractions, mainly provide the antioxidant activity to the product. Kefir is a fermented carbonated milk beverage used as a natural probiotic. Probiotics generally contain beneficial bacteria for human gut health. Consumption of kefir helps to reduce metabolic disorders, atherosclerosis and allergic diseases, promote bowel movement, reduce flatulence and create a healthier digestive system. The antioxidant properties of kefir produced from goat milk had been checked by DPPH and ABTS method during different stages of fermentation and storage period. The antioxidant activity and total phenolic content of buffalo milk kefir fermented with grains or a commercial starter culture as well as characterization of the amino acid profile was evaluated by Ozcan *et al.* (2019). Mainly in this study a detailed investigation had been performed into the antioxidant capacity of buffalo milk kefir made using different starter culture. Fermentation and storage process

helped to enhance production of natural antioxidative components. The demand of antioxidant rich foods has increased and become very popular. Authors suggested that buffalo milk kefir is capable to reduce oxidative stress and considered as potential antioxidant agent (Ozcan *et al.*, 2019).

Faki *et al.*, 2022 worked on incorporation of lyophilized grape seed extract powder into ayran drinks at various concentration (i.e. 4,5,6 mg/100 ml). They also evaluated different physico-chemical parameter, total phenolic content and antioxidant activity values. They found that incorporation of grape seed extract powder (GSEP) has no effect on physico-chemical properties of the final product. Addition of (GSEP) into ayran drink was successful to increase antioxidant activity of the final product. Addition of 4 mg lyophilized grape seed extract powder per 100 ml ayran drink was more suitable than other combination.

2.4 Nutritional and Therapeutic Value of Whey

Whey protein is widely popular for their high nutritious value and versatile functional properties in food products. It is the collection of globular proteins isolated from whey. Eighty percent casein and 20% whey protein are available in cow milk. Whey protein contains β -lactoglobulin (48-58%), α -lactalbumin (13-19%), bovine serum albumin (6%) immunoglobulin and proteose peptones.

The amino acid cysteine in whey protein is a substrate for the synthesis of glutathione in the body, which is a ubiquitous cellular antioxidant. Laboratory experiments have suggested that whey protein and its components might reduce the risk of cancer in animals, suggesting an avenue for future medical research (Parodi, 2007). Whey has discovered approximately 3000 years ago. Besides from its use as a therapeutic agent up to 18th centuries, whey has been recognized as a byproduct of the dairy Industry (Smithers, 2008).

Whey was used as animal feed in the past. The production of whey is estimated at about 165 million tones in the world in which cheese whey contributes about 95%. The production of channa and paneer whey contributes major portion of total whey production (Macwan *et al.*, 2016). In the absence of systematic survey/statistics the estimated production of whey is about 5 million ton per annum (Gupta, 2008). The

utilization was 75% in Europe and probably less than 50% in the rest of the world and as a result a very large amount of material with potential value as a food or feed is wasted (Aneja *et al.*, 2002). Whey is serious pollutant as it imposes a very high BOD of 30000-50000 mg/lit and COD 60000-80000 mg/lit.

Expulsion of whey also contains a significant loss of potential nutrients and energy has been looked upon seriously by the environmentalists and technologists due to polluting strength. Several treatments which are incurred in proper disposal of whey are the main reason of economic blow in case of dairy industry (Macwan *et al.*, 2016). Although several possibilities of cheese whey utilization have been explored, a major portion of the world cheese whey production is discarded as effluent. Its disposal as waste poses serious pollution problems for the surrounding environment.

The main predominant ingredient in the whey is lactose. It helps in absorption of the calcium from the food in the human body. Through lactic acid production lactose reduces the pH, improves calcium for transport. α -lactalbumin which is a part of whey protein (previously mentioned) is also an excellent source of essential amino acid. α -lactalbumin is only whey protein that can bind calcium and make it crucial for release of calcium to the babies and foetus, it also gives the antitumor effect. β -lactoglobulin, the another part of whey protein helps to bind the fat soluble retinol binding protein, and thus delivers vitamin A to the new born, makes them more accessible to the body. In infant formulae, the hydrolysed forms of β -lactoglobulin are commonly used to decrease the possible allergic responses (Bajaj and Sangwan, 2002 and Tariq *et al.*, 2013).

Cheese whey surplus is utilized as drinking water for farm animals. As whey is full of nutrients it provides high quality protein, lactose, calcium, phosphorus, sulphur and water soluble vitamins in proper amount to the animal (Siso,1996). Whey has also been used as an agricultural fertilizer but with the drawback that it leaves high saline deposits. In any case, transport of liquid cheese whey is very expensive.

Lactose fermentation by the treatment of whey for production of ethanol has received wide attention to date and its production in various large scales have also been developed. Production of ethanol from whey by several distilleries is in commercial

operation in Ireland. Production of ethanol from cheese whey in New Zealand is around 50% (Mawson, 1994).

Whey derived bio-active compounds and certain amino acids provide various health benefits to active people beyond body composition (Ha and Zemel, 2003). Gastro-intestinal disorders are the risk factors of the human gut health. Cramps, bloating, and diarrhea etc. are experienced due to gastro-intestinal disorders mainly in case of athletes during or after marathon competition. Glycomacropeptide - milk derived bio-active peptide that comprises 15-20% of proteins present in whey helps to exhibit prebiotic or probiotic activity and it also stimulate cholecystokinin. Cholecystokinin has toxin binding properties and it may release from intestinal cells (Walzem *et al.*, 2002).

In fermentation process, whey is used directly for fermentation using lactose-fermenting micro-organism for its bioconversion. Bio-active peptides are formed by transformation of whey protein through enzymatic or fermentation process. The demand of milk derived products is increasing day by day which leads to increased production of whey. It creates a serious management problem. Different value added products like whey powder, whey protein, whey permeate, bio-ethanol, bio-polymers, hydrogen, methane, electricity bio-protein (single cell protein) and probiotics. The transformation of whey into proteinaceous products is attractive and demanding among all value added products. Transformation of whey into proteinaceous products generally recognized as safe (GRAS) regulatory status of whey is the main important factor. On the other hand whey protein concentrate, whey protein isolate and individual whey protein can be directly processed from whey. These whey proteins are utilized or transformed into bio-active peptides via enzymatic or fermentation process. The proteinaceous products have application as functional, nutritional and therapeutic commodities (Yadav *et al.*, 2015).

Branched chain amino acids (BCAA) which are metabolized directly into the muscle, such as isoleucine, leucine and valine. Whey protein is the rich source of those branched chain amino acid. These are first amino acids used during periods of exercise and resistance trainings (Sherwood *et al.*, 2007).

Whey protein is the potential source of electrolytes including sodium and potassium. These can be helpful for recovering from disease like diarrhea. Apart from those minerals like calcium, magnesium are present in trace amount in whey which are beneficial for binding proteins. Zinc is also present in very low amount in whey. Lactose comprises about 70% of the total solids of whey. It promotes absorption of Mg and zinc ions which helps in better diarrheal management

The water soluble vitamins are transferred into a whey in a varying extent 40-70% of vitamin B12, 55-75% of vitamin B6 and pantothenic acid, 70-80% of riboflavin and biotin, 80-90% of thiamine, nicotinic acid, folic acid and ascorbic acid during the manufacturing process. Vitamin B12 was transferred into whey during a rennet coagulation rather than acid coagulation was used. From old people to little children all type of consumers prefer to have whey based beverages because of its health benefits and also helpful for curing diseases like phenylketonuria (Miller, 2005).

Among all minerals present in whey, potassium is involved in the transmission of nerve impulses and muscular contractions. Magnesium depolarizes the nerve or muscle causing relaxation and help to lower blood pressure and supports and alkaline tissue pH, maintain bone density, cell wall integrity and nerve impulses. Lactoperoxidase inhibits the growth of bacteria (including pathogenic bacteria) and fungi also regulate iron absorption and bio-availability. Whey also possesses the vitamins necessary for its utilization. It contains vitamins A, B1, B2, B3, B5, B6, C, D and E.

Whey and whey derived products besides being a nutritional ingredient in various foods can also be used as functional ingredient supplying flavor, texture, colour and certain aeration properties in variety of foods. Whey proteins possess very good functional properties, such as solubility, foaming, emulsifying, gelling and water binding. The solubility of whey proteins is high at all pH values when compared to other proteins like caseins and soy protein which are highly pH dependent. Whey protein from gels and bind considerable amounts of water when heated and this were found important in baked foods, meat products and processed foods. Whipping and foaming properties of whey proteins was excellent and they were found next only to egg white, whey proteins were also found to be very good emulsifying agents.

Greatest Protein Digestibility Corrected Amino Acid Score (PDCAAs) is showed by whey protein. Whey protein had greater PDCAAs value than meat protein, wheat protein and nuts protein. Egg protein, whey protein, casein protein, milk protein and soy protein showed the value 1, whereas meat protein and wheat protein showed 0.92 and 0.25, respectively. The biological value is also more in whey protein compare to egg protein, beef protein, and soy protein. Egg protein, meat protein, casein protein, soy protein and wheat protein, milk protein showed biological value 104, 80, 77, 74, 64, 91, respectively, whereas whey protein had the biological value was 104 (Smithers, 2008 and Smithers, 2015).

Table 2.3 Type and composition of whey

Parameter	Amount (%)	
	(Source: Monika Rani <i>et al.</i> , 2018)	(Source: Gupta, 2008)
Moisture	93.30	93.50
Total Solids	6.70	6.50
Lactose	4.88	5.00
Protein	0.68	0.40
Fat	0.65	0.50
Ash	0.49	0.50

Table 2.4 Vitamin Content of Whey

Vitamins	Content (mg/100g)
Thiamine	0.04
Pantothenic acid	0.4
Nicotinic acid	0.2
Riboflavin	0.14
Vitamin B6	0.05
Vitamin B12	0.0002
Folic acid	0.001

(Source: Zadow, 1991)

Table 2.5 Whey classification based on pH and Acidity

Parameter	Sweet whey	Medium acid whey	Acid whey
Titratable acidity (% LA)	0.10-0.20	0.2-0.4	Minimum 0.40
pH	5.8-6.6	5-5.8	Maximum 5.0

(Source: Bund and Pandit, 2005)

Table 2.6 Comparison of proximate composition of milk and whey

Component	Content (%W/V)	
	Milk	Whey
Total solids	12.8	6.3
Fat	3.7	0.1
Lactose	4.9	4.9
Casein protein	2.8	<0.1
Whey protein	0.7	0.7
Ash	0.7	0.5

(Source: Smithers, 2008)

Table 2.7 Mineral content in cheese and paneer whey

Minerals	Paneer whey (mg/L)	Cheese whey (mg/L)
Citrate	6750	2452
Potassium	1300	1300
Chloride	1349	1167
Calcium	480	291
Sodium	350	260
Magnesium	59	36
Zinc	0.28	0.21

(Source: Nupur and Gandhi, 2009)

Table 2.8 Whey protein and their biological function

Whey protein	Function
α -lactalbumin	It kills cancer cells. It also has antibacterial actions on respiratory system, as well as preventive effects on the gastrointestinal mucosa.
β -lactoglobulin	It binds retinol and enhances absorption of retinol by the gut. Excellent binding ability with long-chain fatty acids.
Lactoferrin	It modulates iron absorption through the intestines, inhibits the certain microorganisms, and maintains an immune reaction of immuno competent cells.
Serum albumin	It binds and transfers fatty acids and bile pigment.
Immunoglobulin A	Inhibits growth and activity of different bacteria by condensing, prevents microorganisms from adhering to the mucosal surface, and removes toxins generated by viruses and bacteria.
Immunoglobulin M	Functions similar to IgG but has higher bioactivity.
Lactoperoxidase	Catalyzes the reaction of generating cyanogen ion having effective antibacterial activity from cyanic ion and hydrogen peroxide inside the body
Lysozyme	Bacteriolysis effect by cell wall destruction.
Immunoglobulin G	Involves bactericidal actions and inhibits microorganisms from adhering to tissues, neutralization of toxin and virus

(Tsutsumi and Tsutsumi 2014)

Table 2.9 Whey protein constituents and its composition

Whey protein constituent	Concentration (g/L)	Molecular weight (KDa)	Amino acids residues
α -Lactalbumin	1.2	14,775	123
β -Lactoglobulin	1.3	18,277	162
Bovine serum albumin	0.4	66,267	582
Immunoglobulin (A,M and C)	0.7	25000 (light chain) 50000-70000 (heavy chain)	-
Bovine Lactoferrin	0.1	80,000	700
Glycomacropeptide	1.2	6700	64
Bovine Lactoperoxidase	0.03	70,000	612

(Source: Minj and Anand, 2020)

2.5 Utilization of whey in dairy and food products

Concentrated paneer whey was utilized for preparing multigrain bread dough using different grains flour including wheat, maize, sorghum, flaxseeds and oats and concluded that whey with 15% total solids can completely replace water for multigrain bread dough (Paul *et al.*, 2016).

For dahi preparation concentrated paneer whey was utilized. Milk was replaced by concentrated paneer whey (11.5% total solids) in ratio of milk to whey as 1:1, 2:1 and 3:1. The author proposed that to manufacture dahi of acceptable quality, concentrated paneer whey might be used at a rate of 25%. From storage study it was found that dahi manufactured from mixture of milk and concentrated whey (3:1 ratio) maintained the overall sensory acceptability and physico-chemical attributes of control dahi samples and had an 8-day shelf life at 4°C (Raghabendra *et al.*, 2017).

Parekh *et al.* (2017) used fermented paneer whey to manufacture cultured butter milk. It provides practical and commercial application of paneer whey. The addition of fermented paneer whey increased the bio-functional properties of cultured butter milk significantly.

Procedure for utilization of paneer whey in symbiotic sherbet like candy with added soy protein was developed. Researcher found that addition of 87.50% paneer whey, 1.28% soy protein, 4.0% fructo-oligosaccharide and 0.025% probiotic culture into symbiotic sherbet candy produced more nutritional and functional product without altering its sensory properties. Whey protein concentrate was added in misti dahi for

development of protein rich misti dahi by Shree *et al.* (2017). Misti dahi with 2% WPC had overall acceptability score due to its good body and texture and some improvement in functional characteristics such as water binding capacity, gelation, emulsification and viscosity of the product.

Different rate of pineapple pulp in the range of 5 to 15% of channa whey, sugar @ 8.0% were used for developing whey beverage. Author reported that pineapple flavoured whey beverage prepared with incorporation of 5% pineapple pulp of channa whey and 8% of sugar considered optimum (Bhavsagar *et al.*, 2010).

Lactose hydrolysed paneer whey was utilized by Kalariya (2020) for preparation of fruit based probiotic candy. It was prepared using sapota pulp, pomegranate powder and beetroot powder @ 14, 6 and 5% of total whey.

To improve physico-chemical characteristics, sensory attributes and melting resistance moringa pod powder was added into whey based candy according to Panchal (2019). The level of nutritionally valuable minerals like calcium, magnesium, potassium, sodium etc. can be enhanced by moringa pod powder.

Jan *et al.* (2021) developed ice pops by utilizing whey blended with jamun juice and sucralose was used as a sweetener.

2.6 Antioxidative activity of whey

The production of reactive oxidative species (ROS) helps to enhance the oxidative metabolism associated with exercise. ROS accumulation is one of the main reason for delaying muscle recovery and may impair performance (Lands *et al.*, 1999). Two minor proteins in whey protein i.e. lactoferrin and lactoferricin act as antioxidant through their iron binding capacity. In native state, lactoferrin is 8-30% in saturated condition, this condition enables chelation of iron and subsequent inhibition of bacterial growth or oxidative reactions. The antioxidant activity is enhanced by whey by contributing cysteine rich proteins which are pivotal in the synthesis of glutathione, a major intracellular antioxidant (Walzem *et al.*, 2002).

Possible antioxidant mechanisms of whey include chelation of transition metals by lactoferrin and serum albumin and free radical scavenging by amino acids such as tyrosine and cysteine. Oxidative stress can cause cell destruction and infuriated

several chronic diseases like neurological disorders, cancer, and liver injury and ageing and seems to elevate risk of cystic fibrosis, chronic pancreatitis, diabetes and cardiovascular disease. Free radicals which are mainly responsible for oxidative stress, are precursor of degradation of essential biological molecule such as lipids, proteins, DNA and others. Cells which create a shield against oxidative damage follow many mechanisms such as prevention, repair, antioxidant synthesis and absorption of dietary antioxidants or their precursors. Antioxidants are major weapon to combat free radicals leading to reduced oxidative stress, also helpful for reducing different diseases. β -lactoglobulin and α -lactalbumin have shown antioxidant properties by their several synthetic peptides. Whey derived from bovine species and individual whey protein exhibits antioxidant properties. Antioxidant activity of whey protein is an excellent establishment of scientific aspect. Lipid oxidation which is a vital problem can be inhibited by antioxidant activity of whey protein. Chelation of transition metals by lactoferrin shows the antioxidant ability of whey protein. Sulphur containing amino-acids are capable to scavenge the free radicals which are responsible for lipid oxidation. To increase the oxidative stability, whey protein is added to the soya bean oil emulsion. Lactoferrin and casein may hinder lipid peroxidation, generation of peroxide radicals, thiobarbituric acid reacting substances, oxygen absorption and emergence of iron oxide free radicals. Foods which contains whey protein have showed higher antioxidant activity (Khan *et al.*, 2019).

α -lactalbumin has greater antioxidant activity than β -lactoglobulin among all whey components. Due to sulfhydryl group β -lactoglobulin has low antioxidant activity. Despite the absence of free sulfhydryl groups, α -lactalbumin showed higher antioxidant activity though the reason remained unclear. Lactoferrin possess iron binding ability and thereby it shows antioxidant activity (Tong *et al.*, 2000).

β -lactoglobulin free milk had roughly 50% lower antioxidant activity than skimmed milk as reported by Stobiecka *et al.* (2022).

The antioxidant capacity of whey is due to presence of whey proteins, urate and residual vitamin C as molecular weight component. The antioxidant activity from acid whey is due to chelation of iron and hydrogen donation capacity.

Zulueta *et al.* (2009) reported that casein fractions were the main contributor of the antioxidant activity in case of whole milk while albumin was the primary contributor of whey protein. Hydrophilic compounds such as vitamin C, uric acid etc. were the principal factor to provide antioxidant ability of the deproteinized milk. Milk samples have better antioxidant activity than whey because albumins are more sensitive to heat treatment. The author also found the deproteinized milk showed lowest antioxidant capacity for containing water soluble antioxidant compounds.

Ashoush *et al.* (2013) reported that whey powder showed the 72.15% scavenging activity by using DPPH free radicals in their studies of evaluation of whey powder. Whey powder could be used to improve functional property of food by their natural antioxidant capabilities.

Waychal *et al.* (2022) worked on paneer whey beverage added with strawberry pulp in different proportion i.e. 100:0, 98:6, 96:8 and 94:10, respectively. The product was subjected for antioxidant activity measured by Trolox Equivalent Antioxidant Capacity (TEAC). The antioxidant activity was increased as the level of strawberry pulp increased as the level of strawberry pulp increases in paneer whey due to high antioxidant potential of strawberry.

2.7 Antioxidant activity by ABTS method:

In ABTS assay (2, 2-azinobis-(3-ethylbenzothiazoline-6- sulfonic acid) the capacity of compound to scavenge ABTS radical ($\text{ABTS}^{\bullet+}$) is measured. The original $\text{ABTS}^{\bullet+}$ assay was based on the activation of met myoglobin with hydrogen peroxide in the presence or absence of antioxidants. The improved technique for the generation of $\text{ABTS}^{\bullet+}$ described here involves the direct production of blue/green $\text{ABTS}^{\bullet+}$ Chromophore through the reaction between ABTS and potassium persulfate. This method based on one of the spectrophotometric methods that have been applied to the measurement of the total antioxidant activity of solutions of pure substances, aqueous mixtures and beverages. This assay clearly improves the original TEAC assay (The ferryl myoglobin/ ABTS assay) for the determination of antioxidant activity in a number of ways. First, the chemistry involves the direct generation of the ABTS radical monocation with no involvement of an intermediary radical. Second, it is a decolorization assay, thus the radical cation is pre-formed prior to addition of

antioxidant test systems, rather than the generation of the radical taking place continually in the presence of the antioxidant. Hence the results obtained with the improved system may not always be directly comparable with those obtained using the original TEAC assay. Third, it is applicable to both aqueous and lipophilic systems (Re *et al.*, 1999).

The antioxidant activity of two symbiotic dairy products viz. symbiotic lassi with honey and whey based synbiotic drink with inulin and orange juice along with their physico-chemical and microbiological activity during storage period at 734 nm (Shah *et al.*, 2016). Antioxidant activity of raw materials such as milk, honey, inulin, orange juice and whey (samples prepared using suitable diluents) was determined by hydroxyl radical scavenging activity and ABTS assays, which is measurable spectrophotometrically. The ABTS radical scavenging activity (%) was calculated using the following.

The (%) ABTS activity = $(\text{Absorbance of blank} - \text{Absorbance of test}) \times 100 / (\text{Absorbance of blank})$

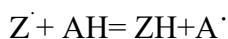
2.8 Antioxidant activity by DPPH method

The antiradical activities of various antioxidants were determined using the free radical, 2,2-Diphenyl-1-picrylhydrazyl (**DPPH**). In its radical form DPPH has an absorption band of 515 nm which disappears upon reduction by an antiradical compound. This method is widely used to evaluate antioxidant activities, because of its simplicity, rapidity, sensitivity and reproducibility compared with other methods (Milardovic *et al.*, 2006).

A number of protocols have been followed for DPPH antioxidant assays, resulting in variation in the results of different laboratories. A perspective of the protocols followed by different workers with incongruity in their results and recommended a standard procedure within the sensitivity range of spectrophotometry, besides sensitivity of DPPH to light, pH and solubility of the compound (Moharram and Youssef, 2014).

When a solution of DPPH is mixed with that of a substance that can donate a hydrogen atom, then this gives to the reduced form with the loss of this violet colour

(although there would be expected to be a residual pale yellow colour from picryl group still present). Representing the DPPH radical by **Z** and the donor molecule by AH, the primary reaction is



Where ZH is the reduced form and A is the free radical produced in this step. The latter radical will then undergo further reactions which control overall stoichiometry.

The methanolic crude extracts of some commonly used medicinal plants were screened for their free radical scavenging properties using ascorbic acid as a standard antioxidant. Free radical scavenging activity was evaluated using 1, 1-diphenyl-2-picryl hydrazyl (DPPH) free radical. All the methanolic extract exhibited antioxidant activity significantly. The overall antioxidant activity of green tea (*Camellia sinensis* Linn.) was the strongest, followed in descending order by black tea (*Camellia sinensis* Linn.), *Eugenia caryophyllus* (Spreng.) Bullock and Harrison, *Piper cubeba* Linn, *Zingiber Officinale* Roscoe and *Piper nigrum* Linn. *Trigonella foenum graecum* Linn., *Elettaria cardamomum* Linn. and Maton showed weak free radical scavenging activity with the DPPH method (Khalaf *et al.*, 2007). Radical scavenging assay of DPPH radical was determined according to Mau *et al.* (2004) with minor modifications. The methanolic extracts of red and white mycelia of *Antrodia camphorata* (100, 200, 250, 500, 1000 µg in 1 mL methanol) was mixed with 4 mL of 0.004% methanolic solution of DPPH. The mixture was shaken vigorously and left to stand for 30 min in 30°C, and the absorbance was then measured at 517 nm.

(%) of Inhibition= (absorbance of control-absorbance of sample)/absorbance of ascorbic acid (control)×100 [determination of indophenol method by AOAC (1997)]

CHAPTER-3

Materials and Methods...

3. MATERIALS AND METHODS

This chapter focuses on the materials, experimental methodologies adopted in the present investigation.

3.1 Materials

The particulars regarding the materials used in the present investigations are delineated hereunder.

3.1.1 Collection of samples

Lassi available in **tetrapak** packaging and **polypouch** film were collected from different shops in West Bengal, to determine physico-chemical characteristics and antioxidant activity of those collected samples during the month of May and June.



Fig. 3.1 Lassi in tetrapak packaging



Fig. 3.2 Lassi in polypouch film

3.1.2 Whey fortified lassi samples

Whey based lassi samples available in PET bottle collected from experiential learning unit of Milk Parlour, Students' Dairy, WBUAFS, Mohanpur Campus, Nadia, WB during the month of March, September and December.



Fig. 3.3 Lassi available in PET bottle

3.1.3 Chemicals

All the chemicals used during investigation were of analytical grade and obtained from standard suppliers.

3.1.3.1 Reagents for ABTS method

Potassium Persulfate, ABTS, Methanol were procured from Nice Chemicals Pvt. Ltd., Tokyo Chemical Industry Co. Ltd. and Avarice Industries, respectively.

3.1.3.2 Reagents for DPPH method

DPPH, Methanol were procured from Sigma-Aldrich, Germany, Avarice Industries, respectively.

3.1.3.3 Reagents for determination of titratable acidity in terms of lactic acid

N/10 sodium hydroxide solution and Phenolphthalein were procured from Nice Chemicals Pvt. Ltd.

3.1.3.4 Reagents for determination of fat content

Concentrated Ammonia Solution - specific gravity 0.88, Concentrated Hydrochloric Acid, Ethyl Alcohol-95% by volume, Diethyl Ether- specific gravity 0.720, Peroxide free Petroleum Ether – Boiling range 40°-60°C, Diethyl Ether were procured from Nice Chemicals Pvt. Ltd.

3.1.3.5 Reagents for determination of protein content

Concentrated Sulfuric Acid, Copper Sulphate, Potassium Sulphate, Methyl Red, Bromocresol Purple, Ethyl Alcohol, 0.02 N Hydrochloric Acid, Sodium Hydroxide were procured from Nice Chemicals Pvt. Ltd.

3.1.3.6 Reagents for determination of peroxide value

Glacial acetic acid, chloroform, Potassium iodide powder, sodium thiosulphate solution and starch solution were procured from Nice Chemicals Pvt. Ltd.

3.1.3.7 Reagents for determination of tyrosine value

Trichloroacetic Acid, Alkaline Copper Tartarate, Folin-Ciocalteu Reagent, 0.1 N Hydrochloric Acid were procured from Nice Chemicals Pvt. Ltd.

3.2 Analytical methods

Various analytical methods had been followed to analyze the chemical quality of lassi.

3.2.1 Determination of Titratable acidity (% of lactic acid) of lassi samples

A method as described in the method given by Bandyopadhyay *et al.* (2016) was followed to determine the titratable acidity of lassi samples.

Reagents

0.1(N) NaoH solution

Phenolphthalein indicator

Procedure

Ten gram lassi sample was taken. 25 mL distilled water was added to it and titrated against 0.1(N) NaoH solution using phenolphthalein solution as indicator. Result was expressed as (%) of lactic acid.

Calculation

Titratable acidity (% L.A) = $0.9 \times V/W$

Where,

V= mL of 0.1 N NaoH solution used in the titration

W= weight in g of dahi taken for the test

3.2.2 Determination of fat content of lassi samples by Rose Gottlieb method

A method as described by Bandopadhyay *et al.* (2016) was followed to determine fat content of lassi.

Reagents

Concentrated Ammonia Solution - specific gravity 0.88

Concentrated Hydrochloric Acid

Ethyl Alcohol-95% by volume

Diethyl Ether- specific gravity 0.720

Peroxide free Petroleum Ether – Boiling range 40°-60°C

Diethyl Ether

Procedure

Ten gram of lassi was weighed in a beaker and to it 1 mL ammonia & 10 mL Conc. HCl was added. Heated for a while & transferred to Rose-Gottlieb extraction tube. 10 mL ethyl alcohol was added to the beaker then poured into extraction tube & mixed well. 25 mL diethyl ether was added to the extraction tube & stoppered & mixed well. 25 mL petroleum ether was added to the extraction tube then mixed well. The mixture was allowed to stand for 30 minutes till clear layer separation took place. The clear layer was extracted into a pre weighed beaker. Extraction was repeated after adding 5 mL ethyl alcohol, 15 mL diethyl ether & 15 mL petroleum ether. The extracted solvent was evaporated completely on hot plate. Beaker was kept in desiccator and repeated heating & weighing was carried out till successive reading showed difference of 1mg.

Calculation

Fat percent by weight = $100(w_2-w_1)/(w_3-w_1)$

Where,

W1= Weight in g of empty disk.

W2= Weight in g of disk with dried fat.

W3= Weight in g of disk before drying.

3.2.3 Determination of protein content in lassi samples

The protein content of the product was determined by measuring the total nitrogen content as described by AOAC (1995) and multiplying the total nitrogen content with the factor of 6.38. About 2 g of sample was accurately weighed into 100 mL kjeldahl flask followed by addition of 20 mL concentrated sulfuric acid in such a way as to wash any sample particles on the body of flask. The contents of the flask were digested after adding 1 g of digestion mixture ($\text{CuSO}_4:\text{K}_2\text{SO}_4 = 1:4$) till the clear remnant was obtained. After digestion has cooled to room temperature, 85 mL of distilled water was added and mixed with the contents. The contents were distilled using steam. Fifty mL of saturated boric acid with 4 drops of mixed indicator (1 part of methyl red prepared by dissolving 0.2 mg methyl red and diluted to 100 mL in 95% ethyl alcohol with 5 parts of bromocresol green prepared by dissolving 1 mg bromocresol green and diluted to 500 mL in 95% ethanol) solution was taken in flask

and placed under condenser tip so that tip is well below boric acid solution surface. Fifty five mL of 40% sodium hydroxide solution was placed in alkali tank of distillation unit and digestion tube containing diluted digest was attached to the distillation unit. Distillation continued till about 150 mL distillate was collected in the conical flask. The distillate collected in the conical flask was titrated against 0.02 N HCL to first trace of pink. A blank determination was also carried out using distilled water instead of sample.

Percentage total protein was calculated using the following formula

Calculation

$$\text{Protein (\%)} = 1.4007 \times (V_s - V_b) \times N \times 6.38$$

Where,

V_s = Volume of HCL used for titration of sample (mL)

V_b = Volume of HCL used for titration of blank (mL)

N = Normality of HCL solution

W = Weight of the sample (g)

3.2.4 Determination of pH of the lassi samples

The pH of lassi samples was determined by using digital pH meter following the procedure stated in IS: 1479 (Part II), 1961. In potentiometric method pH had been measured.

Procedure

Standardize the instrument against a buffer solution of known pH and check against another buffer of different pH. Then connect the saturated calomel and milk half cells to the potentiometer. Balance the potentiometer to make the reading. Equilibrium occurs almost instantaneously and no drift is experienced. The pH is indicated directly on the instrument.



Fig. 3.4 Auto pH meter (SYSTONIC)

Indication

In normal milk, the pH is well below 6.9. On an average, cow milk gives a pH of 6.6, and buffalo milk 6.8. Milk of pH over 6.9 should be regarded with suspicion as indication of some diseases of the udder, or late lactation milk. In case of Lassi sample the measurement had been followed this same procedure.

3.2.5 Determination of ash content of lassi sample

Taken accurately weighed 10 mL of sample in a previously weighed dry silica-dish. It was then kept in a muffle furnace maintained $550 \pm 1^\circ\text{C}$ for 5 hours until the ash is free from carbon. The dish was removed and cooled in a desiccator and weighed immediately (Bandopadhyay *et al.*, 2016).

Calculation

Percentage of ash = $(W_2 - W) \times 100 / (W_1 - W)$

Where,

W = Weight in grams of empty crucible.

W₁ = Weight in grams of sample crucible.

W₂ = Weight in grams of sample with crucible after ashing.

3.2.6 Determination of total solids of lassi sample

The total solids content was determined as per BIS (1989) recommended for milk, using 10 mL of sample. Take 10 mL sample in a previously weighed dry aluminum dish. It was then kept in a hot air oven maintained at $101 \pm 1^\circ\text{C}$ for 2 hours to get the content dried and cooled in a desiccator, and weighed. The process of heating, cooling and weighing was repeated until the difference between two consecutive weights was less than 1 mg.

Calculation

Total solid percentage = $100 \times W / W_1$

Where,

W = Weight in g residue after drying

W₁ = Weight in g of sample taken for the test

3.2.7 Determination of sucrose content of lassi sample

Sugar content (Sucrose) percent contained in Lassi samples was worked out by subtracting reducing sugar (lactose) from total sugar of lassi.

Lactose was determined as per Lane-Eynon method given in IS: 1479-PART-II (1961).

Total sugar of lassi samples was determined as per procedure of Lane Eynon (1923) and modified by Ranganna (1977).

3.3 Analysis of lassi for physico-chemical analysis

Physico-chemical characteristics were analysed for the storage study. The lassi samples were kept for the storage study along with controls at ambient temperature. The treated samples as well as control were for their sensory attributes and chemical changes at different intervals viz., 0, 3, 5 and 7 days.

3.3.1 Determination of peroxide value of lassi samples

The peroxide value was determined following the method as given in ISI (1981) SP: 18 (Part XI).

Reagents

Solvent mixture: Mixture of glacial acetic acid and chloroform (2:1)

Potassium iodide powder

5% Potassium iodide solution

0.002N sodium thiosulphate solution

1% starch solution

Procedure

Taking the fat (1g) extracted by Rose-Gottlieb method in a test tube 1g KI and 20 ml solvent mixture was added. The solution was boiled for not more than 30 seconds. The solution was poured into conical flask containing 20 mL KI solution. The tube was washed with 25 mL distilled water than poured into the previous conical flask .Titrated against 0.002 N sodium thiosulphate solution using starch as indicator till the color of solution changed from black to colorless. The mL of sodium thiosulphate

used for titration was recorded. Similarly blank test was carried out using the same procedure without fat.

Calculation

Peroxide value = $(T-t) / W$

Where,

T = Volume in mL of 0.002N sodium thiosulphate solution required for sample

t = Volume in mL of 0.002N sodium thiosulphate solution for blank

W = Weight in g of sample

3.3.2 Determination of tyrosine value of lassi sample

The extent of protein breakdown was estimated in terms of tyrosine value as delineated by modified method of Juffs (1973).

Procedure

Five g of lassi sample was weighed in a beaker followed by addition of 5 mL distilled water and 30 % (w/v) tri-chloroacetic acid (TCA) each. The contents of the beaker were mixed well and kept undisturbed for few minutes (5 min) and thereafter filtered through Whatman No.42 filter paper. Then 0.1 mL of filtrate was transferred to 10 mL volumetric flask followed by addition of 5 ml alkaline copper tartarate and kept undisturbed for 10 min. Subsequently 0.5 mL of Folin-Ciocalteau reagent (0.667 N in 0.1 N HCl) was added and kept undisturbed for 30-45 min at 25-30°C. Afterwards distilled water was added to make up the volume and extinction was measured at a wave length of 700 nm. Extinction was equated to tyrosine concentration by means of a standard curve.

Preparation of Tyrosine Standard curve

Five mg of tyrosine was dissolved in 100 mL distilled water and different concentration of this solution (0.1 to 1 mL) was taken in different test tubes (Table 3.1). To this, 5 ml of alkaline copper tartarate solution (prepared by mixing 50 mL of reagent I and 1 ml of reagent II. Reagent I was prepared by dissolving 2 g of sodium carbonate in 100 ml of distilled water. Reagent II was prepared by dissolving 0.5 g of copper sulphate and 1 g of sodium potassium tartarate in 10 mL of distilled water) was added, mixed well and kept at room temperature for 10 min. To that 0.5 mL of Folin-Ciocalteau reagent was added and mixed well. This was kept at 25-30 °C for

30-45 min and the next in ction was measured at 700 nm. A standard curve was drawn, absorbance against tyrosine concentration (Fig.3.6). Tyrosine content of the sample was obtained from the standard curve and expresses in mg of /100 mL of filtrate as mentioned below

$$\text{Tyrosine (mg /100 mL of filtrate)} = \text{Tyrosine content in mg per 0.1 mL of filtrate} \times 100 / (0.1)$$



Fig. 3.5 Different tyrosine concentration for preparation of Tyrosine standard curve

Table 3.1 Standard Tyrosine concentration with respective optical density

Standard	
Optical Density	Tyrosine (mg/mL)
0	0
0.13	0.01
0.248	0.02
0.367	0.03
0.47	0.04
0.573	0.05

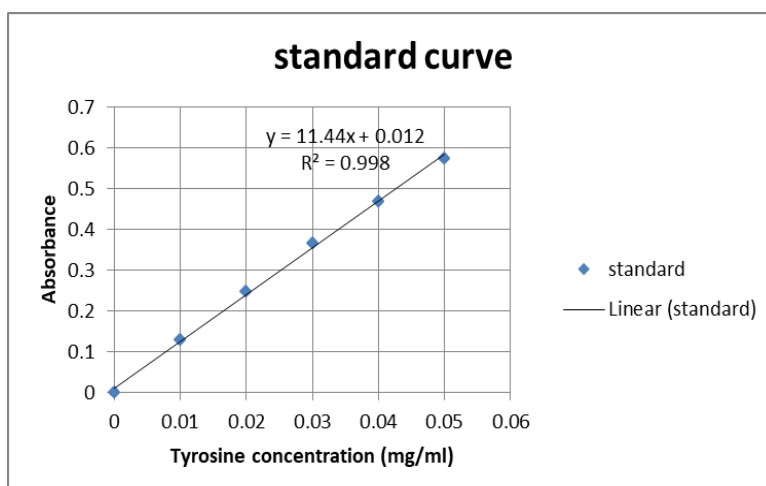


Fig. 3.6 Tyrosine standard curve

3.3.3 Determination of antioxidative activity of lassi samples

First prepare supernatants for determination of antioxidative characteristics of lassi samples. Hundred gram of lassi samples were taken in the centrifuge tubes and then centrifuged for 30 min (3000r.p.m), filtered (using whatman no.40) aseptically in sterile test tubes for analysis.

To determine antioxidative activity, two methods were followed, these are;

- ABTS [2,2'-azinobis (3 ethyl benzothiazoline)-6-sulfonic acid] method.
- DPPH (2,2 diphenyl-1-picryl hydrazyl) method.



Fig. 3.7 Filtration of supernatants after centrifugation

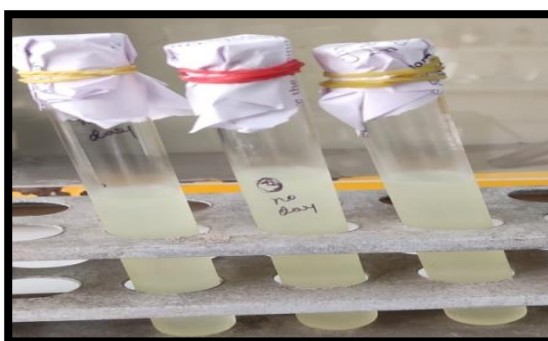


Fig. 3.8 Sample Supernatants after filtration

3.3.3.1 Measurement of antioxidant activity by ABTS method

Antioxidative activity of dahi was analysed following the spectrophotometric method given by Re *et al.*, 1999.

Reagents

Potassium Persulphate solution (2.45mM): Potassium Persulphate (13.2mg) was weighed and dissolved in 10 mL of double distilled water

ABTS solution (7Mm): ABTS (80 mg) was weighed and dissolved in 10 mL of double distilled water.

ABTS stock solution: The ABTS cation radical was produced by the reaction between 2.45mM potassium persulfate and 7mM ABTS in 1:1 ratio and stored in the dark at room temperature for 12-16 hrs.

ABTS working solution: 1 mL of ABTS stock solution was diluted with methanol till it gave an absorbance of 0.70 ± 0.02 at 734 nm. The stock solution of ABTS was stable up to 2 days for analytical purpose.

Procedure

ABTS working solution (3.9 mL) and 0.1 mL of appropriate diluted sample was mixed and kept at room temperature for 5 min and absorbance at 734 nm was recorded using spectrophotometer. Methanol was used as blank. Standard Trolox curve against ABTS is given in Fig. 3.9

Calculation

% ABTS free radical inhibition activity = $(\text{Blank abs} - \text{Sample abs}) \times 100 / \text{Blank abs}$

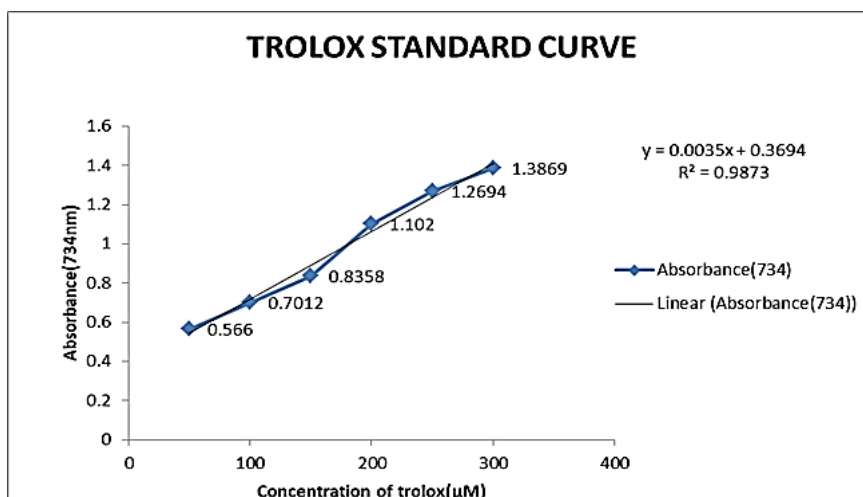


Fig. 3.9 Standard Trolox Curve (ABTS)

3.3.3.2 Measurement of antioxidative activity by DPPH method

Reagents

Methanolic extract preparation of sample: The extraction procedure was carried out based on method given by Mbunde *et al.* (2018) with slight modifications. 5g of lassi sample was extracted in 50 mL methanol for 48 hours at 37°C. It was then filtered through Whatman No. 1 filter paper. 0.5 mL filtrate was taken in test tube, To determine antioxidant activity, the methanolic extract of the sample was prepared

Antioxidant activity of extracts of lassi sample was determined by using the DPPH (2, 2- diphenyl-1-picrylhydrazyl) assay (Mbunde *et al.* (2018).

Procedure

Four mL of 0.2 mM DPPH solution was added in 1 mL of methanolic extract of sample followed by 2 mL of methanol, mixed properly and was incubated for 30 min in dark. The absorbance was measured at 517 nm in UV vis spectrophotometer. The control (Ac) consisted of 4 ml DPPH solution and 3 mL methanol. Methanol was used as blank.

Free radical scavenging activity expressed in terms of per cent inhibition was calculated using the formula

$$\text{Inhibition (\%)} = (Ac - A) \times 100 / (Ac)$$

Where, Ac is the absorbance of control and A is the absorbance of test.

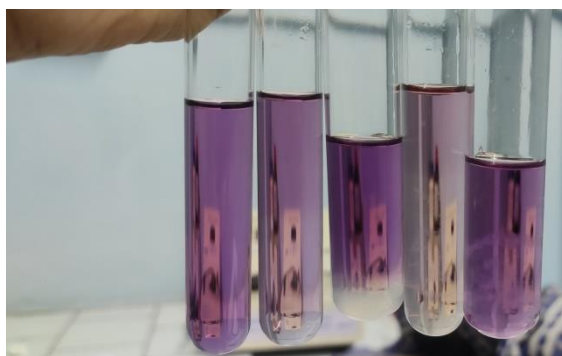


Fig. 3.10 Inhibition of free radical scavenging activity by DPPH method

3.4 Statistical analysis

The statistical analysis of the data obtained during the course of investigation was done for meaningful interpretation of the results. Statistical software IBM SPSS statistics 20 was used for statistical analysis of the results. Least significant difference at ($p < 0.05$) was used to determine significant differences between means from all the treatments.

CHAPTER-4

Results and Discussion...

4. RESULTS AND DISCUSSION

The present study was undertaken to Evaluation of Physico-Chemical Characteristics and Antioxidant Potential of Lassi samples available in the market of West Bengal simultaneously antioxidative activity of fermented ,non-fermented whey fortified samples and along with control (without addition of whey) samples were also evaluated. The results are described as follows.

4.1 Physico Chemical quality of lassi samples available in West Bengal Market

Five different types of lassi collected from West Bengal market were analyzed for fat, TS (total solid), ash, titratable acidity, protein, sucrose, pH, ABTS, DPPH and tyrosine value (Table 4.1). As no legal standard is available for lassi, so the results of the market sample could not be compared with any standard.

4.1.1 Fat content

Fat content of the lassi samples which were collected from market in West Bengal listed in Table 4.1. The average fat content of market lassi sample was observed to be in the range between $1.53\pm 0.01\%$ to $2.12\pm 0.01\%$. The values of fat content were significantly ($p < 0.05$) different among the samples. Bhoir *et al.* (2012) found that fat content of lassi samples is in the range of 3.26% to 2.88%. The finding of Bhoir *et al.* (2012) is slightly lower than the result of present study.

4.1.2 Total solids content

The average total solids content of lassi samples were observed to be in the range between $18.95\pm 0.05\%$ to $20.92\pm 0.01\%$. The range of total solids varied significantly ($p < 0.05$) among the five different samples of lassi from market. Different amount of total solids in lassi samples might be due to the curd quality, different manufacturing conditions. In case of market sample of lassi, various flavouring compounds that are used for test enhancing attributes might be responsible for difference in total solid content. With regard to total solids content of lassi, the results of present study were in agreement with those reported by Pagote and Balachandran (1993), Jadhav (1991) and Kalokhe (1991).

Table 4.1 Physico-chemical characteristics of five different types of lassi samples available in West Bengal market

Parameters	Market Sample				
	MS1	MS2	MS3	MS4	MS5
pH	4.28±0.02 ^b	4.13±0.05 ^c	4.44±0.04 ^a	4.20±0.02 ^{bc}	4.24±0.05 ^{bc}
Titrateable acidity (% LA)	0.57±0.02 ^a	0.56±0.03 ^a	0.54±0.01 ^a	0.46±0.01 ^b	0.43±0.01 ^b
Fat (%)	1.53±0.01 ^d	1.70±0.01 ^c	1.79±0.01 ^b	2.12±0.01 ^a	1.21±0.01 ^c
Protein (%)	2.36±0.02 ^a	1.29±0.01 ^d	2.12±0.01 ^b	2.08±0.04 ^b	1.53±0.01 ^c
Sucrose (%)	11.45±0.04 ^b	10.43±0.01 ^e	11.91±0.02 ^a	11.33±0.01 ^c	10.54±0.01 ^d
Total solids (%)	18.95±0.05 ^d	19.89±0.02 ^b	19.93±0.01 ^b	20.92±0.01 ^a	19.33±0.01 ^c
Ash (%)	0.44±0.01 ^d	0.43±0.01 ^b	0.56±0.01 ^b	0.67±0.01 ^a	0.45±0.01 ^c
ABTS [concentration of ABTS (µM) absorbed in terms of TEAC]	73.98±4.62 ^c	99.16±7.11 ^a	76.83±12.60 ^{ab}	77.37±0.94 ^{ab}	75.73±2.09 ^b
DPPH (% of inhibition)	9.65±0.37 ^{bc}	15.60±0.09 ^a	13.52±1.20 ^a	9.12±0.54 ^d	11.36±0.62 ^b
Tyrosine (mg/100 mL)	4.73±0.14 ^{ab}	4.72±0.06 ^b	3.80±0.12 ^c	4.47±0.55 ^b	5.34±0.04 ^a

Types of market samples is 5, each types of sample collected for 3 times (i.e. n = 15)

Each time analysis is done in triplicate

Values are mean of three replicates along with standard error

a,b,c,d,e different superscripts within a row denotes significant differences (p<0.05) among market samples

4.1.3 Ash content

The ash content of market samples of lassi have been shown in table 4.1. The average ash content of lassi was observed to be in the range between $0.43\pm 0.01\%$ to $0.67\pm 0.01\%$. The average value of ash differs significantly ($p < 0.05$) among lassi samples. The average values for total ash content in market lassi are in the range from 0.38% to 0.90% as reported by Ghule *et al.* (2016). The ash content of lassi as reported by Ghule *et al.* (2016) is lower than the present finding of the market sample analysis.

4.1.4 Titratable acidity (in terms of lactic acid)

Titrateable acidity of different market samples of lassi showed approximately non-significant ($p > 0.05$) difference. The lactic acid percent of MS1, MS2, and MS3 varied with each other non-significantly ($p > 0.05$), whereas MS4 varied non-significantly ($p > 0.05$) only with MS5. The titrateable acidity of samples varied from $0.43\pm 0.01\%$ lactic acid to $0.57\pm 0.02\%$ lactic acid. This result is lower than that reported by Bhoir *et al.* (2012), who observed the titrateable acidity (in terms of lactic acid) as 0.60% to 0.99%. Somewhat similar observation on acidity of lassi was recorded by De (1980), Jadhav (1991), Rathaur and Solankey (2002).

4.1.5 Protein content

The protein content of the five types of market lassi samples was analyzed. All the values are listed in Table 4.1. The average protein content of different types of lassi was observed to be in the range from $1.29\pm 0.01\%$ to $2.36\pm 0.02\%$. The protein content of lassi samples showed significant differences ($p < 0.05$) with each other. With regards to protein content in lassi, variable results were recorded by Pagote and Balachandran (1993). Chaudhari (1959) observed that protein in skim milk lassi ranges from 3.3 to 3.5%, whereas Laxminarayana and Shankar (1980) mentioned that protein content of lassi is 3.4%.

4.1.6 Sucrose content

The average sucrose content of different types of lassi samples were observed to be in the range from $10.43 \pm 0.01\%$ to $11.91 \pm 0.02\%$ (Table 4.1). Significant variation ($p < 0.05$) had been observed among all the samples. Differences in sucrose content might be due to the different milk composition, different amount of sugar added for enhancement of flavour and improvement of textural property, and for different processing conditions. Bhoir *et al.* (2012) reported a wide variation of sucrose content i.e. from 8.51% to 9.46% for lassi samples.

4.1.7 pH

The pH of most of the lassi samples available in market were significantly ($p < 0.05$) different. The average pH of lassi samples varied from 4.13 ± 0.05 to 4.44 ± 0.04 . The differences in pH might be due to the methods of preparation of each sample, the acidity of curd which was used, the amount of starter culture added during fermentation time indirectly affects to the pH of the final product. The pH in lassi sample was ranged from 3.83 to 4.34 as reported by Ghule *et al.* (2016).

4.1.8 Antioxidative activity

Lassi samples were analyzed to determine the antioxidative activity by ABTS [2,2'-azinobis (3 ethyl benzothiazoline)-6-sulfonic acid] and DPPH (2, 2 diphenyl-1-picryl hydrazyl) methods.

4.1.8.1 Antioxidant activity by ABTS [2,2'-azinobis (3 ethyl benzothiazoline)-6-sulfonic acid] method

Antioxidative activity of market lassi samples determined by ABTS method is given in Table 4.1. The antioxidative activity i.e. concentration (μM) of ABTS absorbed in terms of TEAC varied from 73.98 ± 4.62 to 99.16 ± 7.11 (Table 4.1). Antioxidative activity (by ABTS method) of MS1 varies significantly ($p < 0.05$) from other samples. Different types of starter cultures produce different types of peptides during fermentation, which are directly related with the significant variation in antioxidant activity.

4.1.8.2 Antioxidative activity by DPPH (2, 2 diphenyl-1-picryl hydrazyl) method

Antioxidative activity by DPPH method is given in Table 4.1. The antioxidative activity in terms of % of inhibition of DPPH at 517 nm was found to be in the range from 9.12 ± 0.54 to 15.60 ± 0.09 . Most of the market samples showed significantly ($p < 0.05$) different % of inhibition of DPPH.

No previous work has been found for antioxidant activity of market lassi samples. So the result for antioxidant activity of lassi samples determined by ABTS method and DPPH method could not be suitably compared. However, the significant difference observed in market lassi samples may be attributed to the differences in the number of days of storage on the collection day of different market samples and the packaging type of the samples. Samples collected in tetrapak exhibited higher antioxidant activity.

4.1.9 Tyrosine value

Tyrosine value of market lassi sample MS3 showed significant differences ($p < 0.05$) from other sample. Table 4.1 demonstrated the tyrosine value of market lassi samples. The average tyrosine value of lassi samples was observed to be in the range from 3.80 ± 0.12 mg of tyrosine/100mL to 5.34 ± 0.04 mg of tyrosine/100mL. Proteinase enzymes are of great importance regarding the changes in physico-chemical properties of lassi because they are responsible for proteolysis at low temperatures. Proteolysis might be attributed to the processing temperatures that cause unfolding of protein molecules thereby creating larger surface area or sites of action of bacterial enzymes. No research work has been done in tyrosine value of market lassi samples.

4.2 Physico-Chemical quality of whey fortified lassi sample

Three types of lassi samples such as lassi fortified with fermented whey (T1), lassi fortified with non-fermented whey (T2) and control lassi i.e. without whey (T3) were evaluated for the pH, titratable acidity, protein, sucrose, total solids and ash content (Table 4.2).

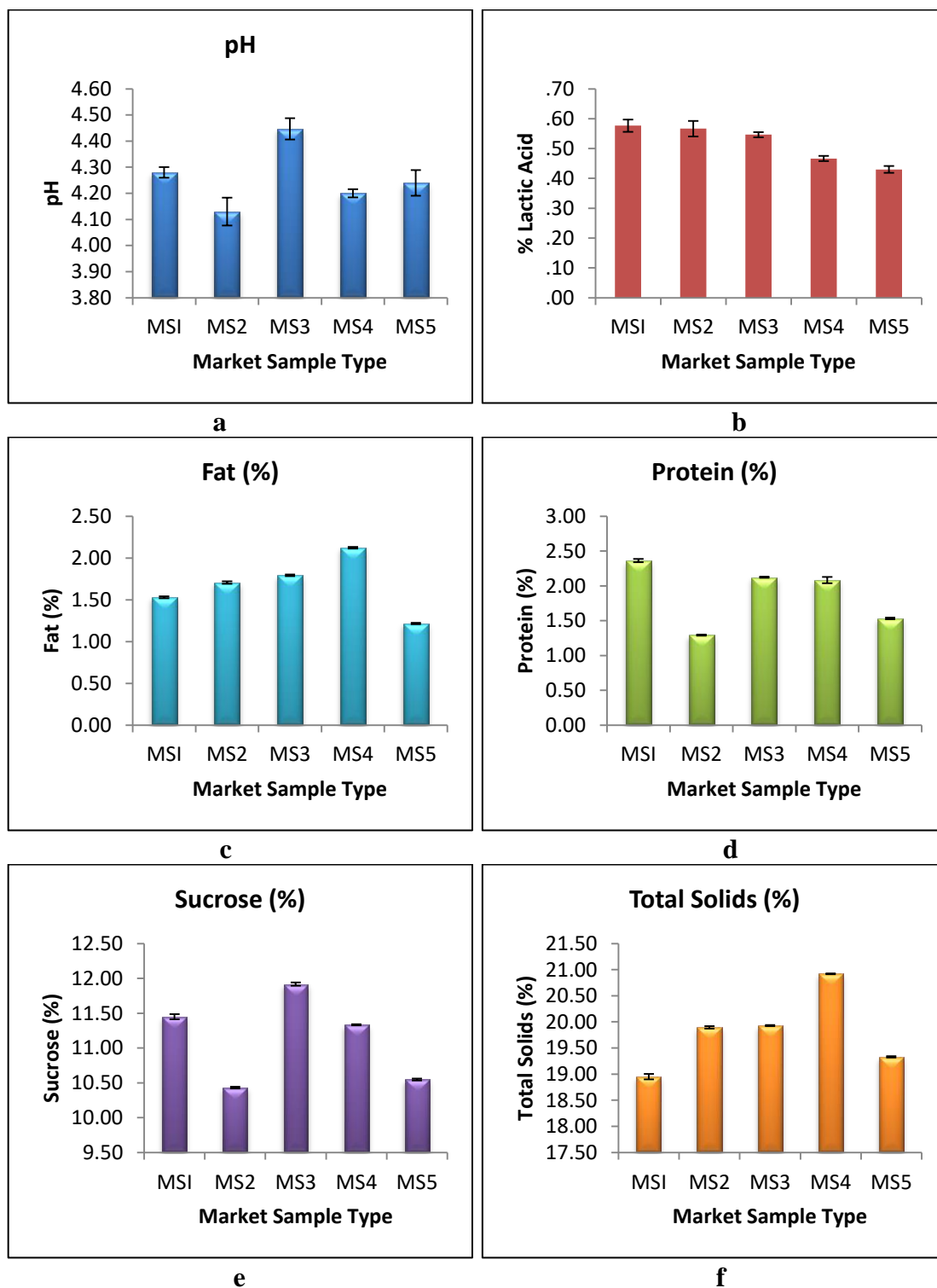


Fig. 4.1 (a) pH, (b) Titratable Acidity, (c) Fat content, (d) Protein content, (e) Sucrose content, (f) Total solids content of market sample of lassi

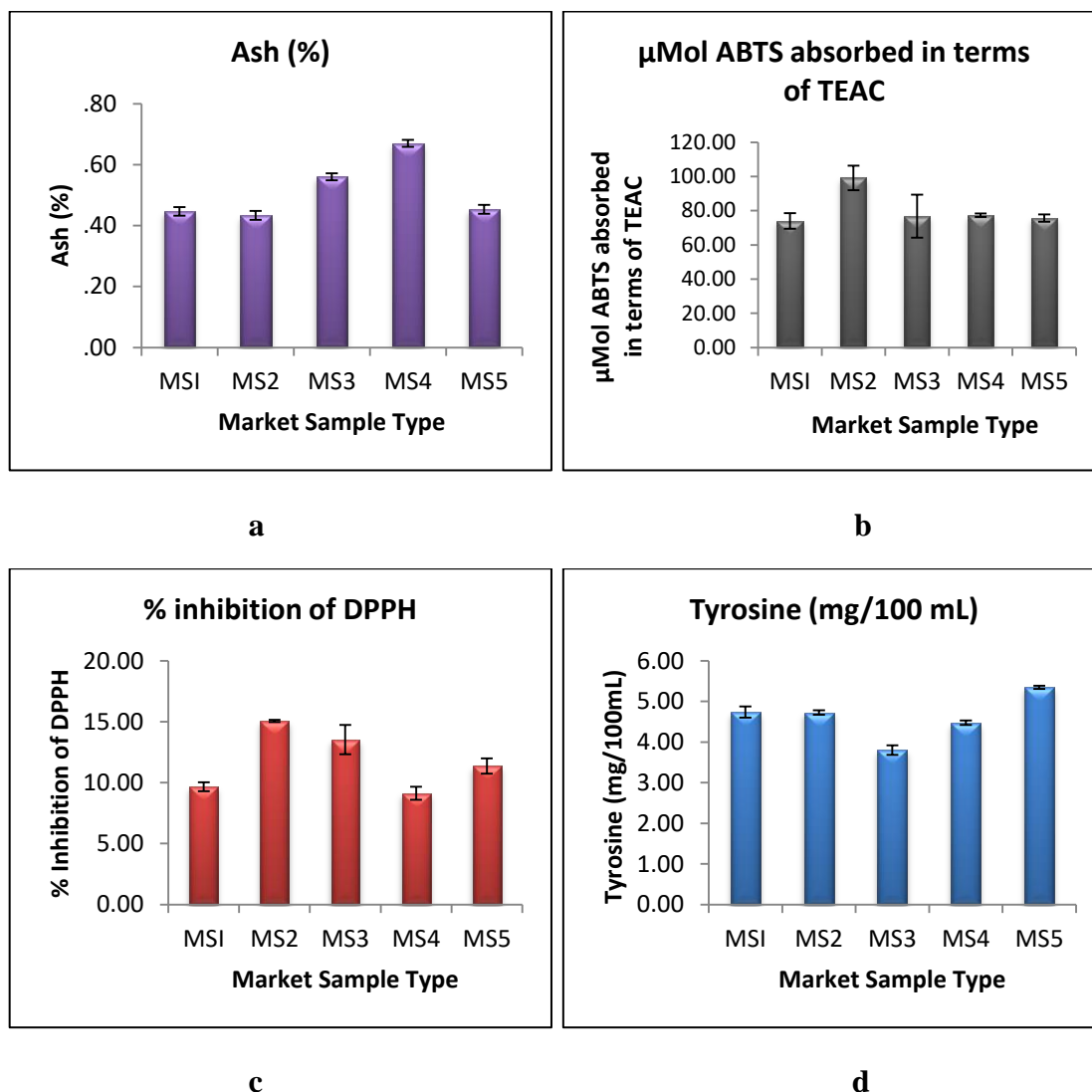


Fig. 4.2 (a) Ash content, (b) Antioxidant activity by ABTS, (c) Antioxidant activity by DPPH, (d) Tyrosine of market sample of lassi

4.2.1 pH

The pH of T1, T2 and T3 varied significant ($p < 0.05$). The average pH of T1 was observed to be 4.29 ± 0.04 , while for T3 it was only 4.56 ± 0.03 (Table 4.2). The differences in pH might be due to the methods of preparation of each sample, the acidity of curd which was used, the amount of starter culture added during fermentation of milk and whey, extent of fermentation.

4.2.2 Titratable acidity (in terms of lactic acid)

Titratable acidity of T1 differed significantly ($p < 0.05$) from T2 and T3. The average titratable acidity of partially fermented whey fortified lassi samples was observed to be $0.59 \pm 0.01\%$ lactic acid, while it was $0.50 \pm 0.02\%$ and $0.51 \pm 0.02\%$ of lactic acid for T2 and T3, respectively (Table 4.2). The difference in acidity among these three types of samples might be due to the fermentation process which was done in whey, the different acidity developed in curd due to starter culture activity.

Table 4.2 Physico- Chemical quality of whey fortified lassi samples

Parameters	T1	T2	T3
pH	4.29 ± 0.04^c	4.41 ± 0.01^b	4.56 ± 0.03^a
Titratable acidity (% LA)	0.59 ± 0.01^a	0.50 ± 0.02^b	0.51 ± 0.02^b
Fat (%)	1.85 ± 0.03^a	1.78 ± 0.01^a	1.49 ± 0.10^b
Protein(%)	2.25 ± 0.01^a	2.13 ± 0.01^b	1.78 ± 0.02^c
Sucrose (%)	11.28 ± 0.07^b	11.27 ± 0.03^b	11.91 ± 0.03^a
Total solids (%)	20.50 ± 0.02^a	19.42 ± 0.07^b	19.03 ± 0.20^c
Ash (%)	0.35 ± 0.01^b	0.35 ± 0.02^b	0.45 ± 0.01^a

T1: Partially fermented whey fortified lassi

T2: Non-fermented whey fortified lassi

T3: Control lassi without whey

Values are mean of three replicates along with standard error.

a,b,c different superscripts within a row denotes significant differences ($p < 0.05$) among samples.

4.2.3 Fat content

Whey fortified lassi samples T1 and T2 differed non-significantly ($p > 0.05$) from control lassi sample T3 (Table 4.2). The values were $1.85 \pm 0.03\%$, $1.78 \pm 0.01\%$ and $1.49 \pm 0.10\%$ for T1, T2 and T3, respectively (Table 4.2).

4.2.4 Protein content

The protein content of the whey fortified lassi sample (T1 and T2) and control sample (T3) were shown in Table 4.2. A significant difference ($p < 0.05$) was observed among these three types of samples. The reasons of differences in protein content in three type lassi might be due to the fermentation carried out for whey. Fermented whey fortified lassi produced more peptides than non-fermented whey fortified lassi and lassi without addition of whey sample.

4.2.5 Sucrose content

The average sucrose content of control lassi sample which was $11.91 \pm 0.03\%$ differed significantly ($p < 0.05$) from T1 and T3. The sucrose content of fermented whey fortified lassi sample and non-fermented whey sample was $11.28 \pm 0.07\%$ and $11.27 \pm 0.03\%$, respectively (Table 4.2).

4.2.6 Total solids content

The average total solids content of lassi samples were observed to be $20.50 \pm 0.02\%$, $19.42 \pm 0.07\%$ and $19.03 \pm 0.20\%$ for T1, T2 and T3, respectively as shown in Table 4.2. The range of total solids varied significantly ($p < 0.05$) among three samples. The reasons of various significance is due to addition of different amount of various components in lassi which is directly responsible for enhancing the amount of total solids to get the desirable taste and mouthfeel, texture of different three products.

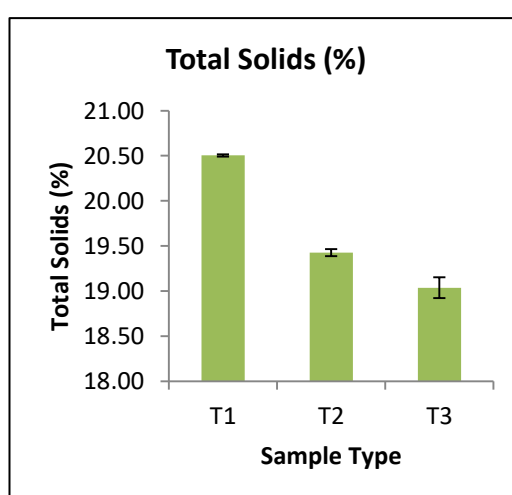


Fig. 4.3 Total Solids content of fermented fortified lassi (T1), non-fermented whey fortified lassi (T2), control lassi sample (T3)

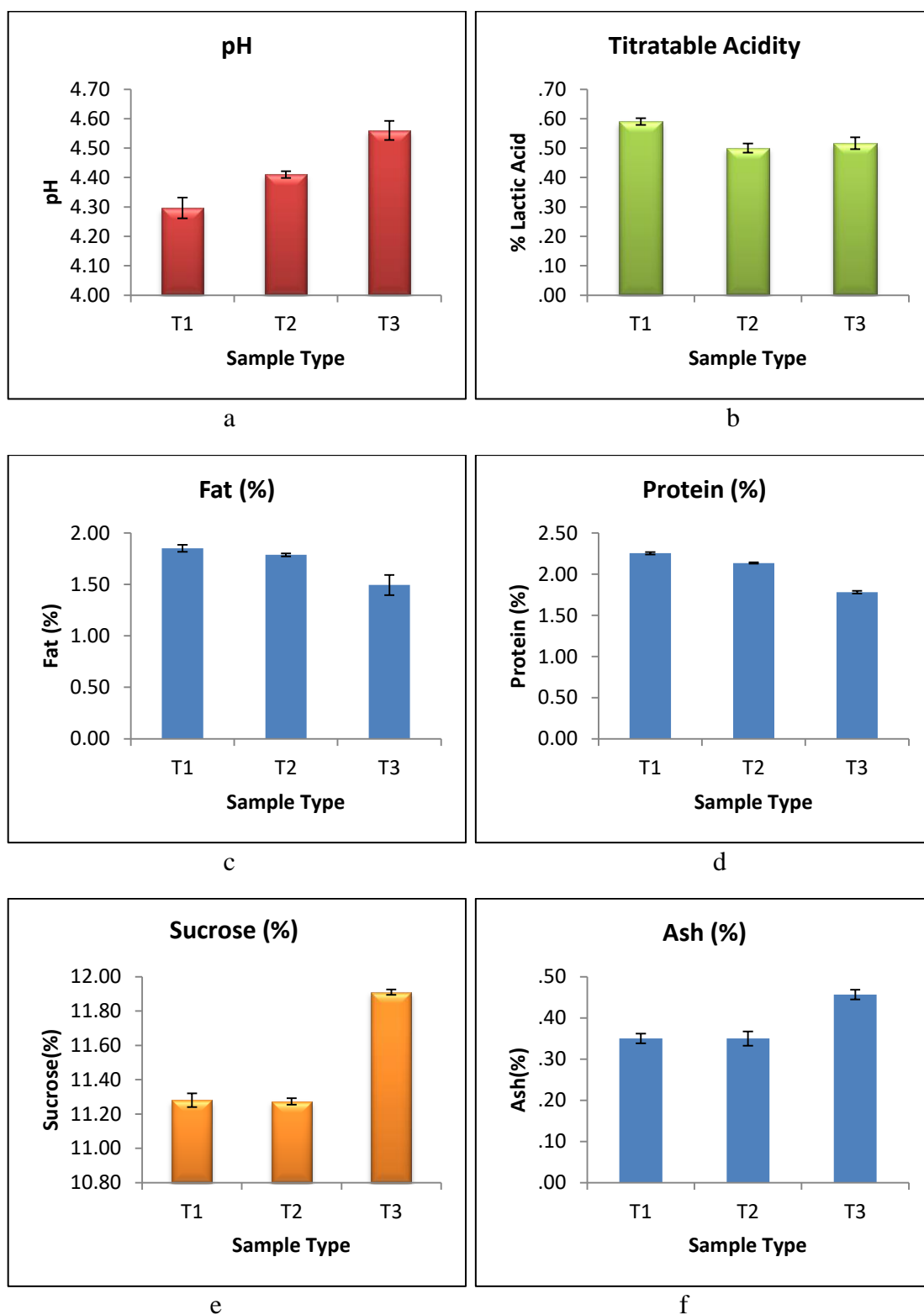


Fig. 4.4 (a) pH, (b) Titratable Acidity (in terms of lactic acid), (c) Fat content, (d) Protein content, (e) Sucrose content, (f) Ash content of fermented whey fortified samples (T1), non-fermented whey fortified samples (T2), control sample (T3)

4.2.7 Ash content

The ash content is shown in Table 4.2. The average ash content of lassi samples was observed to be $0.35\pm 0.01\%$ and $0.35\pm 0.02\%$ for T1 and T2, respectively. The ash content of both T1 and T2 differed significantly ($p < 0.05$) from T3. The ash content provided an idea of mineral content present in lassi samples.

4.3 Changes in physico-chemical characteristics and antioxidant activity of whey fortified lassi and control lassi during storage at $7\pm 2^\circ\text{C}$

Fermented whey and non-fermented whey fortified lassi (T1 and T2, respectively) and control sample without whey (T3) was stored at $7\pm 2^\circ\text{C}$ and evaluated for its peroxide value, tyrosine value and antioxidant activity during storage of 7 days at 2 days interval.

4.3.1 Changes in peroxide value of whey fortified lassi and control lassi during storage at $7\pm 2^\circ\text{C}$

The peroxide value of any product is directly related with the secondary phase of lipid oxidation. Peroxide value of whey fortified lassi and control lassi measured the extent of lipid oxidation. Peroxide value of whey fortified lassi increased with the progression of storage non significantly ($p > 0.05$) (Table 4.3). The peroxide value of all the samples differed non-significantly ($p > 0.05$) with each other during storage. At the 7th day of storage peroxide value was 0.52 ± 0.02 mEq/kg of fat, 0.48 ± 0.02 mEq/kg of fat and 0.48 ± 0.17 mEq/kg of fat for T1, T2 and T3, respectively (Table 4.3).

During storage fat became rancid due to the oxidative rancidity in fat. Peroxide value mainly varies with the age of the product. No research work has been done on peroxide value on fermented and non-fermented whey fortified lassi during storage period. The average peroxide value of dahi sample was found to be 0.344 ± 0.009 mEq/kg of fat (Chatterjee *et al.*, 2022). However, no such findings are available on peroxide value of lassi during storage.

Table 4.3 Changes in peroxide value of whey fortified lassi and control lassi during storage at 7±2°C

Days	Peroxide value (mEq/kg of fat)		
	T1	T2	T3
0	0.48±0.02 ^{aA}	0.42±0.02 ^{bA}	0.42±0.02 ^{abA}
3	0.52±0.02 ^{aA}	0.49±0.01 ^{aA}	0.42±0.02 ^{abB}
5	0.52±0.04 ^{aA}	0.51±0.02 ^{aA}	0.38±0.02 ^{bB}
7	0.52±0.02 ^{aA}	0.48±0.02 ^{aA}	0.48±0.17 ^{aA}

T1: Partially fermented whey fortified lassi

T2: Non-fermented whey fortified lassi

T3: Control lassi without whey

Values are mean of three replicates along with standard error.

A,B different superscript within a row denotes significant (p<0.05) difference among sample types

a,b different superscripts within a column denotes significant (p< 0.05) differences among day.

4.3.2 Changes in tyrosine value of whey fortified lassi and control lassi during storage at 7±2°C

Proteolysis is one of the main key to identify the spoilage of the product. Proteinase enzymes which are responsible for proteolysis have the great impact on physico chemical properties of lassi as they cause proteolysis at low temperature. Processing temperature might cause unfolding of protein molecules and thereby create larger surface area or sites of action for bacterial enzymes. Table no 4.4 demonstrated the tyrosine value of sample which varied significantly (p< 0.05) day wise as well as type wise also. The average tyrosine value was observed to be 4.88±0.035, 4.29±0.03 and 3.68±0.02 mg of tyrosine/100 mL for T1, T2 and T3, respectively. Greater the tyrosine value, greater the degree of proteolysis and vice versa. The tyrosine value increases with the increase of days. Significantly (p<0.05) higher proteolysis was observed in fermented whey fortified sample than other. The average tyrosine value

of dahi sample was observed to be 0.276 ± 0.008 mg of tyrosine/mL according to Chatterjee *et al.* (2022). No findings are available the tyrosine value of lassi.

Table 4.4 Changes in tyrosine value of whey fortified lassi and control lassi during storage at $7 \pm 2^\circ\text{C}$

Days	Tyrosine (mg/100 mL)		
	T1	T2	T3
0	$4.12 \pm 0.03^{\text{dA}}$	$3.08 \pm 0.02^{\text{dB}}$	$2.85 \pm 0.02^{\text{dC}}$
3	$4.18 \pm 0.01^{\text{cA}}$	$4.00 \pm 0.06^{\text{cB}}$	$2.92 \pm 0.01^{\text{cC}}$
5	$4.61 \pm 0.02^{\text{bA}}$	$4.13 \pm 0.01^{\text{bB}}$	$3.16 \pm 0.01^{\text{bC}}$
7	$4.88 \pm 0.04^{\text{aA}}$	$4.29 \pm 0.03^{\text{aB}}$	$3.68 \pm 0.02^{\text{aC}}$

T1: Partially fermented whey fortified lassi, T2: Non-fermented whey fortified lassi

T3: Control lassi without whey

Values are mean of three replicates along with standard error.

A,B,C different superscript within a row denotes significant ($p < 0.05$) difference among sample types; a,b,c different superscripts within a column denotes significant ($p < 0.05$) differences among day.

4.3.3 Changes in antioxidative activity of whey fortified lassi and control lassi as measured by ABTS [2,2' - azinobis - (3-ethylbenzothiazoline – 6 - sulfonic acid)] method during storage at $7 \pm 2^\circ\text{C}$

Antioxidative activity of whey fortified lassi along with control lassi sample determined by ABTS method is given in Table 4.5. Table 4.5 demonstrated the antioxidative activity in ABTS method with an average value of 67.01 ± 1.10 , 44.61 ± 1.49 , 73.49 ± 0.54 [in terms of concentration (μM) of ABTS absorbed in terms of TEAC] for T1, T2 and T3, respectively at 7th day. Whey fortified lassi along with control lassi samples varied significantly ($p < 0.05$) during storage period. These antioxidant properties of the samples increased gradually with storage upto certain extent and then again decreased with progression of storage. Different types of starter culture produced different types of peptides during fermentation of whey and

throughout the storage period of lassi, which might be the reason for significant ($p < 0.05$) variation in antioxidant activity. Whey proteins donate hydrogen to reduce free radicals and free sulfhydryl groups from cysteine, are effective at inhibiting lipid auto-oxidation and, the presence of antioxidant enzyme (e.g., superoxidimutase and catalase) and non-enzymatic antioxidants (e.g., tocopherols, carotenoids, citrate, phosphate and ascorbic acid) in lassi might also contribute to the overall antioxidative effect.

4.3.4 Changes in antioxidative activity of whey fortified lassi and control lassi as measured by DPPH (2, 2 diphenyl-1-picryl hydrazyl) method during storage at $7 \pm 2^\circ\text{C}$

Antioxidative activity by DPPH method is given in Table 4.6. The average antioxidative activity in terms of (% of inhibition) DPPH at 517 nm was found to be 15.90 ± 0.08 % for T1, 15.63 ± 0.03 % for T2 and 12.41 ± 0.02 % for T3 at 7th day. The antioxidant activity of T1 was the highest and T3 exhibited the lowest value at 7th day of storage. The antioxidant activity of those samples increased during storage period.

No previous work has been found for antioxidant activity of whey fortified lassi samples (fermented, non-fermented along with control). So the result for antioxidant activity of lassi samples determined by ABTS method and DPPH method could not be suitably compared. However the significant difference observed in whey fortified lassi and control lassi samples may be attributed to the differences in the number of days of storage at the 2 days of interval. Samples with partially fermented whey fortified lassi should have higher antioxidant activity.

Significant differences of antioxidant activity between control, fermented and non-fermented fortified whey may be attributed to the fermentation in the whey in case of fermented whey fortified samples and the inherent antioxidant activity of non-fermented whey in case of non-fermented whey fortified samples.

Table 4.5 Changes in antioxidative activity of whey fortified lassi and control lassi as measured by ABTS method during storage at 7±2°C

Days	In terms of concentration (µM) of ABTS by TEAC		
	T1	T2	T3
0	77.22±0.50 ^{bAB}	80.78±4.43 ^{aA}	70.05±0.67 ^{dB}
3	87.23±0.92 ^{aA}	80.87±2.62 ^{aB}	76.29±0.66 ^{bB}
5	78.63±0.51 ^{bA}	62.39±2.12 ^{bB}	79.19±0.53 ^{aA}
7	67.01±1.10 ^{cB}	44.61±1.49 ^{cC}	73.49±0.54 ^{cA}

T1: Partially fermented whey fortified lassi, T2: Non-fermented whey fortified lassi

T3: Control lassi without whey

Values are mean of three replicates along with standard error.

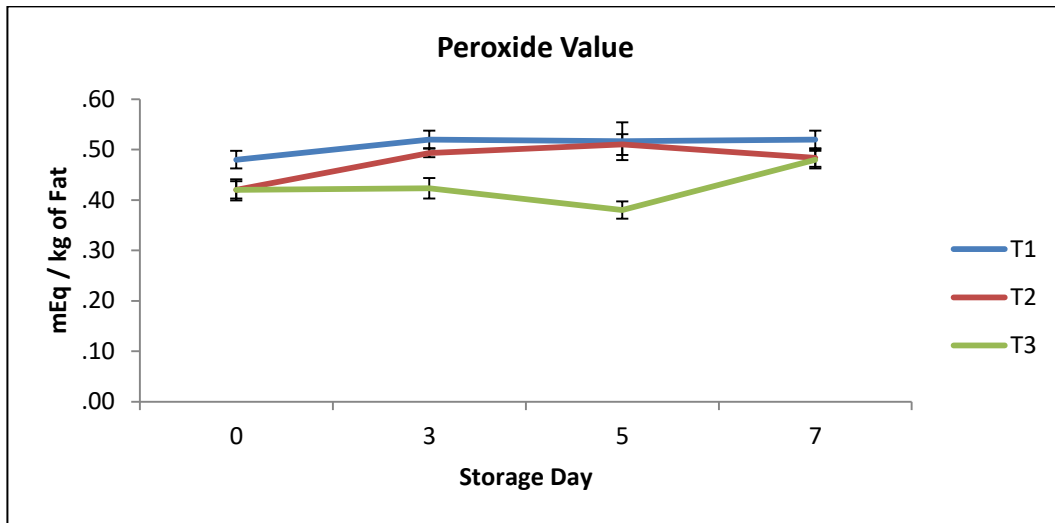
A,B,C different superscript within a row denotes significant (p<0.05) difference among sample types; a,b,c different superscripts within a column denotes significant (p< 0.05) differences among day.

Table 4.6 Changes in antioxidative activity of whey fortified lassi and control lassi as measured by DPPH (2, 2 diphenyl-1-picryl hydrazyl) method during storage at 7±2°C

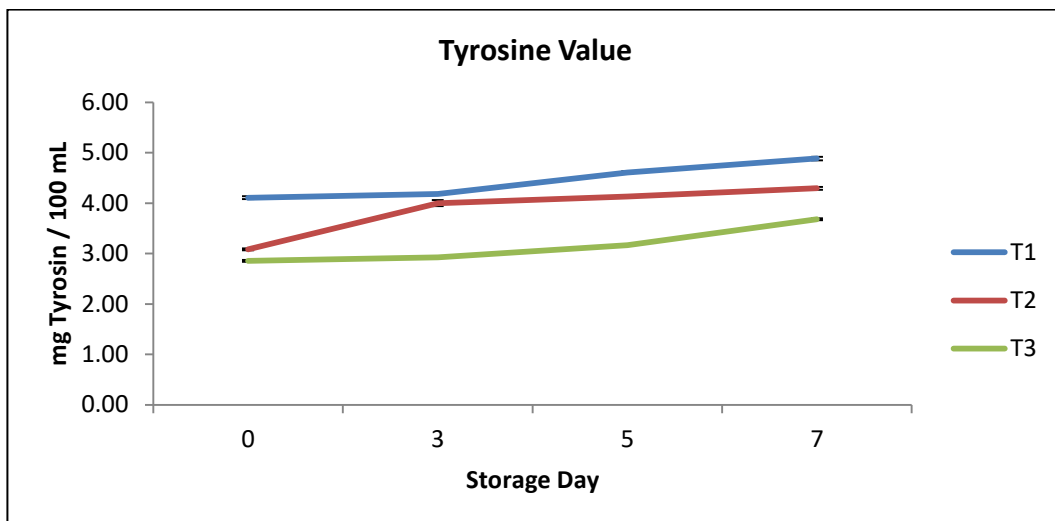
Day	DPPH (% of inhibition)		
	T1	T2	T3
0	11.94±0.05 ^{dA}	10.21±0.03 ^{dC}	11.57±0.01 ^{aB}
3	13.85±0.03 ^{cA}	13.52±0.25 ^{cA}	11.77±0.01 ^{bB}
5	15.25±0.03 ^{bA}	14.59±0.57 ^{bA}	12.13±0.07 ^{cB}
7	15.90±0.08 ^{aA}	15.63±0.03 ^{aB}	12.41±0.02 ^{aC}

T1: Partially fermented whey fortified lassi, T2: Non-fermented whey fortified lassi

T3: Control lassi without whey; Values are mean of three replicates along with standard error. A,B,C different superscript within a row denotes significant (p<0.05) difference among sample types; a,b,c different superscripts within a column denotes significant (p< 0.05) differences among day.

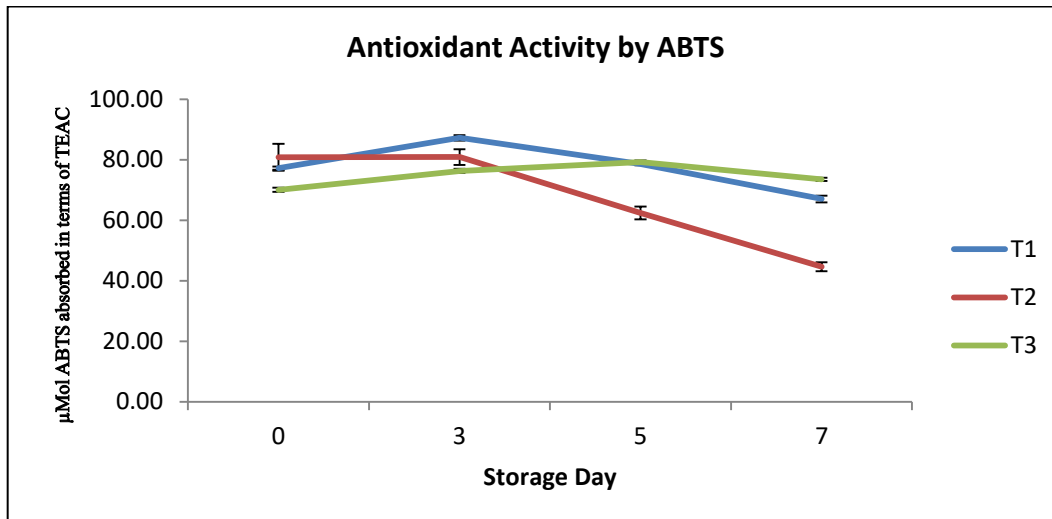


a

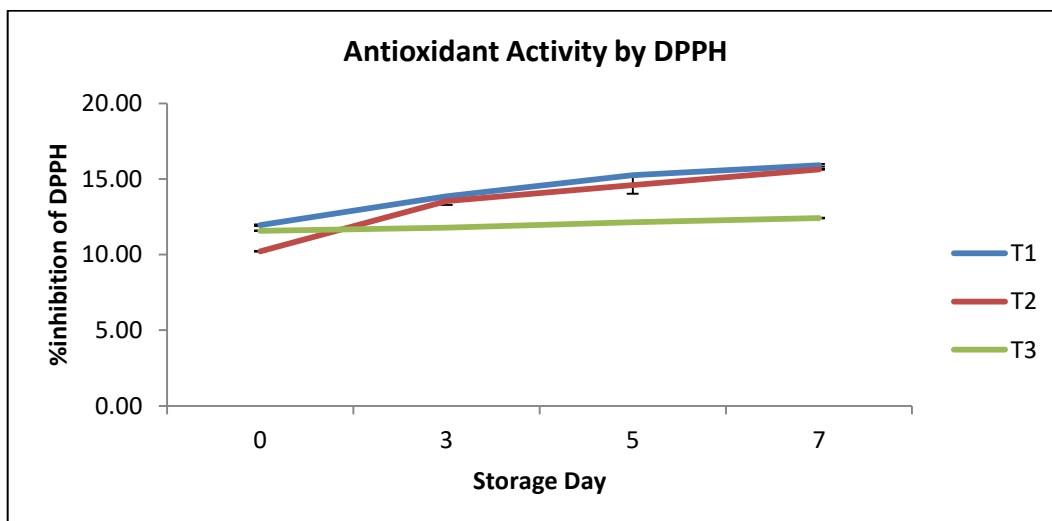


b

Fig. 4.5 Changes in (a) peroxide value and (b) tyrosine value of fermented whey fortified lassi (T1), non-fermented whey fortified lassi (T2) and Control lassi (T3) during storage at $7\pm 2^{\circ}\text{C}$.



a



b

Fig. 4.6 Changes in antioxidant activity of fermented whey fortified lassi (T1), non-fermented whey fortified lassi (T2) and Control lassi (T3) by (a) ABTS method and (b) DPPH method during storage at $7\pm 2^\circ\text{C}$.

CHAPTER-5

Summary and Conclusion...

5. SUMMARY AND CONCLUSION

Lassi is a fermented thrust quenching beverage especially in summer and very popular throughout India. It provides various functional and nutritious ingredients to human diet. A Large population of people consumes lassi in summer according to research study.

In this study, 5 types of different Lassi samples were collected from the market of West Bengal and three types of samples such as partially fermented whey fortified lassi, non-fermented whey fortified lassi and control lassi without whey were collected from experiential learning unit (POS) with prior information.

Salient features of study are

The market lassi samples were collected from different shops of West Bengal market and analyzed for Physico-chemical, antioxidative properties.

1. The average fat, protein, ash, total solids and sucrose content, pH and titratable acidity of lassi samples was observed to be in the range between $1.53\pm 0.01\%$ to $2.12\pm 0.01\%$, $1.29\pm 0.01\%$ to $2.36\pm 0.02\%$, $0.43\pm 0.01\%$ to $0.67\pm 0.01\%$, $18.95\pm 0.05\%$ to $20.92\pm 0.01\%$ and $10.43\pm 0.01\%$ to $11.91\pm 0.02\%$, 4.13 ± 0.05 to 4.44 ± 0.04 and $0.43\pm 0.01\%$ lactic acid to $0.57\pm 0.02\%$ lactic acid, respectively. Statistically significant variation was observed in terms of fat, protein, ash, sucrose, total solids content, pH and titratable acidity among all 5 different types of market samples.

2. Market lassi samples were analyzed to determine their antioxidative activity by ABTS and DPPH methods. Antioxidative activity of market lassi samples determined by ABTS method in terms of concentration (μM) of ABTS varies from 73.98 ± 4.62 μM to 99.16 ± 7.11 μM . Antioxidative activity by DPPH method in terms of (% of inhibition of DPPH) at 517 nm found to be in the range from $9.12\pm 0.54\%$ to $15.60\pm 0.09\%$. The tyrosine value of market lassi samples which was estimated varied significantly ($p < 0.05$) from 3.80 ± 0.12 mg of tyrosine/100ml to 5.34 ± 0.04 mg of tyrosine/100ml.

3. Three types of lassi samples such as lassi fortified with fermented whey, lassi fortified with non-fermented whey and control lassi i.e. without whey were evaluated for the pH, titratable acidity, protein, sucrose, total solids and ash content.
4. The pH of all samples varied significantly ($p < 0.05$) with each other. The average pH of partially fermented whey fortified lassi was observed to be 4.29 ± 0.04 , while for control sample it was only 4.56 ± 0.03 .
5. The average titratable acidity of partially fermented whey fortified lassi samples was observed to be $0.59 \pm 0.01\%$ lactic acid, while it was $0.50 \pm 0.02\%$ and $0.51 \pm 0.02\%$ of lactic acid for non-fermented whey fortified lassi and control sample, respectively.
6. Whey fortified lassi samples differed non-significantly ($p > 0.05$) from control lassi sample for their fat content. The values were $1.85 \pm 0.03\%$, $1.78 \pm 0.01\%$ and $1.49 \pm 0.10\%$ for lassi fortified with fermented whey, lassi fortified with non-fermented whey and control lassi, respectively.
7. The protein content of lassi fortified with fermented whey, lassi fortified with non-fermented whey and control lassi were $2.25 \pm 0.01\%$, $2.13 \pm 0.01\%$ and $1.78 \pm 0.02\%$, respectively.
8. The average sucrose content of control lassi sample which was $11.91 \pm 0.03\%$ differed significantly ($p < 0.05$) from other samples. The sucrose content of fermented whey fortified lassi sample and non-fermented whey sample was $11.28 \pm 0.07\%$ and $11.27 \pm 0.03\%$, respectively.
9. The average total solids content of lassi samples were observed to be $20.50 \pm 0.02\%$, $19.42 \pm 0.07\%$ and $19.03 \pm 0.20\%$ for lassi fortified with fermented whey, lassi fortified with non-fermented whey and control lassi, respectively. The range of total solids varied significantly ($p < 0.05$) among three samples.
10. The average ash content of lassi samples was observed to be $0.35 \pm 0.01\%$ and $0.35 \pm 0.02\%$ for lassi fortified with fermented whey, lassi fortified with non-fermented whey, respectively.

11. Fermented whey and non-fermented whey fortified lassi and control sample without whey was stored at $7\pm 2^{\circ}\text{C}$ and evaluated for its peroxide value, tyrosine value and antioxidant activity during storage of 7 days at 2 days interval.

12. Peroxide value of whey fortified lassi increased with the progression of storage non significantly ($p>0.05$). The peroxide value of all the samples differed non-significantly ($p>0.05$) with each other during storage. At the 7th day of storage peroxide value was 0.52 ± 0.02 mEq/kg of fat, 0.48 ± 0.02 mEq/kg of fat and 0.48 ± 0.17 mEq/kg of fat for fermented whey and non-fermented whey fortified lassi and control sample, respectively.

13. It was observed that the tyrosine value of samples varied significantly ($p< 0.05$) day wise as well as type wise. The average tyrosine value was observed to be 4.88 ± 0.035 , 4.29 ± 0.03 and 3.68 ± 0.02 mg of tyrosine/100 ml for fermented whey and non-fermented whey fortified lassi and control sample, respectively.

14. The antioxidative activity as determined by ABTS method was 67.01 ± 1.10 , 44.61 ± 1.49 , 73.49 ± 0.54 μM of ABTS absorbed in terms of TEAC for fermented whey and non-fermented whey fortified lassi and control sample, respectively at 7th day of storage. Whey fortified lassi along with control lassi samples varied significantly ($p<0.05$) during storage period.

15. The average antioxidative activity in terms of (% of inhibition) DPPH at 517 nm was found to be 15.90 ± 0.08 % for fermented whey fortified lassi, $15.63\pm 0.03\%$ for non-fermented whey fortified lassi and $12.41\pm 0.02\%$ for control sample at 7th day of storage. The antioxidant activity of fermented whey fortified lassi was the highest and control sample exhibited the lowest value at 7th day of storage. The antioxidant activity of those samples increased during storage period.

Conclusion

Lassi is a popular traditional fermented dairy product, prepared by various methods in the market. The study on market sample analysis revealed the variation of compositions, physico-chemical and antioxidant properties of the lassi samples. The potential of lassi with fortified whey was observed during the investigation as it

exhibited more nutritional value and antioxidant activity than the plain lassi. The fortification of lassi with fermented whey will open a wide market scope due to the increased awareness of the consumer, willingness to buy functional and nutritious products. The development of whey fortified lassi will also enhance the interest for utilization of whey, which will be beneficial in terms of socio-economical point of view and helpful for minimization of the environmental hazards.

CHAPTER-6

Future Scope of Research...

6. FUTURE SCOPE OF RESEARCH

There are various areas where thrust can be provided for further research.

- Determination of ACE inhibitory activity, OPA activity, antihypertensive activity and other functional properties of whey fortified lassi sample and market lassi sample available in West Bengal.
- Determination of antioxidant activity of lassi sample (market sample as well as whey fortified sample) by other two methods viz. ORAC and FRAP method.
- Determination of antimicrobial activity of lassi sample available in West Bengal market and whey fortified lassi sample.
- Isolation and characterization of starter cultures collected from lassi samples.
- Preparation of lassi from the isolated starter cultures.

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