DEVELOPMENT OF TEA WHITENER USING CONCENTRATED WHEY AND MILK SOLIDS

THESIS SUBMITTED TO THE

NATIONAL DAIRY RESEARCH INSTITUTE

(DEEMED UNIVERSITY)

IN PARTIAL FULFILMENT OF THE REQUIREMENTS

FOR THE AWARD OF THE DEGREE OF

MASTER OF TECHNOLOGY
IN
DAIRY TECHNOLOGY

By

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B.Tech. (Dairy Science &Technology)

DIVISION OF DAIRY TECHNOLOGY
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BANGALORE - 560 030, INDIA

2012

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I

DEDICATE

MY THESIS

TO

MY FAMILY, FRIENDS &

TEACHERS........

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Approved By

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CERTIFICATE

This is to certify that the thesis entitled "DEVELOPMENT OF TEA WHITENER USING CONCENTRATED WHEY AND MILK SOLIDS", submitted by MR. JYOTHIS JOY M. towards the partial fulfilment for the award of the degree of MASTER OF TECHNOLOGY in DAIRY TECHNOLOGY of the NATIONAL DAIRY RESEARCH INSTITUTE (DEEMED UNIVERSITY), Karnal (Haryana), India, is a bonafide research work carried out by him under my supervision and guidance and no part of the thesis has been submitted for any other degree or diploma.

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Date:

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ABSTRACT

Whey is an underutilized dairy by-product with superior nutritional and health promoting properties. Cost of whitening beverages like tea and coffee can be reduced if the whey solids are used effectively. Suitability of whey as ingredient for dairy whitener is investigated in the study. Cheese whey with pH 6.2±0.1 was concentrated to 12.0% solids in a vacuum evaporator, pasteurized at 72±2°C for 15 s and stored for 16-18 hours at 5-7°C. The concentrate was diluted to 11.5% solids by addition of potable water. It was then cold blended with toned milk of 11.5% solids. Blends containing whey to toned milk in ratios of 75:25, 50:50 and 25: 75 were prepared and homogenized at 1200- 1500 psi. Decoction was prepared from 2.5 g tea powder per 100 ml water. The samples were compared with unhomogenised and control sample for sensory acceptance. The whitener with 25% whey and subsequently homogenized was of acceptable quality. The final tea beverage was evaluated for sensory parameters and physico-chemical quality attributes. Tea prepared from blends containing 25% whey and which was homogenized was found to give good mouth feel and flavour. The colour of the beverages were analyzed by reflectance meter and computer vision, and found to have significant difference with the control. Among the treatments tea prepared from homogenized 25% blend had the highest reflectance value and lowest browning index. Tea prepared from 50% blends also scored high on sensory aspects but fared poorly with respect to colour. The viscosity results indicated that homogenization had a positive impact on the viscosity. Coffee stability test was done to assess the stability of whitener under acidic conditions and it was observed that blend with 25% whey was comparable to toned milk. Standard plate count, Coliform count and presumptive coliform test were done to assess the microbiological quality of the whitener. The whitener was observed to have a shelf life of 2 days at 7±2°C. Prominent whey flavour was noticed beyond 2nd day storage which limited its shelf life. Based on the observations it is concluded that a satisfactory tea whitener can be prepared by blending whey with toned milk in 25:75 proportion and homogenization as one of the processing parameters.

सारांश

मट्ठा बेहतर पोषण और स्वास्थ्य को बढ़ावा देने के गुण के साथ उत्पाद द्वारा एक underutilized डेयरी है. अगर मट्ठा ठोस प्रभावी ढंग से इस्तेमाल कर रहे हैं चाय और कॉफी जैसे पेय पदार्थ दांत की लागत कम किया जा सकता है. अध्ययन में डेयरी व्हाइटनर के लिए घटक के रूप में मट्ठा की उपयुक्तता की जांच की है. पीएच 6.2 के साथ प्रयोगात्मक डेयरी संयंत्र ± 0.1 से पनीर मट्ठा एक वैक्यूम उद्वाष्पक में 12.0% ठोस, 72 पर 15 s pasteurized का ध्यान केंद्रित किया गया था ± 2/15 डिग्री सेल्सियस और 5-7 डिग्री सेल्सियस पर 16-18 घंटे के लिए भंडारित किया ध्यान केंद्रित के पीने के पानी के अलावा 11.5% ठोस पतला था. यह तो था ठंड toned 11.5% ठोस दूध के साथ मिश्रित. Toned 75:25, 50:50 और 25 के अनुपात में दूध मट्ठा युक्त मिश्रणों: 75 तैयार किया गया और 1200 में homogenized - 1500 साई. काढ़ा बनाने का कार्य 100 मिलीलीटर पानी प्रति 2.5 ग्राम चाय पाउडर से तैयार किया गया था. नमूने संवेदी स्वीकृति के लिए unhomogenised और नियंत्रण नमूना के साथ तुलना की गई. 25% मट्ठा और बाद में homogenized साथ व्हाइटनर स्वीकार्य गुणवत्ता की थी. अंतिम चाय पेय संवेदी मापदंडों और भौतिक रासायनिक गुणवत्ता विशेषताओं के लिए मूल्यांकन किया गया था. चाय 25% मट्ठा युक्त मिश्रणों से तैयार है और जो अच्छा मुँह लग रहा है और स्वाद दे पाया था homogenized गया था. पेय पदार्थ के रंग reflectance मीटर और कंप्यूटर दृष्टि से विश्लेषण किया गया, और नियंत्रण के साथ महत्वपूर्ण अंतर पाया गया. उपचार homogenized मिश्रण 25% से तैयार चाय के अलावा उच्चतम reflectance के मूल्य और सबसे कम भूरे सूचकांक था. 50% से तैयार चाय भी मिश्रणों उच्च संवेदी पहलुओं पर रन बनाए लेकिन रंग के लिए सम्मान के साथ खराब प्रदर्शन किया. चिपचिपापन परिणाम संकेत दिया कि homogenization दलदलापन पर सकारात्मक प्रभाव पड़ा. कॉफी स्थिरता परीक्षण अम्लीय शर्तों के तहत व्हाइटनर की स्थिरता का आकलन किया गया था और यह देखा गया है कि 25% मट्ठा साथ मिश्रण टोन किया हुआ दूध के बराबर था. मानक थाली गिनती, Coliform गिनती से प्रकल्पित और कॉलिफोर्म परीक्षण के व्हाइटनर की सूक्ष्मजीवविज्ञानी गुणवत्ता का आकलन किया गया. व्हाइटनर 7 ± 2 सी. ° 2 दिन की शैल्फ जीवन है मनाया गया प्रमुख मट्ठा स्वाद 2 दिन भंडारण जो अपनी शैल्फ जीवन सीमित परे देखा था. टिप्पणियों पर आधारित निष्कर्ष निकाला है कि एक संतोषजनक चाय व्हाइटनर toned 25:75 और प्रसंस्करण मानकों के रूप में अनुपात homogenization में दूध के साथ मिश्रण मट्ठा द्वारा तैयार किया जा सकता है.

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LIST OF ABBREVIATIONS AND SYMBOLS

%	:	Percentage
”	:	Inch
°	:	Degree
°Brix	:	Degree Brix
°C	:	Degree Celcius
ANOVA	:	Analysis of Variance
AOAC	:	Association of Official Analytical Chemists
BCAA	:	Branched-Chain Amino Acids
BI	:	Browning Index
BIS	:	Bureau of Indian Standards
BOD	:	Biological Oxygen Demand
cfu/ml	:	Colony Forming Units Per Millilitre
CHD	:	Coronary Heart Disease
CIELAB	:	Commission Internationale de L’Eclairage
COD	:	Chemical Oxygen Demand
cP	:	Centipoise
DPPH	:	Diphenyl Picrylhydrazyl
Fig.	:	Figure
FRAP	:	Ferric Reducing Antioxidant Power
g	:	Gram
g/ Kg	:	Gram per Kilogram
g/L	:	Gram per Litre
Hg	:	Mercury
Hrs	:	Hours
k	:	Kappa
Kcal	:	Kilocalorie
Kcal/litre	:	Kilocalorie per Litre
Kg	:	Kilogram
KJ	:	Kilo Joule
KWh	:	Kilo Watt Hour

L	:	litre
LA	:	Lactic Acid
LDPE	:	Low Density Poly Ethylene
litres/hour	:	Litres per Hour
Mg	:	milligram
Mg/kg	:	milligram per kilogram
min	:	Minute
ml	:	Millilitres
mm	:	millimeter
Mmol	:	Milli Mole
Mn kg	:	Million Kilogram
Mn	:	Million
N	:	Normal
PCT	:	Presumptive Coliform Test
psi	:	Pounds per Square Inch
rpm	:	Rotation per Minute
s	:	Second
SMP	:	Skim milk powder
SNF	:	Solids not fat
SPC	:	Standard plate count
TA	:	Titratable Acidity
TM	:	Toned milk
TS	:	Total solids
v.	:	Version
V/V	:	Volume by Volume
W/V :		Weight by volume
WMP	:	Whole milk powder
WPC	:	Whey protein concentrate
α	:	Alpha
B	:	Beta

1. Introduction

Whey is the serum portion of the milk, containing constituents which are in true solution; predominantly lactose, whey proteins and minerals. Whey is obtained as byproduct in the manufacture of *paneer*, chhana, cheese, casein and is one of the major by-products of dairy industry in the world as about 27.47% of total milk production is used for cheese production (IDF, 2009). About 9 litres of whey is generated for every kilogram of cheese manufactured, and a large cheese making plant can generate over 1 million litres of whey daily (Jelen 2003).

India is the leading milk producing country in the world, accounting for more than 13% world's total milk production. Indian dairy market is growing at an annual rate of 7% with annual milk production of 108.5 million tonnes in 2008-09 (National Dairy Development Board, 2010). It is estimated that about 2% of milk produced in India is converted to *paneer* and chhana (Aneja *et al.*, 2002), and production of whey is estimated to be around 270 million kg per annum, which contains about 16.2 million kg of valuable milk solids. Large quantity of whey is unutilized and disposed off through effluent treatment systems. Whey is a potent pollutant with a biological oxygen demand (BOD) of 35–45 g/L. 4,000 L of whey, the output of a small creamery, has the polluting strength of the sewage of 1,900 people (Marwaha and Kennedy 1988). In order to treat this whey to the statutory pollution control norms, about 0.4 kWh of energy per liter at a cost of a 40-45 paisa is incurred. Thus, disposal of whey not only causes the loss of nutritionally rich milk solids but also puts additional financial burden on the dairies. Whey not used for humans was fed to pigs or other livestock, spread as fertilizer, or simply thrown out. Whey proteins are a rich source of all essential amino acids. Relative to other protein sources, whey has a high concentration of branched-chain amino acids (BCAAs) – leucine, isoleucine, and valine. BCAAs, particularly leucine, are important factors in tissue growth and repair. Whey proteins are also rich in the sulphur containing amino acids cysteine and methionine. With a high concentration of these amino acids, immune function is enhanced through intracellular conversion to glutathione (Marshall, 2004). Efficient utilization of whey is the key to economic prosperity and nutritional security. Nowadays whey is evolving into a sought-after product because of the lactose, minerals, and proteins it contains as well as the functional properties it imparts to food (Onwulata and Tomasula, 2004). In the past few technologies have been developed to utilize whey solids by extracting whey proteins (Werner, 1981), cream yoghurt (Kulkarni *et al*., 1990), coffee drink (Dhaka *et al*., 2002), whey soups (Arora and Jha, 2005), whey beverages (Keerthana and Reddy, 2006), whey jaggery (Pankaj, 2011) etc. Although dedicated efforts have been made in this direction no products of mass acceptability have been developed restricting whey utilization to laboratory and experimental purposes. Development of a methodology to utilize whey with minimal processing and technological intervention and low cost will benefit the dairy industry at large.

Tea is the most commonly consumed beverage in the world. Black, Oolong and Green teas are all produced from the leaves of the plant *Camellia sinensis* using different processing methods. Black tea infusions contain small amounts of potassium, magnesium, fluoride and phosphorus, and if consumed with milk, provide a range of additional nutrients in small amounts, including some calcium, B vitamins and energy. A mug of tea with semi-skimmed milk and no sugar provides approximately 18 kcal (73 kJ) (Benelam and Wyness, 2010).

Milk and milk products form essential constituents of the food basket in the Indian families. In the low and middle income segments of the society, milk is essentially used as whitener in the preparation of tea and coffee. It is estimated that almost 30% of the milk purchased by the consumer is utilized for whitening tea and coffee. In recent times, the cost of milk has increased considerably and the trend is continuing, resulting in additional economic burden on the low income group consumers.

The economic loss occurring to dairy plants due to the treatment of whey can be minimized by recovering and utilizing the whey solids appropriately with minimum processing operation. Further, the cost of whitening of beverages like tea and coffee can also be reduced if these whey solids are used effectively in developing a low cost whitener. The use of whey also would add to the nutritional superiority since it contains many health promoting constituents. Based on the above hypothesis, a project is proposed with the following objectives:

1. Optimization of process to develop a suitable tea whitener using whey and milk solids.
2. Evaluation of physico-chemical, microbiological and sensory characteristics of optimized tea whitener.

2. Review of Literature

The literature relating to the topic of present investigation has been reviewed under the following groupings.

2.1 Whey - General

In India, whey is produced as a byproduct of cheese (enzyme coagulation), channa, *paneer*, casein (acid coagulation) and *chakka* (fermented coagulated milk product). Though cheese is the major product in the world utilizing around 27% of milk, *paneer* and channa of traditional dairy sector are the major contributors to Indian whey production contributing around 270 Mn Kg of whey. Stringent environmental policies in India have made manufacturers to establish and process costly whey disposal and treatment process systems. BOD of whey is about 36000 mg/Kg which is very high (Keerthana and Reddy, 2006). Proper effluent treatment and disposal of whey mandates significant cost which is a burden on the dairy processing sector.

Organized dairy sector is continuously looking for new whey utilization and processing technologies for economic viability. A commercially viable technology which can generate sufficient demand to use all available whey will be a boon for dairy industry and also addresses the environmental issues.

2.2 Composition of Whey

Since the time of Hippocrates and Gelan and through middle ages physicians have been known to utilize whey for its therapeutic value (Whittier, 1944). Whey contains around 40-50% of total milk solids including approximately 80-90% lactose, 20% protein, 70% minerals and almost all water soluble vitamins. Even now whey is looked upon as a burden to be disposed. Only a couple of potential uses have been developed to the point of commercial reality.

Whey contains milk constituents which are in true solution i.e. minerals, lactose, water-soluble proteins and vitamins. The composition of whey varies marginally depending upon the type of milk, type of product made, type of coagulating agent etc. Acid whey obtained as a by-product of cottage cheese or *paneer* manufacture has a low pH and consequently longer storage life than “sweet whey” obtained from cheddar and other types of cheeses (Chandra, 1980). The classification of whey based on acidity and pH is illustrated (Table 2.1) below

Table 2.1. Classification of whey based on acidity and pH

Type	Titratable acidity (% LA)	pH
Sweet	0.10 - 0.20	5.8 - 6.6
Medium acid	0.20 - 0.40	5.0 - 5.8
Acid	0.40 - 0.60	4.0 - 5.0

(Bund and Pandit, 2005)

In the production of cheese or casein, about 80 to 90% of original milk used yields whey as a by-product, which contains about 50% of the milk constituents (Jayaprakasha, 1992). The composition of whey proteins in these two types of whey is not identical. The rennet whey contains a well-defined part of k-casein, the casein macropeptide, as a result of action of chymosin. The whey proteins are composed of α- lactalbumin (about 1.0 g / kg whey), β-lactoglobulin (about 3.3 g / kg whey), immunoglobulin (about 0.7 g / kg whey) etc. The typical composition of sweet whey, medium acid and acid whey are given (Table 2.2) below.

Table 2.2. Composition of different types of whey (g/litre)

Constituents	Sweet whey	Acid whey
Total solids	63-70	63-70
Lactose	46-52	44-46
Protein	6-10	6-8
Calcium	0.4-0.6	1.2-1.6
Phosphate	1.3	2.0-4.5
Lactate	2.0	6.4
Chloride	1.1	1.1

Jelen (2002)

2.3 Physical properties of whey

2.3.1 Color

Cheddar whey is a thin liquid with greenish yellow tinge. The color of whey is a decisive factor in the sensory acceptance of products manufactured from it. Bleaching is an excellent method to improve color but have deleterious effects in the flavour of the product. Whey was bleached using hydrogen peroxide and benzoyl peroxide prior to spray drying for the manufacture of whey protein concentrate. Whey protein concentrate produced from unbleached control sample was more yellow than bleached samples. Flavour deterioration was higher in bleached samples due higher proportion of oxidation products. Hydrogen peroxide samples were whiter but poor in flavour than benzoyl peroxide sample and unbleached control whey (Crossiant *et al*., 2009)

2.3.2 Viscosity

The viscosity of unconcentrated cheese whey manufactured commercially using different microbial strains varied between 1.7Cp and 2.2 Cp. Higher viscosity was observed when exopolysaacharide producing ropy strains were used. The viscosity of whey samples were in direct correlation with the type of strain used for cheese production. Fivefold concentration of the above whey resulted in increase of viscosity to 2.1Cp-3.1Cp (Petersen *et al*., 2000).

The viscosity of cheddar and cottage cheese whey was unaffected by pH values and the effect of pH on the viscosity of commercial unprocessed whey appeared insignificant under the conditions likely to be encountered during whey processing (Delaveau and Jelen, 1979).

2.4 Nutritional and Biological Importance of Whey

Whey contains 6-7% of total milk solids and is a rich source of lactose, proteins, minerals and vitamins. Lactose constitutes more than 75% of dry matter, and protein accounts for another 12%. Significant quantities of micronutrients like milk salts, water soluble vitamins, peptides, immunoglobulins and lactoferrin are present in whey making it nutritionally important (IDF, 2003).

In recent times milk constituents are being used in the nutraceutical and pharamaceutical industry widely. Lactose and milk proteins are considered to have preboitic effect and confer benefits to the consumer by modulating and promoting growth and activity of natural colon microflora. Invasive species and pathogenic species are also suppressed due to the prebiotic ingredients. The energy value of whey is 210Kcal/litre (Swaminathan, 1982).

2.5 Therapeutic Value of Whey

Cheese whey beverage was used for treating human ailments and conferring therapeutic benefits to humans since the time of Hippocrates. Whey was prescribed by doctors for various diseases in the medieval periods and by the mid 19th century whey cures were so established validated by operation of over 400 whey houses in Western Europe. Ailments like dyspepsia, uraemia, arthritis, gout, liver disease, anaemia and tuberculosis were treated with injection of up to 1500 gram whey per day (Holsinger *et al*., 1974). In the past, whey was considered a cure-all used to heal ailments ranging from gastrointestinal complaints to joint and ligament problems. Presently whey is a popular dietary protein supplement purported to provide antimicrobial activity, immune modulation, improved muscle strength and body composition, and to prevent cardiovascular disease and osteoporosis (Marshall, 2004). Shahani *et al*. (1978) reported that whey and whey protein concentrates could be extensively used for a number of formulations in human foods. Incorporation of whey protein concentrates in infant food formulations, geriatric foods, and specialized diets benefits the consumer due to therapeutic nature and high nutrient profile of the same. Due to the fact that whey proteins have much higher digestibility than casein, they are often used in production of infant formulae and to improve the nutritional value not only of dairy products but also of many other food products. Immunoglobulins and other glycoproteins (lactoferin transferin) and enzymes (lysozyme, lactoperoxidase) are immune enhancing factors present in whey. These whey components have potential antimicrobial properties with ability to reduce or even inhibit allergic reactions (Tratnik, 2003).

2.6 Functional Properties of Whey

Functional properties of whey can be chiefly attributed to its whey protein content. The beneficial functional properties have a role to play in enhancing the quality of dairy, bakery and confectionery products. Functional properties include of whey protein inclusion are solubility, water binding/absorption, viscosity, gelation, emulsification/ stabilizing effects, texture modification, aeration/ whipping, flavor enhancement, cohesion, adhesion and elasticity etc. Incorporation of whey solids in small amounts can modify the food properties to a great extent. Lactose also imparts functional characteristics when incorporated into various products. When added to bakery products it lowers the sweetness, increases browning, higher moisture retention, enhanced emulsification, enhanced flavor and non hygroscopicity (Burrington, 1999).

2.7 Environmental Impact of Whey Disposal

Whey is a by-product of cheese, *paneer* and casein manufacture. 100 litre milk yields approximately; 10 kg of cheese and 90 litres of whey, 15-18 kg *paneer* and 82-85 litres of whey and 4 kg of green casein curd and 96 liters of whey. Whey cannot be disposed as such due to its polluting nature. Old age practices of spraying into open fields, draining to water bodies are no longer practiced due environmental concerns and stringent regulations. The practical difficulty in transporting and distributing to dairy farms and the problems associated with it hinders the plan to utilize whey as an animal feed extensively.

The biological oxygen demand and chemical oxygen demand of whey is very high due to its carbohydrate and protein content. Specially designed anaerobic fermentors or reactors are required to treat whey and bring down BOD and COD levels to the permissible limits. The cost of treatment of whey per litre is about 0.40-0.45 paise which accumulate to a huge expenditure even for a plant with installed capacity of 5 tonnes of cheese per day. This in turn creates a financial burden which strangles the future developmental projects of the processing plant.

In light of the global food shortage, the most logical use would be to return whey to the human food chain in a palatable form. Utilization of whey as human food also controls the pollution woes. (Papinwar, 2010)

2.8 Utilization of Whey

2.8.1 Whey Solids in Human Foods

2.8.1.1 Whey Solids in Dairy Products

Rasogolla was prepared using whey as coagulant, cooking medium and dipping medium and effect on quality was studied. Sour whey as coagulant, fresh whey without sugar as cooking medium and fresh whey syrup (50°Brix) was suitable for rosogolla preparation (Dabur and Prakash, 2007).

Sweet whey, whey protein isolates and whey protein concentrates are commonly used in ice cream and frozen dessert formulations. Incorporation of whey solids in the formulations adds functional properties and brings cost efficiency. The functional benefits include emulsification properties, water binding, bulking, whipping or foaming ability, viscosity, color and appearance modification, and modification flavor profile and freezing point. Dry sweet whey and whey protein concentrates can be added from 2.5-3% levels and 0.65-1.6% levels respectively for good quality characteristics (Young, 1999).

Whey products can be used to provide solids non fat milk solids in yoghurt formulas. The advantages of such incorporations include texture improvement, reduced curd syneresis, prebiotic effect, nutraceutical benefits, flavor modification and extended shelf life. Whey proteins are generally of bland taste, so they have fewer tendencies to mask added flavors. Stabilizing effect of whey proteins helps to remove starch or other thickeners which further improves the flavor. Viscosity of whey solids added samples are also higher which gives better texture (Hugunin, 1999).

2.8.1.2 Whey Solids in Candies and Confectionaries

Plain condensed, sweetened condensed or dried whey may be used as a source of whey solids. Sweet rennet-type whey is preferred to neutralize acid whey because of its superior flavour. Candies such as fudge, caramel and toffee can be made with a whey solids content of 14 to 40 per cent (Langwill, 1941). Whey is especially useful in fudge; the lactose on crystallizing contributes to the desired grainy texture. Whey caramels should be fortified with casein in order to produce the characteristic chewy body. Whipped sweetened condensed whey can be used to incorporate air in special types of candies. Whey solids have been favored as an ingredient for formulation of confectionary products. Whey formulae make caramels with a fine flavour. It is possible to make a light caramel with little or no colour when only sweetened condensed whey is used. Caramels produced by using sweetened condensed whey and soya proteins closely resemble those produced from skim milk or whole milk. Confectionary coatings prepared by using whey in the formulations have enhanced the texture, flavour, and colour characteristics of the finished product and preserved freshness in year-round usage (Mathur and Shahani, 1979).

Table 2.3. Recommended levels of addition as percentage of total formula

Product	Sweet whey	WPC 34 - WPC 50	WPC 80	Demineralized whey, Modified whey
White bread	1-5%	1-4%	1-3%	2-6%
Sweet Rolls	2-5%	1-4%	1-3%	2-6%
Cookies & Biscuits	1-5%	1-5%	1-4%	2-5%
crackers	1-5%	1-4%	1-3%	2-6%
Pizza dough	1-5%	1-4%	1-3%	2-6%
cakes	1-6%	1-4%	1-3%	1-6%
Icings & Fillings	1-3%	1-2%	1-2%	1-3%
Low fat, low sugar baked goods	2-10%	3-9%	3-5%	2-10%

**** Replacement of up to 50% egg white.**

*** Replacement of up to 50% fat.**

~ Replacement of up to 25% sugar.

(Burrington, 1999)

2.8.1.3 Whey Solids in Beverages

Whey or whey derivatives derived from cheese, casein, *paneer* etc., can be used for beverage preparation. Deproteinization and lactose are generally done to increase the acceptance of whey based beverages.

Whey is used in the formulation of nutritive soft drinks (alcoholic and non alcoholic) or high protein beverages with or without the addition of fruit juices. (Zadow, 1983). A variety of beverages including plain, carbonated, alcoholic and fruit flavored are popular across the globe (Clark, 1987). Technology development for the production of protein beverage with whey as base ingredient can combat malnutrition in underdeveloped countries (Holsinger *et al*., 1974). Whey beverages are genuine thirst quenchers and are light, refreshing, and less acidic than similar fruit based beverages (Prendergast, 1985). Utilization of whey for beverage production yields good profit margins to the processing plant.

2.8.1.4 Whey Solids in Dairy Whitener Formulations

Acid whey, ion exchanged to 90% reduction in minerals and ultrafiltered to concentrate total solids in retentate to 16.6% to 22.6% and which contained protein at 50% of solids in retentate was used as total replacement for sodium caseinate. Optimal stability and functionality were obtained at 1.5% acid whey proteins. Dipotassium phosphate was added to the formulation to prevent protein precipitation in hot coffee. A mixed emulsifier consisting of polyoxyethylene sorbitan monostearate and sodium stearoyl lactylate gave a coffee whitener formulation of optimum quality. 0.5% titanium dioxide was added to the formulation to give optimum whitening ability and was estimated using a spectrophotometer. Demineralized acid whey protein was an acceptable replacement for sodium caseinate in spray-dried coffee whiteners and can replace sodium caseinate at a 1:2 ratio (Gruetzmacher and Bradley, Jr., 1991).

Succinylated whey concentrate was substituted for sodium caseinate in the formulation of coffee whitener and the effects on quality and acceptance of coffee beverage were determined. Succinylated whey concentrate has emulsification properties suitable for preparation of stable coffee whitener. The resultant coffee whitener had with higher viscosity, greater stability, lower whitening power, and greater acceptability than the control sample made with sodium caseinate. Replacement of sodium caseinate in coffee whitener with succinylated whey concentrate appears to be feasible (Thompson and Reniers, 1982).

A powdered beverage whitener composition comprising, about 50 - 80% of a milk powder, 5 - 30% of whey solids, 10 - 30% of one or more added sugars on dry basis was developed. The beverage whitener comprises of, 10 to 60% of milk proteins and 25 to 85% of sugars, wherein the milk proteins include added whey proteins. Preferably the whitener comprises less than about 2% by weight of fats. The whitener compositions are especially suitable for preparing foamy beverages, such as cappuccino coffee (Charman, 2004).

A liquid coffee whitener formulation containing whey protein hydrolysate instead of sodium caseinate was developed. The liquid coffee whitener contained 6.8% vegetable fat, 4.0% corn syrup solids, 8.8% whey protein hydrolysate, 0.2% emulsifier, 0.2% lecithin and 80% water. The liquid coffee whitener had good functional and organoleptic characteristics comparable with conventional formulations containing sodium caseinate (Rasilewicz, 1991)

A coffee drink with whey as base ingredient was formulated. Cheese whey with a pH 6.0±0.2 was used in the trials; calcium hydroxide was used to adjust pH to the above mentioned levels. Effect of levels of whey addition, milk and sugar on sensory attributes was studied. The optimized beverage contained 120 ml whey, 60 ml standardized milk, 15.3 g sugar, 0.9 g sodium alginate and 0.5 g coffee powder in a serving size of 200 ml (Dhaka *et al*., 2002).

Whey-polyphosphate can be used to replace sodium phosphate in whole or in part. A liquid coffee whitener formulation and dry blend mix was developed with dry whey phosphate mixture as base ingredient. The liquid coffee whitener contained 9.5% phosphate-whey mixture, 3.9% corn syrup solids, 10% hydrogenated vegetable shortening, 0.4% stabilizer-emulsifier and about 75% water. The dry coffee whitener formulation contained 38% phosphate-whey mixture, 16% corn syrup solids, 44% hydrogenated vegetable shortening, 1.5% stabilizer-emulsifier. Feathering or fat separation was not observed when the whitener was added to hot coffee at 82°C (Ellinger *et al*., 1971).

2.8.2 Whey Utilization in Animal Feeds

Whey not used for humans was fed to pigs or other livestock, spread as fertilizer, or simply thrown out. Whey has supplemented pig feed for centuries, and the growth of computerized systems has allowed for more precise feeding of whey and other liquid feeds to weaned pigs and lactating sows (Meat and Livestock Commission 2003).

Feeding liquid whey to farm animals has been practiced for hundreds of years, but replacing drinking water for farm animals with liquid whey perhaps is relatively new. A large cottage cheese company in the Northeast United States regularly is channeling its acid whey in this manner. It provides the tanks to the farmer and transports the whey every other day to the surrounding farms without charge. Feeding of such cottage cheese whey to farm animals results in a savings of about one-third of the farmers' total feed bill. However, feeding cattle and other farm animals with liquid whey is not practical under all conditions. It requires ready access to rural areas where the animals are located and full cooperation of farmers. Also, the entire financial burden of such whey movement usually falls upon the cheese manufacturer. Essentially, the successful utilization of fluid whey by farm animals depends upon the location of the cheese making site (Kosikowski, 1979).

2.8.3 Whey utilization as Fermentation Media

Whey can be potentially used as a fermentation media for the production of single cell protein, ethanol, baker’s yeast, methane, lactic acid etc. due to its high lactose content (Murdia *et al*., 2005). Lactose-fermenting yeasts produce ethanol utilizing lactose. Deproteinated cheese or acid casein whey is used as the substrate and may be concentrated by reverse osmosis or supplemented by other lactose enriching streams to increase the lactose concentration to 10-13% and yield of the product obtained is around 75-85%. Whey can be used for the production of single cell proteins and one of the most popular processes is the Bel-process of France. Strains of *Kluyveromyces marxianus* var. *lactis* or *marxianus* were grown in whey. Product contained approximately 50% protein, 30% carbohydrate, 6% lipid and 8% minerals on dry matter basis. Diluted whey is one of the best medium for production of β-galactosidase using *Kluyveromyces* species grown on diluted whey. β-galactosidase is used to hydrolyze lactose to overcome the problem of lactose intolerance and to generate syrups, which are sweeter than lactose and which do not crystallize easily. The yeast autolysate may be used to replace yeast extract in microbiological media. Lactic acid commonly used in food industry, ammonium lactate used as animal feed as well as propionic acid, a fungistatic agent, can be produced using whey as medium.

2.9 Tea as a Beverage

2.9.1 Introduction

Tea is a pleasant, popular, socially accepted, economical, and safe drink that is enjoyed every day by hundreds of millions of people across all continents. Tea leaves are primarily manufactured as green or black or oolong, with black tea representing approximately 80% of the tea products consumed (Hakim *et al*., 2000). The global market for hot beverages (coffee and tea) is forecasted to reach US$69.77 billion in value and 10.57 million tons in volume by the year 2015 (GIA, 2011). Amongst tea producing countries, the principal producers are China, India, Sri Lanka, Kenya and Indonesia. These five countries account for 77% of world production and 80% global exports (Majumder *et al*., 2010). There are at least 5 tea varieties depending upon the extent of fermentation viz. white tea, green tea, black tea, oolong tea, yellow tea and post-fermented tea. Tea is graded based upon quality and size of fresh leaf and bud used for tea production.

2.9.2 Types of Tea

- White tea: Wilted and unoxidized
- Yellow tea: Unwilted and unoxidized, but allowed to yellow
- Green tea: Unwilted and unoxidized
- Oolong: Wilted, bruised, and partially oxidized
- Black tea: Wilted, sometimes crushed, and fully oxidized
- Post-fermented tea: Green tea that has been allowed to ferment/compost

2.9.3 Black Tea Grades (Owuor, 2003a)

TGFOP - Tippy Golden Flowery Orange Pekoe

TFOP – Tippy Flowery Orange Pekoe

FOP - Flowery Orange Pekoe

OP - Orange Pekoe

FP- Flowery Pekoe

Broken Grades

TGFBOP - Tippy Golden Flowery Broken Orange Pekoe

GFBOP - Golden Flowery Broken Orange Pekoe

TBOP - Tippy Broken Orange Pekoe

GBOP - Golden Broken Orange Pekoe

FBOP - Flowery Broken Orange Pekoe

TGBOP - Tippy Golden Broken Orange Pekoe

BOP - Broken Orange Pekoe

BP – BROKEN Pekoe

BPS - Broken Pekoe Souchong

PS - Pekoe Souchong

S- Souchong

BM – Broken Mixed

BT – Broken Tea

2.9.4 Tea Composition

Phenolic compounds constitute 25-35% of dry matter content of young, fresh tea leaves. Flavonols are 80% of the phenols. Enzymatic oxidation of flavones during fermentation is responsible for color and flavour of black tea. Theaflavins and thearubigins are responsible for reddish-yellow colour of black tea extract. Flavour intensity of black tea is directly correlated with phenolic contents and polyphenol oxidase activity. Flavnaol oxidation doesn't occur in green tea as the enzymes are inactivated in green tea.

Greenish or yellowish color of green tea is due to flavonols and flavaones. Polyphenolic content of green tea and black tea are 17.5% and 14.4% respectively. Catechins account for 90% of polyphenols in green tea where as it is only 25% in black tea. In fermented teas 38-41% of the dry matter is soluble in hot water (Belitz *et al*., 2004).

Table 2.4. Composition (% dry weight basis) of fresh and fermented tea leaves and of tea brew

Constituent	Fresh flush	Black tea	Black tea brew[a]
Phenolic compounds[b]	30	5	4.5
Oxidized phenolic compounds[c]	0	25	15
Protein	15	5	+[d]
Amino acids	4	4	3.5
Caffeine	4	4	3.2
Crude fiber	26	26	0
Other carbohydrates	7	7	4
Lipids	7	7	+
Pigments[e]	2	2	+
Volatile compounds	0.1	0.1	0.1
Minerals	5	5	4.5

[a] brewing time 3 min. [b] Mostly flavanols. [c] Mostly thearubigins. [d] traces. [e] chlorophyll and carotenoids.

(Belitz *et al*., 2004)

2.9.5 Health benefits of tea consumption

Tea is a pleasant, popular, socially accepted, economical, and safe drink that is enjoyed every day by hundreds of millions of people across all continents. Tea contains caffeine and a variety of polyphenolic compounds (Duthie & Crozier 2003). Tea drinking is associated with a decreased risk of CHD, and this may be because of its polyphenol content. Tea has also been associated with reduced risk of cancer, dental caries and bone loss, although the evidence for these associations is weaker than for CHD (Ruxton, 2008). The major components of black tea are theaflavins (1-2% dry weight) and thearubigins (10-20% dry weight)

The other health benefits reported are (Hakim *et al*., 2000):

- Tea has been known to have various clinical merits, such as antiviral, antibacterial, antipyretic, and anticarcinogenic effects

- Tea polyphenols scavenge active oxygen radicals and chemo carcinogen-induced carcinogenesis
- They also block the inhibition effect of carcinogen in intercellular communication and induce apoptosis
- Tea-derived polyphenols exhibit antimutagenic and genotoxic activities probably associated with anticarcinogenic activity.

2.9.5.1 Health Benefits of Milk Tea

Tea is traditionally drunk with a small amount of milk in India and many other countries. Addition of milk to tea has reduced incidence of esophageal cancer in regular tea drinkers (Morton, 1979). These effects on health and palatability have been ascribed to association between the various polyphenolic tea compounds (tannins) and proteins in the milk, but only recently has a more detailed molecular interpretation been put forward (Luck *et al*., 1994).

Black tea infusions contain small amounts of potassium, magnesium, fluoride and phosphorus, and if consumed with milk, provide a range of additional nutrients in small amounts, including some calcium, B vitamins and energy. A mug of tea with semi-skimmed milk and no sugar provides approximately 18 kcal (73 kJ) (Benelam and Wyness, 2010).
Antioxidant potential of tea and polyphenolic beverages is not altered by addition of milk. An in vitro low-density lipoprotein oxidation model was used to assess the relative antioxidant activity of the polyphenolic beverages tea, coffee, and cocoa on a cup-serving basis. The antioxidant activity as determined by the lag time was in the range of 292−948 min for coffee, 217−444 min for cocoa, 186−338 min for green tea, 67−277 min for black tea, and 6−78 min for herbal tea. Addition of milk did not alter the antioxidant activity (Richelle *et al*., 2001).

Antioxidant activity of black tea enhances and stabilizes with milk or sugar. Four tea brews viz. Plain black tea, black tea with sugar, black tea with milk, black tea with milk & sugar were tested for their radical scavenging activity and antioxidant potential. The radical scavenging and antioxidant activities were estimated using the 1, 1-diphenyl picrylhydrazyl (DPPH) and ß-carotene-linoleic acid model systems, respectively. The test positively confirmed that addition of milk to black tea enhances its antioxidant activity (Sharma *et al*., 2008).

2.9.6 Incorporation of Tea Extracts in Dairy Products

Green and Pu-erh infusions were prepared and added to bulk milk intended for yoghurt preparations at three levels viz. 5%, 10% and 15%. The antioxidant capacity was estimated using diphenyl picrylhydrazyl (DPPH) and ferric reducing antioxidant power (FRAP) methods. The effect of tea infusion on acidity characteristics and bacterial counts during cold storage of the samples were also studied. The average radical scavenging activity was 31 fold and 15 fold higher for yoghurts with Green and Pu-erh infusions in comparison with plain yoghurt. The respective FRAP values were 12 and 5 fold higher. Tea supplementation had a positive influence on initial acidity of yoghurts and count of *Lactobacillus delbrueckii ssp bulgaricus* in comparison with plain yoghurt. Lower pH values were observed for yoghurts supplemented with tea extracts irrespective of the level of fortification. The tea supplemented yoghurts were found to have higher number of viable *Lactobacillus delbrueckii ssp bulgaricus* and *Streptococcus thermophilus* cells. (Lejko *et al*., 2011) This confirms that milk product supplemented with tea extracts can confer additional health benefits.

Skim milk was supplemented to reconstituted skim milk at five levels viz. 2%, 4%, 6%, 8% and 10% and subjected to pasteurization, homogenization and salt addition (Calcium Chloride and Sodium Chloride). The phenol content and antioxidant capacity of skim milk increased by addition of green tea. Pasteurization and addition of Calcium Chloride to green tea supplemented skim milk improved its antioxidant activity. A functional drink which can reduce incidence of degenerative diseases by radical scavenging was developed. (Gad and El-Salam, 2010).

2.9.7 Effect of Polyphenols on Milk Stability

Freeze dried extracts of green tea, black tea, cocoa powder and instant coffee were added at 0.4 g per 100 ml and 0.8 g per 100 ml to skim milk and stability at sterilization temperatures studied. High polyphenolic contents of the substance increased the casein micellar stability significantly (O'Connell *et al*., 1998).

Caffeic acid at 5.5 mmol per litre enhanced the stability of milk at sterilization temperature. Caffeic acid at markedly enhanced the heat stability of milk at 140°C, the minimum was eliminated and stability. "Caffeic acid addition resulted in reduction of reactive lysine and sulphydryl content and inhibited the dissociation of k-casein-rich protein from the casein micelles in milk on heating. It is postulated that on heating in milk, caffeic acid is thermally oxidised to quinones which then interact with nucleophilic amino acid residues to inhibit k-casein dissociation from the casein micelle" (O'Connell and Fox,1999).

2.10 Dairy Whitener Quality

2.10.1 Resistance to Feathering

Dairy whitener formulations should be stable over a range of conditions to be effectively used. Spray dried products must firstly possess instant solubility properties i.e. satisfy the dispersability, wettability and solubility criteria normally required when fat-containing powders are added to water. The second requirement is that the creamer, on dissolving in coffee, should not coagulate or give rise to a sludge-like precipitate or sediment. Terms such as floaters and sinkers are occasionally used to describe the appearance of instability (Teehan *et al*., 1997). Feathering or oiling off refers to unwanted scum formation or precipitation or both which greatly decreases the aesthetic value of the product. Coffee stability of dairy whiteners or dried milks is extremely important from the consumer point of view as they influence the choice of product by the consumer to a great extent (Nath *et al*., 2000).

2.10.2 Whitening Ability

Another important quality of the dairy whitener is its whitening ability. The consumer primarily assesses the richness of tea or coffee containing milk by looking at its colour and appearance. The whitening ability is controlled by the composition of the whitener and the nature of additives incorporated into the product to aid in processing operations. Major constituents like milk proteins, fat decide the color of the beverage made from the dairy whitener.

2.11 Justification of the present project

Whey is an underutilized byproduct of the dairy industry. The prime reason behind the underutilization and disposal of whey is the cost factor and technological proficiency required for its effective utilization. Most of the technologies for whey utilization were limited to the research and laboratory levels due to the above reasons. Utilizing whey through the market milk channel holds great potential. Utilization of whey in such a way does not require any additional infrastructure to the processing entity other than simple equipments for clarification and concentration. Whey utilization through such a mode is easy, compatible and cost effective. The cost of liquid milk has increased considerably in the recent times. Formulation of a tea whitener based on a blend of whey and toned milk costing less than toned milk is in the larger interest of the society. Incorporation of whey proteins also improves the nutritional standards of the product. Based on these factors the present project was initiated.

3. Materials and Methods

3.1 LOCALE OF STUDY

The locale of the present investigation was the Southern Research Station Of National Dairy Research Institute, Adugodi, Bangalore.

3.2 MATERIALS

3.2.1 Ingredients

3.2.1.1 Milk

Milk sourced from the NDRI Experimental Dairy Plant was used for the preparation of *paneer* and cheese.

3.2.1.2 Whey

Microbial enzyme in dried form was used as milk clotting agent during cheese preparation and citric acid (Commercial grade) for coagulating the milk during *paneer* preparation. The whey obtained from these processes as byproduct was used in all trials detailed in the present investigation.

3.2.1.3 Tea powder

In all the trials Taj Mahal brand (Hindustan Unilever Limited) purchased from local market and was used for the preparation of tea extract (decoction).

3.2.1.4 Sugar

Crystalline sugar obtained from the local market was used as the sweetener.

3.2.1.5 Potable Water

Water treated by Kent water purifier systems was used for preparation of decoction and for dilution purposes.

3.2.2 Chemicals and Media

The chemicals of high quality conferring to AR grade were used in the analysis of the products. Dehydrated microbiological media supplied by Himedia, Mumbai was used for the microbial analyses**.**

3.2.3 Equipment

3.2.3.1 Single Effect Evaporator

Single Effect Evaporator (A.P.V., Calcutta) with water evaporation capacity of 20 litres/hour was used for concentrating the whey. Whey was concentrated to 12-15% solids content and further diluted with potable water to 11.5% TS.

3.2.3.2 Digital pH meter

A digital pH meter of the make Digisun electronics, Hyderabad was used for measuring the pH of whey, whey-milk blends, tea decoction and tea.

3.2.3.3 Homogenizer

The Crepaco Homogenizer with 500 lit/hr capacity was used to homogenize milk at 1500 psi, single stage.

3.2.3.4 Reflectance meter

An analog reflectance meter model CL-28 of ELICO PVT. LTD., Hyderabad make was used for measuring colour of decoction and tea.

3.2.3.5 Computer Vision

A flatbed scanner model HP scanjet 3970 was used to scan the samples and the images were imported to a computer installed with Adobe Photoshop 7.0 software and the color parameters of the samples were obtained**.**

3.2.3.6 Gerhardt digestion and distillation assembly

Gerhardt Turbotherm digestion unit and Vapodest distillation unit of Gerhardt, Germany were used to estimate protein contents in ingredients and finished products.

3.2.3.7 Brookfield Viscometer

Brookfield DV Pro II Viscometer with controlled temperature bath was used for testing viscosity of products. Spindle 18 was used during the experiment**.**

3.2.3.8 Centrifuge

G.E centrifuge with capacity to hold 50 ml tubes was used to centrifuge the samples.

3.2.3.9 Glassware

High quality glasswares manufactured by Borosil and Schott Duran were used. All the glasswares were thoroughly washed with detergent, rinsed with distilled water and dried in hot air oven prior to use.

3.3 METHODS

3.3.1 Standardization of Decoction

Black tea was prepared using 2.5 g tea powder per 100 ml water. The tea powder was steeped for 2 min in boiling water and filtered to get the decoction. Taj Mahal brand tea powder was used in all the trials.

3.3.2 Standardization of Tea Beverage

A Control tea was prepared from toned milk (3%fat, 8.5% SNF). Three different levels of TM *viz.* 60 ml, 70 ml and 80 ml for every 100 ml decoction and 8% sugar on total volume basis was tried to optimize the beverage.

3.3.3 Whey Processing

Cheese whey and *paneer* whey were filtered through double layer muslin cloth and was concentrated to 12-15% solids in single effect evaporator at 24” Hg vacuum, corresponding to a temperature of 63-65^{0}C. The concentrated whey was filtered and pasteurized before being cooled to room temperature. The processed product was stored at 5-7^{0}C in the cold store.

3.3.3.1 Blending

Concentrated whey (12-15% TS) was adjusted to 11.5% solids by the addition of calculated quantity of potable water. It was then cold blended with toned milk in three proportions namely 75:25, 50:50 and 25:75 respectively.

3.3.3.2 Homogenization

Whey-milk blends were cold homogenized at 1200- 1500 psi in Single stage. The temperature of the blend was maintained at 5 – 7 ^{0}C. The homogenized blends were immediately pasteurized to avoid microbial proliferation.

3.3.4 Process optimization

Whey (*paneer*/cheese)

↓

Vacuum concentration (12% TS)

↓

Pasteurization (72C°/ 15 sec)

↓

Cold storage (5 -7 C°/ 16-18 hrs)

↓

Dilution to 11.5% solids by addition of potable water

↓

Cold mixing whey and toned milk in 75: 25, 50:50 & 25: 75 ratios

↓ **Unhomogenized blend** → Cold Storage

↓ **Homogenization**
1200- 1500 psi, 1 stage
↓
Pasteurization → Cold Storage

Cold Storage

↓

Whey-milk Tea Whitener

Fig. 3.1 Flow Diagram for the Preparation of Whey-Milk Tea Whitener

Whey-milk Tea Whitener

↓

Heating to boiling point

↓

Addition of sugar (8% of total volume)

↓

Addition of decoction (80-90℃) [80 ml blend to 100 ml decoction]

↓

TEA

Fig. 3.2 Flow diagram for the preparation of tea from Whey-milk Tea Whitener

3.4 METHODS OF ANALYSIS

3.4.1 Preparation of sample

The whey-milk blends were thoroughly mixed in beakers into a homogenous mix. A representative sample was taken from the lots for analysis of chemical parameters.

3.4.2 Physicochemical Analysis

3.4.2.1 Determination of Total Solids (Gravimetric method)

About 5 ml of the prepared whey concentrate, whey-milk blends and milk samples were weighed in a flat bottomed aluminum dish which was previously dried and weighed. The dish containing the sample was dried in an oven maintained at 98 ± 2^{0}C for about 3 hours, cooled in a desiccator and weighed. The process of drying, cooling and weighing was repeated at 30 minutes intervals till the difference between two consecutive weighing was less than 1 mg.

$$\textbf{Solids \% by weight} = \frac{100\,(W_2 - W)}{(W_1 - W)} \qquad \textbf{(3.1)}$$

W_1 = weight in g of the dish with the material before drying

W_2 = weight in g of the dish with the material after drying, and

W = weight in g of the empty dish

The moisture and total solids content in the whey- milk blends were determined by the method as detailed IS: (SP: 18, Part XI) (1981).

3.4.2.2 Determination of moisture (Gravimetric method)

The moisture content was calculated by the following formula

$$\textbf{Moisture \% by weight} = \frac{100\,(W_1 - W_2)}{W_1 - W} \qquad \textbf{(3.2)}$$

W_1 = weight in g of the dish with the material before drying

W_2 = weight in g of the dish with the material after drying, and

W = weight in g of the empty dish

3.4.2.3 Determination of Fat (Mojonnier Fat Extraction Tube)

The fat was extracted and estimated in the whey-milk blends and milk samples using Mojonnier Fat-Extraction apparatus according to the method detailed in IS: (SP: 18, Part XI) (1981), with slight modifications. 11 g of the samples were weighed accurately into the Mojonnier tubes. 1 ml of ammonia solution was added to the tubes and was mixed well in lower bulb. 10 ml of alcohol was added to the tube and mixed well. The tubes were cooled by keeping in chilled water. 25 ml of diethyl ether was added to the tubes followed by 25 ml, of petroleum ether and tubes were shaken vigorously for 1 minute. The tubes were allowed to stand for 30 minutes so a clear ethereal layer is formed. The ethereal layer was decanted to beaker of predetermined weight. Second extraction was carried out using 15 ml diethyl ether and 15 ml petroleum ether. The ethereal layer was decanted into the beaker after 30 minutes.
The third and final extractions were carried out using 15 ml diethyl ether and 15 ml petroleum ether. The ethereal layer was decanted into the beaker after 30 minutes. All traces of the solvent was removed by keeping the beaker in water bath and over hot plate prior to keeping in hot air oven at 98-100° C for 1 hour. The beaker was cooled in a dessicator and weights were noted. Repeated weighing was done till the difference in weights were not more than 1 mg

$$\textbf{Fat \%} = \frac{\textbf{Weight of fat x 100}}{\textbf{Weight of sample}} \qquad \textbf{(3.3)}$$

3.4.2.4 Determination of Ash (Gravimetric Method)

The ash was estimated in the whey-milk blends and milk samples according to the method detailed in IS: (SP: 18, Part XI) (1981), with slight modifications. 10 g of the samples was transferred to a previously weighed silica dish. The sample was dried over a heater coil.

The dish with dried sample was transferred to a muffle furnace for ashing at 550 ° C for 4 hours.

3.4.2.5 Determination of Protein (Kjeldahl method AOAC (2005)

Microkjeldahl method was used to estimate protein content of the samples. 2ml of the sample were added to the 300 ml digestion tube followed by 5g of digestion mixture and 12.5 ml of conc. Sulphuric acid. The samples were digested at temperatures 150 – 300 ° C until a clear filtrate is obtained. The contents were cooled to room temperature. About 20-25 ml of 40% NaOH was added to make the solution alkaline. The contents were steam distilled and the liberated ammonia was collected in 25 ml of saturated boric acid containing 2-3 drops of mixed indicator (10 ml of 0.1% bromocresol green + 2 ml of 0.1% methyl red indicator in 95% ethyl alcohol). Distillation was continued until about 65 ml of distillate was collected and then it was titrated against N/10 H_2SO_4 to end point. The blank was carried out simultaneously using all the reagents except the sample and the per cent protein was calculated as follows:

$$\%\ \text{Nitrogen} = \frac{1.4 \times (S-B) \times \text{Normality of } H_2SO_4 \times 100}{\text{Weight of sample}}$$

$$\%\ \text{Protein} = 6.38 \times \%\ \text{Nitrogen} \qquad \textbf{(3.4)}$$

where, S = Volume of H_2SO_4 used for sample titration

B = Volume of H_2SO_4 used for blank titration

3.4.2.6 Determination of Acidity

The acidity was estimated in the whey-milk blends and milk samples according to the method detailed in IS: (SP: 18, Part XI) (1981), with slight modifications. 10 ml of the sample was mixed with equal quantity of boiled water and added with 1 ml of phenolphthalein indicator and titrated against 0.1 N NaOH solution till faint pink color is obtained. Test was repeated for concordant values.

$$\text{Titratable acidity (as \% lactic acid) \%} = 9\ AN/V \qquad \textbf{(3.5)}$$

Where, A = Volume in ml of standard NaOH for titration

N = Normality of standard NaOH

V = volume of sample taken for test

3.4.2.7 Viscosity (Brookfield Viscometer)

Brookfield DV Pro II Viscometer with controlled temperature bath was used for testing viscosity of products. Spindle 18 was used during the experiment. Tests were done at 30 ° C at 30 rpm. The viscometer was allowed to stabilize for one minute and reading at end of first minute was noted down as the viscosity value.

3.4.2.8 Test for Feathering Resistance (Coffee Stability Test)

Coffee solution at 16 g powder per litre of water was prepared. 17 ml of conc. whey, whey-milk blends and milk samples were added to 50 ml of coffee solution. The samples were blended into homogeneity. The samples were transferred into 50 ml centrifuge tubes and centrifuged at 2000 – 2200 rpm for 5 minutes. The volume of sediment formed was determined after decanting the supernatant liquid. A sedimentation value between 0.75 and 1.25 ml was indicative of good stability.

3.4.2.9 Color Evaluation

3.4.2.9.1 Reflectance

Reflectance of decoction and tea samples was determined using analog reflectance meter; model CL-28 of ELICO PVT. LTD., Hyderabad makes. The reflectance values were determined by keeping reflectance meter on the surface of sample bottles in which the product was filled. Reflectance values along the 4 surfaces of the sample bottles were taken.

3.4.2.9.2 Computer Vision

The colour of the decoction, tea samples and Tea whiteners were measured using a computer based image analysis technique. The samples were placed in petriplates of (3 mm thickness and 75 mm diameter) and scanned using flatbed scanner (Model: HP Scanjet 3970) at 1280 ×720 dpi resolution. The images were then imported into Adobe Photoshop 7.0 software and the ‘L’, ‘a’ and ‘b’ values were obtained from the histogram window. According to International Commission of Illumination (CIE, 1986), a colour can be defined by three parameters namely L*, a* and b*. L* is a measure of lightness or luminance, which ranges from 0 (black) to 100 (white), and a* and b* are the two chromatic components, which range from -120 to 120 (a* from green to red and b* from blue to yellow) (Anonymous Adobe Systems Inc., 1998). The software uses a scale, ranging from 0 to 255, to characterize lightness, 'a' and 'b'. These values were converted into CIELAB L*, a* and b* using the following formulae (Yam & Papadakis, 2004).

$$L^* = \left[\frac{L}{255}\right] \times 100 \quad \textbf{(3.6)}$$

$$a^* = \left[\frac{240a}{255}\right] - 120 \quad \textbf{(3.7)}$$

$$b^* = \left[\frac{240b}{255}\right] - 120 \quad \textbf{(3.8)}$$

The browning index of the samples was calculated using the formulae given by Oliviera *et al*. (2011).

Browning Index (BI) = [100(x-0.31)]/0.17 **(3.9)**

Where, x= (a+1.75L)/ (5.645L+a-3.012b)

3.4.3 Microbial Analysis

3.4.3.1 Total Plate Count

1ml of the samples were transferred to 9 ml saline solution aseptically and first dilution was prepared. Subsequent dilutions upto 10^{-4} were prepared. 2^{nd}, 3^{rd}, 4^{th} dilutions were pour plated using milk agar and incubated at 25° C for 48 hours and observed for colony formation IS: (SP: 18, Part XI) (1981). The colony count was taken after the incubation period.

3.4.3.2 Coliform count

1ml of the samples was transferred to 9 ml saline solution aseptically and first dilution was prepared. Subsequent dilutions upto 10^{-2} were prepared. 1^{st} was pour plated using violet red bile agar and incubated at 37° C for 24 hours and observed for colony formation IS: (SP: 18, Part XI) (1981). The colony count was taken after the incubation.

3.4.3.3 Presumptive Coliform Count

1 ml of sample from the 2nd dilution was transferred to a test tube containing 10 ml Mc-conkey broth with Durhams tube in it. The tubes were incubated at 37° C for 24 hours and observed for color change and gas production (BIS 1981).

3.4.4 Sensory evaluation

The sensory evaluation was done by a select panel of judges on a 9 point scale for appearance, flavour, body & texture and overall acceptability. A score of 9 corresponded to highest acceptability while a score of 1 corresponded to least acceptability of the products.

3.4.5 Shelf life study

200 ml of the samples were filled in LDPE pouches and maintained at 7±2°C and quality parameters were studied.

3.4.6 Statistical Analysis

Statistical analysis of the sensory data and reflectance values were done using SPSS (v. 15.0)

4. Results and Discussion

The results obtained during the present investigation have been compiled, analysed and discussed in the order given below.

1. Standardization of Decoction
2. Optimization of Tea Preparation
3. Selection of Type of Whey for the Preparation of Dairy Whitener
4. Optimization in Relation to Blending Ratio of Whey and Milk
5. Effect of Homogenization on Whitening and Sensory Characteristics
6. Physicochemical Characteristics of Tea Whitener
7. Shelf Life Studies of Tea Whitener

4. 1 Standardization of Decoction

A uniform quality of tea decoction is essential to assess the sensory quality of tea prepared by using the tea whitener. In order to prepare a uniform quality decoction the instructions of the tea blender as indicated on the packaging was adopted. In all the trials the same brand of tea (Taj Mahal brand) was used to prepare the decoction. The decoction was prepared by steeping 2.5 g tea powder for 2 min in 100 ml of boiling water as shown in the flow diagram (Fig 4.1). The decoction was filtered and the total solids were analysed as per AOAC method and was found to be between 0.75-0.82 percent. In all the trials the decoction was prepared in the similar manner to assess the quality of tea whitener. The total solids of the decoction were in agreement with the earlier report (Owuor, 2003b).

Water
↓
Heating to boiling
↓
Addition of tea powder (2.5 g per 100 ml water)
↓
Brewing in boiling water for 2 minutes
↓
Filtering the decoction storing in closed thermos flask
↓
Decoction

Fig. 4.1. Method of Preparation of Tea Decoction

4.2 Optimization of Tea Preparation

The tea beverage was prepared by using the decoction as detailed in Fig.4.1 with different levels of toned milk viz. 60 ml (T_1), 70 ml (T_2) and 80 ml (T_3) for every 100 ml decoction. Based on preliminary trials the sugar level was optimized at 8%. Sensory acceptance of tea prepared from different levels of milk was tested using a 9-point Hedonic scale. The results of the sensory evaluation of the beverage prepared are detailed in table 4.1.

Table 4.1. Sensory scores of tea with different levels of added milk

Parameter	T_1	T_2	T_3
Appearance & color	7.53±0.45[a]	7.71±0.32[a]	7.81±0.34[b]
Body & Texture	7.28±0.42[a]	7.68±0.28[a]	7.79±0.36[b]
Flavor	7.1±0.71[a]	7.27±0.73[a]	7.6±O.62[a]
Overall Acceptability	7±0.58[a]	7.34±0.43[a]	8.16±0.18[b]

T_1: 60 ml TM, T_2: 70 ml T, T_3: 80 ml TM

Scores on 9-point hedonic scale expressed as mean± standard err or

Figures with different superscripts differ significantly (p<0.05)

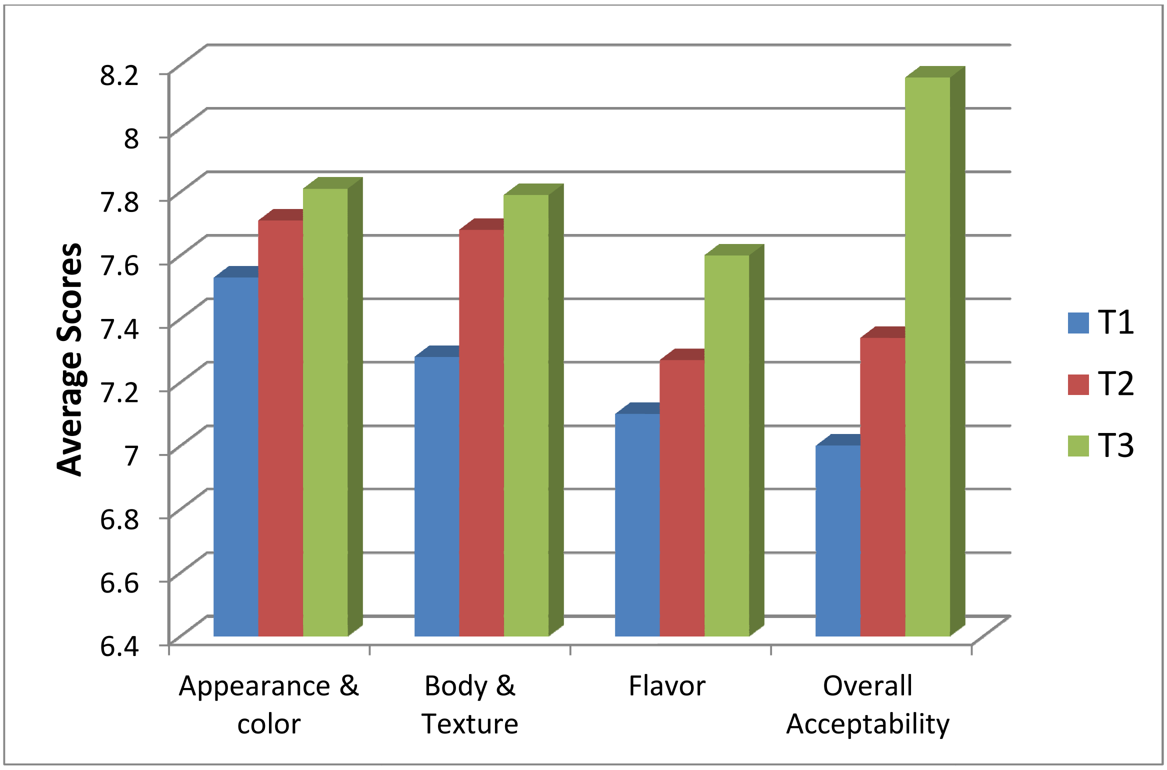

Fig 4.2. Sensory Scores of Tea with Different Levels of Added Milk

It is observed from the table that appearance and color of tea was 7.81±0.34 which was highest for T_3 and the scores were observed to increase gradually with increase in added milk levels. Similarly the body and texture score was also highest for T_3 compared to 7.68±0.28 and 7.28±0.42 respectively for T_2 and T_1. The variation in score was similar to the trend observed for appearance and color. Similarly the flavor score was also observed to increase with the level of milk addition and the highest score was at T_3 with a score of 7.6±0.62. The lowest flavor score was for T_1 at 7.1±0.71 and the score was 7.27±0.73 for T_2. The overall acceptability score was also highest at 8.16±0.18 for T_3 similar to other parameters and a gradual increase in score was observed from T_1 to T_2 to T_3. The scores are graphically represented in Fig. 4.2. Statistical analyses of the sensory data were done using One way ANOVA using SPSS 15.0. It was observed that results differed significantly due to variation in milk levels. There was a significant difference between the overall acceptability scores of T_3 and T_2, T_1. There was no significant difference between the scores of T_2 and T_1 even though an increasing trend in the scores is noticed. The results are tabulated in table 4.2.

Table 4.2. Analysis of Variance for Overall Acceptability

Source of Variation	Sum of Squares	df	Mean Square	F	Sig.
Between Groups (Milk Levels)	8.502	2	4.251	21.26*	.000
Error	6.598	33	.200		
Total	15.100	35			

Significant ($P<0.05$)

Table 4.3. Duncan's Multiple Range Test for Overall Acceptability

			Subset for alpha = .05	
	Milk_level	N	1	2
Duncan	60 ml	12	7.0000	
	70 ml	12	7.3417	
	80 ml	12		8.1583
	Sig.		.070	1.000

There was a significant difference in the overall acceptability of tea with respect to milk content in the beverage. To assess which milk level caused the significant difference, posthoc tests using Duncan's Multiple Range Test (DMRT) was done and results are tabulated (Table 4.3). The difference solely arised due to the higher scores of T_3. The mean scores of T_1 and T_2 were not significantly different. The sensory panel scored the T_3 sample higher than T_1 and T_2 to due to its superior quality attributes. The mere 20 ml & 10 ml increase in milk quantity in T_3 than T_1 and T_2 had major implications on the acceptability. Based on the results it is statistically concluded that optimum quality of tea requires 80 ml of milk in 100 ml decoction and 14.4 g sugar. Based on this information it is inferred that 1 litre of TM yields about 14 cups (160 ml serving size) of good quality tea. This is in agreement with earlier report that each litre of milk can produce about 12-15 cups of tea (Kulkarni *et al*., 2001). Based on the above results in all future trials for every 100 ml decoction about 80 ml of whitener was used for the preparation of tea beverage.

4.3 Selection of Type of Whey for the Preparation of Dairy Whitener

For the development of whitener two types of whey which are common byproducts of Indian dairy industry viz. cheese and *paneer* whey were tried for blending with toned milk for the production of tea whitener. It was observed during preliminary trials that blending concentrated *paneer* whey with toned milk even at 10% level was not suitable as during pasteurization curdling occurred indicating low stability. Even when mix was cold blended and added to tea decoction and heated to above 60±5°C there was flocculation indicating *paneer* whey was unsuitable for the development of tea whitener. This observation could be attributed to the lower pH of 5.4±0.1 and titratable acidity 0.35% lactic acid for *paneer* whey, and upon concentration the pH further dropped to 4.8 ± 0.10. The effect of lower pH on the stability of milk system is well documented (Swaisgood, 2008).

It was further observed that mixing of concentrated cheddar cheese whey (11.5% TS) with toned milk even upto 75 parts of the milk system did not result in any coagulation upon pasteurization reflecting the stability of tea whitener. The pH of fresh cheese whey was 6.2±0.1 and corresponding titratable acidity was 0.13±.05 % lactic acid. The pH of concentrated whey with 11.5% solids was 6.2±0.1 and titratable acidity was 0.30 ± 0.015 % lactic acid. The relatively lesser effect of blending cheese whey with milk on the pH and acidity could be attributed to better stability of blended system. Jelen (1992) has reported that milk systems prepared with retentates from sweet whey were heat stable with no coagulation at 90°C for 30 minutes, while acid whey retentates showed variable stability. In this experiment on thermo stability of milk systems with modified casein to whey ratio , 50:50 blends of skim milk and whey UF retentate had a heat coagulation time of 8 minutes, where as a 70:30 blend of skim milk and whey UF retentate had a heat coagulation time of 30 minutes or higher. When milk is subjected to heat treatment below 100°C it causes whey protein denaturation leading to exposure of their sulfydryl groups and non polar residues. The denatured whey proteins interact with casein micelles which improves the heat stability of concentrated milks (Singh, 1992). Heat induced association of whey proteins especially β-lactoglobulin with casein micelles alters the micelle properties leading to increased heat stability (Swaisgood, 2008).

Based on the above observation in all further trials cheese whey was selected for blending to develop a tea whitener. The flow diagram adopted for optimization is detailed in Fig 4.3. Earlier, use of cheese whey solids in the development of coffee drinks and tea/ coffee whitener has been reported by Dhaka *et al.* (2002) and Thompson and Reiners (1982).

4.4 Optimization in Relation to Blending Ratio of Whey and Milk

In the trials concentrated cheese whey (11.5% TS) was blended with toned milk to produce a Tea whitener. The Tea whitener was prepared by following the procedure outlined in Fig. 4.3. Three different blends in whey to milk ratio 75:25 (T_1), 50:50 (T_2) and 25:75 (T_3) were prepared to optimize the blending proportions. The blends prepared were used in the preparation of Tea as detailed in Fig. 4.4. A control tea was prepared from toned milk (T_4) and sensory scores of tea prepared from the blends were compared against the control. The tea prepared was subjected to sensory evaluation on a 9 point Hedonic scale. The mean scores of sensory evaluation are graphically presented in Fig. 4.3 and are presented in table 4.4.

Table 4.4. Sensory Score of Tea Prepared from Tea Whitener

Parameter	T1	T2	T3	T4
Appearance & color	7.79 ± 0.23^b	7.88 ± 0.13^b	8 ± 0.12^a	8.14 ± 0.09^a
Body & Texture	7.9 ± 0.20^a	8 ± 0.17^a	8.13 ± 0.14^a	8.08 ± 0.08^a
Flavor	7.74 ± 0.21^b	8.03 ± 0.09^a	8.13 ± 0.12^a	8.03 ± 0.09^a
Overall Acceptability	7.72 ± 0.30^b	7.98 ± 0.18^a	8.11 ± 0.10^a	8.12 ± 0.10^a

All ratios are Whey: Milk, *Similar superscripts indicate non significant difference

T_1: 75:25, T_2: 50:50, T_3: 25:75, T_4: Control (TM)

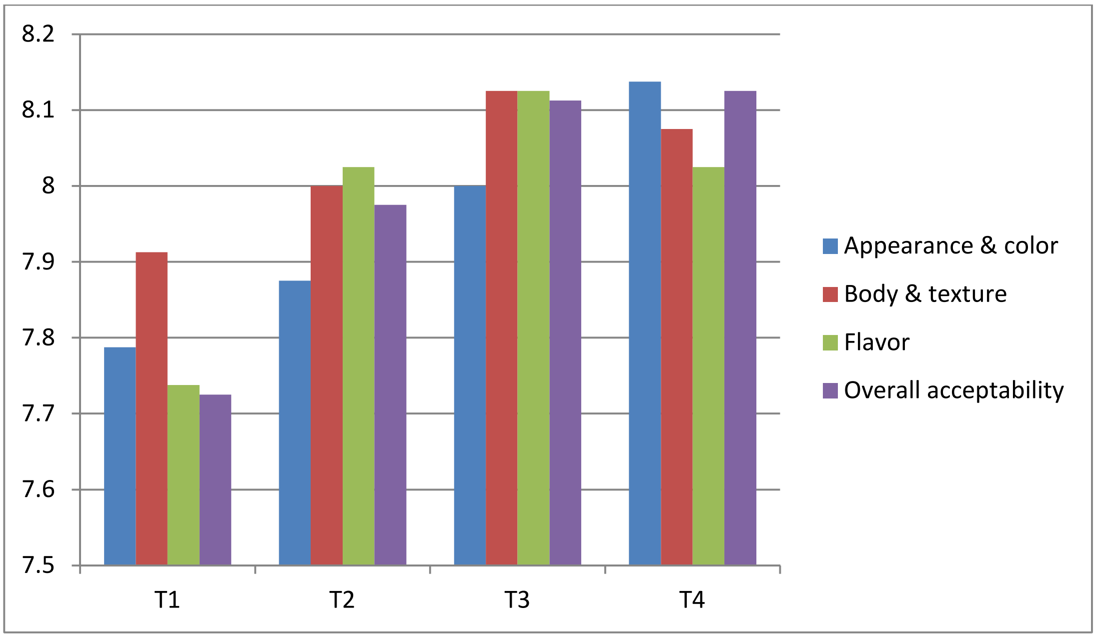

Fig 4.3. Sensory Scores of Tea Prepared using Various Tea Whitener Blends

Sensory scores were statistically analyzed by One-way ANOVA using SPSS 15.0 and were found to have significant difference between the samples. Post hoc tests using Dunnett's two sided t test was done to assess the treatment which contributed to the difference. All the treatments were tested for similarity against the control tea prepared from toned milk.

It is observed from the results that the blending had a decreasing effect on the color and appearance score and it was found to decrease gradually from 8.14 to 7.79 with increase in the level of whey in the blend (Table 4.4). However the difference in the scores between T_3 as T_4 (control) was observed to be statistically non significant.

The ANOVA results and post hoc test results are presented in tables 4.5 and 4.6.

Table 4.5. Analysis Of Variance of Color and Appearance Scores of Tea using Tea Whitener

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	0.558	3	0.186	7.202*	0.001
Within Groups	0.723	28	0.026		
Total	1.280	31			

Significant (P<0.05)

Table 4.6. Multiple Comparisons of Tea Color Assessed by Dunnett's Two Sided t Test

(I) Whey_level	(J) Whey_level	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
					Lower Bound	Upper Bound
T1	T4	-.350 *	.0803	.000	-.5494	-.1506
T2	T4	-.2625 *	.0803	.008	-.4619	-.0631
T3	T4	-.1375	.0803	.228	-.3369	.0619

*. The mean difference is significant at the .05 level.
Dunnett t-tests treat one group as a control, and compare all other groups against it.

Blending had only a marginal effect on the body and texture scores. The scores ranged from 7.9±0.20 for the tea prepared using blend T_1 and 8.08±0.08 for the tea prepared using T_4 (control). The scores for T_2 and T_3 were 8±0.17 and 8±0.14 respectively. The variation among the scores was statistically non significant (Table 4.4).

The flavor scores of tea prepared from the blends varied from 7.74±0.21 to 8.12±0.08 for the treatments and 8.03 ±0.09 for the control. The flavor scores varied significantly between T_1 and T_4. There was no significant difference in flavor scores between T_2 and T_4 and T_3 and T_4. The ANOVA and post hoc test results are presented below in tables 4.7 and 4.8 respectively.

Table 4.7: Analysis Of Variance of Flavor Scores of Tea Prepared using Tea Whitener

	Sum of Squares	**df**	**Mean Square**	**F**	**Sig.**
Between Groups	0.831	3	0.277	12.771*	0.000
Within Groups	0.607	28	0.022		
Total	1.439	31			

** Significant (P<0.05)*

Table 4.8. Multiple Comparisons of Tea Flavor Assessed by Dunnett's Two Sided t Test

(I) Whey_level	(J) Whey_level	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
					Lower Bound	Upper Bound
T1	T4	-.2875 *	.07095	.001	-.4637	-.1113
T2	T4	.0000	.07095	1.000	-.1762	.1762
T3	T4	.1000	.07095	.371	-.0762	.2762

*. The mean difference is significant at the

Dunnett t-tests treat one group as a control, and compare all other groups against it.

The flavour score of T_3 was found to be marginally higher than control even though it was not significant. This may be due to masking of whey flavour effectively by tea flavour and sugar. Based on the results it can be inferred that blending of milk with whey at 25% level (T_3) is feasible and blend can result in Tea whitener which can be used to prepare tea which is of comparable quality to of toned milk.

The overall acceptability scores ranged from 7.72±0.30 to 8.12±0.10 and the highest score amongst the treatments was for T_3 and the lowest for T_1. The score of T_1 was observed to be significantly lower than T_2, T_3 and control. However, the scores did not differ significantly between T_2, T_3 and control. The results of ANOVA and post hoc tests are presented below in tables 4.9 and 4.10.

Table 4.9. Analysis Of Variance of Overall Acceptability Scores of Tea using Tea Whitener

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	0.828	3	0.276	6.588*	0.002
Within Groups	1.174	28	0.042		
Total	2.002	31			

**Significant (P<0.05)*

Table 4.10: Multiple Comparisons of Tea Overall Acceptability Assessed by Dunnett's Two Sided t Test

(I) Whey_level	(J) Whey_level	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
					Lower Bound	Upper Bound
T1	T4	-.4000 *	.10237	.002	-.6542	-.1458
T2	T4	-.150	.10237	.341	-.4042	.1042
T3	T4	-.0125	.10237	.999	-.2667	.2417

*. The mean difference is significant at the .05 level.

a. Dunnett t-tests treat one group as a control, and compare all other groups against it.

The results based on individual characteristics and overall acceptability indicated that the treatment T_3 i.e. the blend of concentrated whey and TM in the ratio 25:75 is a feasible combination to produce a good quality tea whitener. Tea whitener with 50% whey also produced tea beverage with similar overall acceptability scores. Earlier Charman (2004) has reported that whey solids could be incorporated between 5 and 30% to produce a good quality whitener. Based on the results in all the trials Tea whitener was produced by blending concentrated whey and toned milk in 25:75 and 50:50 ratios. In order to further improve the overall acceptability of the Tea Whitener, selected blends were subjected to homogenization and the results are discussed in the subsequent chapter.

4.5 Effect of Homogenization on Whitening and Sensory Characteristics

Homogenization was used as one of the processes to further stabilize the quality parameters of the tea whitener, as it has been reported homogenization had a significant effect on the stability and whitening ability of concentrated milk (Kulkarni *et al*., 2001). The mean scores of the sensory characteristics are presented in Fig 4.4.

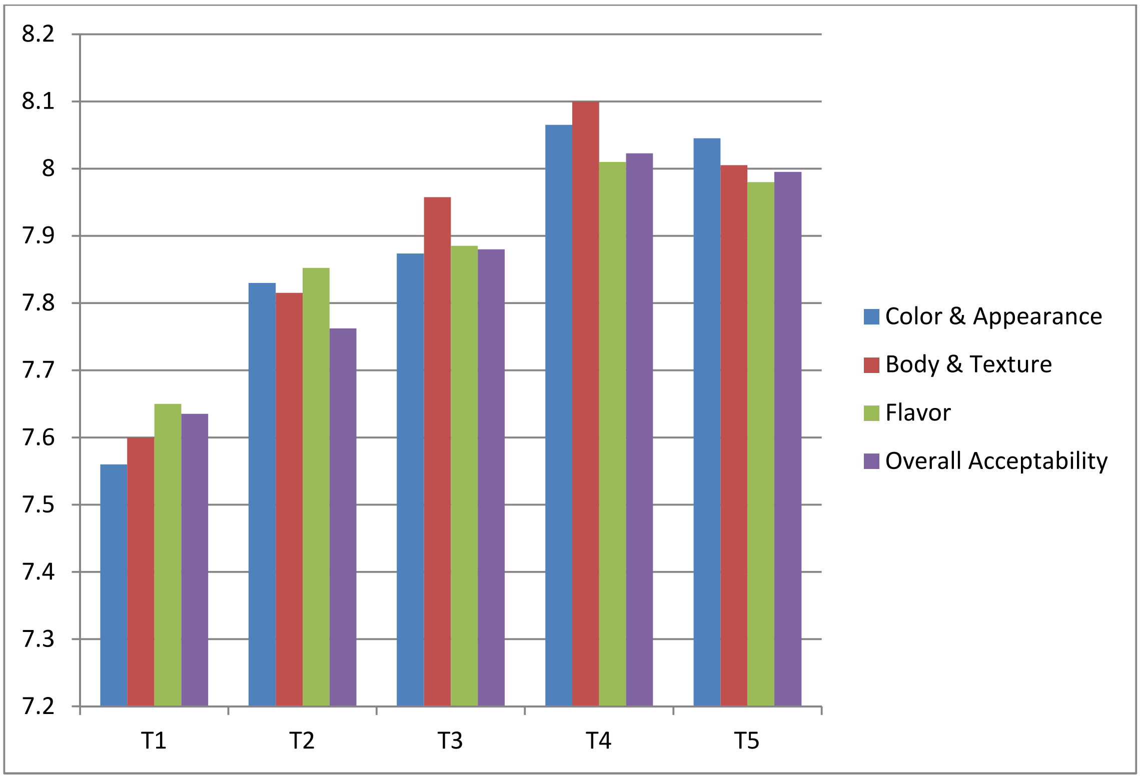

Fig 4.4. Sensory Scores of Tea Prepared from Homogenized and Unhomogenized Tea Whiteners

* All ratios are Whey: Milk

T_1: 50:50 Unhomogenised
T_2:25:75 Unhomogenised
T_3 : 50:50 Homogenised
T_4 : 25:75 Homogenised
T_5 : Toned milk (Control)

Table 4.11. Sensory Scores of Tea Prepared from Homogenized and Unhomogenized Tea Whiteners

Parameter	T_1	T_2	T_3	T_4	T_5
Appearance & color	7.56±0.31^{a}	7.83±0.23^{b}	7.87±0.27^{b}	8.07±0.15^{c}	8.01±0.29^{c}
Body & Texture	7.60±0.47^{a}	7.82±0.29^{b}	7.96±0.31^{bc}	8.10±0.13^{c}	8.00±0.33^{bc}
Flavor	7.65±0.31^{a}	7.85±0.26^{b}	7.89±0.22^{b}	8.01±0.24^{b}	7.98±0.31^{b}
Overall Acceptability	7.64±0.28^{a}	7.8±0.29^{ab}	7.84±0.28^{b}	8.02±0.16^{c}	8.0±0.30^{c}

*Similar superscripts indicate non significant difference

The appearance and color scores for the unhomogenised samples with whey to toned milk ratio of 50:50 (T_1) and 25:75(T_2) were 7.56±0.31 and 7.83±0.23 respectively. Upon homogenization the scores increased to 7.87±0.27 (T_3) and 8.07±0.15 (T_4) indicating the positive influence of homogenization on the appearance and color scores. The results of sensory data were statistically analysed, by One way ANOVA using SPSS 15.0 and post hoc tests were done using Duncan's Multiple Range Test (DMRT) to assess the difference between control tea and the tea prepared from the blends are presented in tables 4.12 and 4.13 respectively.

Table 4.12. Analysis of Variance of Color & Appearance Scores of Tea Prepared from Homogenized and Unhomogenized Tea Whiteners

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	3.325	4	.831	12.257*	.000
Within Groups	6.443	95	.068		
Total	9.768	99			

**Significant (P<0.05)*

Table 4.13. Multiple Comparison of Color & Appearance of Tea using Duncan's Multiple Range Test

Beverage_type	N	Subset for alpha = .05		
		1	2	3
T1	20	7.5600		
T2	20		7.8300	
T3	20		7.8750	
T5	20			8.0450
T4	20			8.0650
Sig.		1.000	.586	.809

Means for groups in homogeneous subsets are displayed.
a. Uses Harmonic Mean Sample Size = 20.000.

The control sample of the tea prepared from unhomogenised toned milk (T_5) had a score of 8.01±0.33 and the difference in scores between T_4 and T_5 were not significant and similarly there was no significant difference between T_2 and T_3. However, the scores for T_1 at 7.56±0.31 were significantly lower than the other treatments and control. The scores of T_4 and T_5 were significantly higher than T_2 and T_3. Trout (1950) had reported that light scattering and light reflecting ability of homogenized milk is noticeable when added to coffee which validates the above results. The results indicated a positive influence of homogenization on the color and appearance scores confirming the earlier reports of Kulkarni *et al* (2001).

The body and texture scores of the treatments ranged from 7.6±0.47 to 8.1±0.13 against 8.00±0.33 for the control.

Table 4.14. Analysis of Variance of Body and Texture Scores of Tea Prepared from Homogenized and Unhomogenized Tea Whiteners

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	3.029	4	0.757	6.773*	0.000
Within Groups	10.621	95	0.112		
Total	13.650	99			

Significant (P<0.05)

Table 4.15. Multiple Comparison of Tea Body & Texture of Tea using Duncan's Multiple Range Test

Beverage type	N	Subset for alpha = .05		
		1	2	3
T1	20	7.6000		
T2	20		7.8150	
T3	20		7.9575	7.9575
T5	20		8.0050	8.0050
T4	20			8.1000
Sig.		1.000	.092	.208

Means for groups in homogeneous subsets are displayed.

Uses Harmonic Mean Sample Size = 20.000.

The body and texture score for T_1 was 7.6±0.47 while the score for T_2 was significantly higher at 7.82±0.29. However, the scores between T_2 and T_3 were observed to be statistically non significant. Amongst the treatments the body and texture scores of T_4 at 8.1±0.13 was observed to be significantly higher than T_1 and T_2 and there was no significant difference between T_3 and T_4. The score for control at 8.01±0.33 was observed to be significantly higher than T_1. The results reflected that homogenization did have a positive effect on the body & texture characteristics but however the difference was only marginal. Similar observations on the effect of homogenization on the body and texture scores have been reported earlier (Kulkarni *et al*., 2001).

The homogenization was observed to have minimal effect on flavour scores as observed from the results.

Table 4.16. Analysis of Variance of Flavor Scores of Tea Prepared from Homogenized and Unhomogenized Tea Whiteners

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	1.610	4	0.402	5.181*	0.001
Within Groups	7.378	95	0.078		
Total	8.987	99			

**Significant (P<0.05)*

Table 4.17. Multiple Comparison Flavor of Tea using Duncan's Multiple Range Test

Beverage_type	N	Subset for alpha = .05	
		1	2
T1	20	7.6500	
T3	20		7.8850
T2	20		7.9100
T5	20		7.9800
T4	20		8.0100
Sig.		1.000	.185

Means for groups in homogeneous subsets are displayed.

a. Uses Harmonic Mean Sample Size = 20.000.

The scores for treatment T_1 were 7.65±0.31 which was observed to be significantly lower than other treatments and control. However flavour scores between T_2, T_3, T_4 and T_5 were observed to be not significant. The homogenization resulted in marginal increase in scores which was significant between T_1 and T_3 but, the scores between T_2 and T_4 were observed to be non significant. The inconsistent effect reflected in flavour score can be ascribed to the compositional variations T_1 and T_2. At higher ratio of whey incorporation there was a significant effect of homogenization on the flavour score while the difference was observed to be insignificant at lower levels of blending whey with milk.

The overall acceptability scores was analysed for statistical significance and the results are presented in tables 4.18 and 4.19.

Table 4.18. Analysis of Variance of Overall Acceptability Scores of Tea Prepared from Homogenized and Unhomogenized Tea Whiteners

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	1.982	4	0.495	6.775*	0.000
Within Groups	6.947	95	0.073		
Total	8.928	99			

**Significant (P<0.05)*

Table 4.19. Multiple Comparison of Overall Acceptability of Tea using Duncan's Multiple Range Test

Beverage_type	N	Subset for alpha = .05		
	1	2	3	1
T_1	20	7.560		
T_2	20		7.8300	
T_3	20		7.8750	
T_5	20			8.0450
T_4	20			8.0650
Sig.		1.000	0.586	0.8090

Means for groups in homogeneous subsets are displayed.

a Uses Harmonic Mean Sample Size = 20.000.

The results indicate that homogenization had a significant positive influence as it is observed that scores of T_3 at 7.84±0.28 was significantly higher than 7.64±0.29 for T_1. Similarly the scores for T_4 at 8.02±0.16 were significantly higher than the scores 7.8±0.29 for T_2. The overall acceptability of control sample was 7.99±0.30 was observed to be significantly higher than T_1, T_2, T_3 and was not different from the score of T_4 at 8.02±0.16.

The results also indicated that homogenization is an important process which can be used effectively for improving the overall acceptability scores of Tea whitener prepared by blending whey and toned milk. The homogenization pressure in the present investigation varied widely between 1200 and 1500 psi due to technical limitations. Effect of homogenization with pressure and temperature variations needs further investigation. Thus based on the results, in the optimization of development of tea whitener by blending whey with milk, homogenization was used as one of the processing parameter and the shelf life studies of the final product were done including this processing parameter. The physicochemical characteristics and microbiological quality of the optimized product are described in the next chapter.

4.6 Physicochemical Characteristics and Microbiological Quality of Tea Whitener

4.6.1 Physicochemical Characteristics

Table 4.20. Proximate Composition of Concentrated Whey and Whey-Milk Blends (%)

Parameter	Concentrated whey	Whey: Milk blends	
		50:50	25:75
Total solids	11.40 -11.60	11.50 -11.60	11.50 -11.60
Protein	1.50-1.60	2.20 - 2.40	2.70 - 2.90
Fat	0.20-0.30	1.60 - 1.70	2.30 - 2.40
Ash	0.84-0.90	0.79 - 0.82	0.75 - 0.80
Acidity	0.29-0.31	0.20 - 0.22	0.17 - 0.18

The various physicochemical characteristics of the concentrated whey and whey-milk blends are presented in tables 4.20 to 4.28. In all the trials concentrated whey with a solids content of 11.5% was blended with toned milk of same solids content to produce the Tea whitener. It is observed from the table 4.20 that the TS of whey varied between 11.4 and 11.6 and is within a narrow range. Accurate solids levels could be maintained in the whitener as the whey was concentrated to a slightly higher level and then diluted to 11.5% by addition of calculated quantity of water. The protein content in concentrated whey was observed to range between 1.5-1.6%, fat content between 0.2-0.3% and minerals assessed as ash content was observed to be in the range of 0.84-0.90%. The acidity expressed as percent lactic acid ranged between 0.29-0.31percent. The whey composition results are in tune with the earlier results reported by Pankaj (2011) when calculated to equivalence of solids as in the present case.

The composition of the blends selected were analysed and results are presented in Table 4.20. The total solids content in both cases ranged from 11.5-11.6%. The solids content were maintained constant throughout the period of investigation. The protein contents ranged from 2.2-2.4 and 2.7-2.9% for 50% and 25% blends respectively. Similarly the fat content ranged from 1.6-1.7% and 2.3-2.4% for the blends and higher fat content represented higher levels of toned milk incorporation. The ash content ranged from 0.79-0.82% and 0.75-0.80% for the 50% and 25% whey blends respectively. These results are in tune with the anticipated values by calculation.

Table 4.21. pH Characteristics

Liquid Type	pH
Concentrated whey (11.5% T.S.)	6.20-6.25
75:25 (Whey: TM)	6.25-6.30
50: 50	6.30-6.40
25:75	6.35-6.50
Decoction	5.40-5.70
Tea (50:50)	6.20-6.30
Tea (25:75)	6.30-6.40
Tea (TM)	6.40-6.50

The pH characteristics of different ingredients are listed in table 4.21. The pH of whey concentrated to 11.5% solids ranged between 6.20-6.25 while that of tea decoction was observed in the range of 5.4-5.7. The pH of three different blends was observed to be 6.25-6.30, 6.30-6.40 and 6.35-6.70 for whey incorporation of 75, 50 and 25 parts respectively. The treatments which were selected for production of Tea whitener (50:50 and 25:75) had a pH ranging from 6.30-6.50. The pH observed for the blends were marginally lower than pH of milk which is about 6.70 at room temperature (Walstra *et al.*, 2006).

Table 4.22. Reflectance characteristics of Decoction and Tea samples

Type	Reflectance
Decoction	4.50 - 5.00
T_1	33.0 - 36.0
T_2	35.0 - 38.0
T_3	36.0 - 40.0
T_4	43.0 – 46.0
T_5	45.0 – 49.0

T_1: 50:50 Unhomogenised T_2:25:75 Unhomogenised T_3 : 50:50 Homogenised
T_4 : 25:75 Homogenized T_5: Control (toned milk)

The reflectance characteristics are an important parameter which gives necessary information on whitening ability. It is observed from table that the reflectance values of tea from toned milk ranged from 45-49 and of the decoction ranged from 4.5-5.0. Increasing the proportion of toned milk in Tea whitener increases the reflectance scores towards the control tea. The reflectance values of tea prepared from 50% blend ranged from 33-36 for the unhomogenised sample which increased to 36-40 upon homogenization. Similarly, for the tea prepared from 25% blend reflectance values ranged from 35-38 for the unhomogenised sample which increased to 43-46 upon homogenization. The results clearly demonstrated that homogenization had a positive effect on whitening of tea which was also confirmed the sensory results of color and appearance (Table 4.11).

Similar observation on improvement of whitening ability by homogenization had been reported earlier (Trout, 1950). However the earlier studies have not reported reflectance values in support of these findings. Statistical analysis of the reflectance values by One way ANOVA was done using SPSS 15.0 to assess the difference between beverage types.

Table 4.23. Analysis of Variance of Reflectance Values of tea prepared from Tea whiteners

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	1865.050	4	466.263	294.017*	.000
Within Groups	118.938	75	1.586		
Total	1983.988	79			

**Significant (P<0.05)*

Dunnett's two sided test was done to compare the reflectance values of tea preparations against the control and results are presented in table 4.24.

Table 4.24. Multiple Comparisons of Tea Reflectance Values Assessed by Dunnett's Two Sided t Test

(I) Beverage_Type	(J) Beverage_Type	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
					Lower Bound	Upper Bound
T1	T5	-12.875 *	.44523	.000	-13.9858	-11.7642
T2	T5	-10.060 *	.44523	.000	-11.1733	-8.9517
T3	T5	-8.875 *	.44523	.000	-9.9858	-7.7642
T4	T5	-2.500 *	.44523	.000	-3.6108	-1.3892

*. The mean difference is significant at the .05 level.

a. Dunnett t-tests treat one group as a control, and compare all other groups against it.

All the tea samples prepared from whitener containing 25% and 50% concentrated whey incorporated into it differed significantly with the tea prepared from toned milk in reflectance characteristics. Homogenization improved the color and appearance scores, but the compositional difference markedly influenced the reflectance characteristics. Whey incorporation even at 25% levels darkens the color of beverage prepared from it. Zadow (1971) has reported maximum reflectance for ultra high treated (UHT) milk at pH of 6.70. Reflectance dropped rapidly with decrease in pH. Gruetzmacher and Bradley, Jr (1991) reported coffee whitener formulation with acid whey solids replacing sodium caseinate produced darker colored coffee beverage and addition titanium dioxide was required to improve color of the beverage which matched with conventional formulations using sodium caseinate as base ingredient. Thompson and Reniers (1982) reported that succinylnated whey concentrate used in coffee whitener formulations produced darker color beverage than conventional formulations. The findings of the present study are in tune with the earlier reports stated above.

The color of the samples analysed by computer vision lightness values for tea whitener are tabulated in table 4.25.

Table 4.25. Lightness Values of Concentrated Whey, Tea Whitener and Decoction

Sample	**L***
Decoction	22.65
T_1	90.20
T_2	92.94
T_3	93.83
T_4	96.86
T_5	97.82
Conc. Whey	86.00

**Mean of three trials*

T_1: 50:50 Unhomogenised T_2:25:75 Unhomogenised T_3 : 50:50 Homogenised
T_4 : 25:75 Homogenized T_5: Control (toned milk)

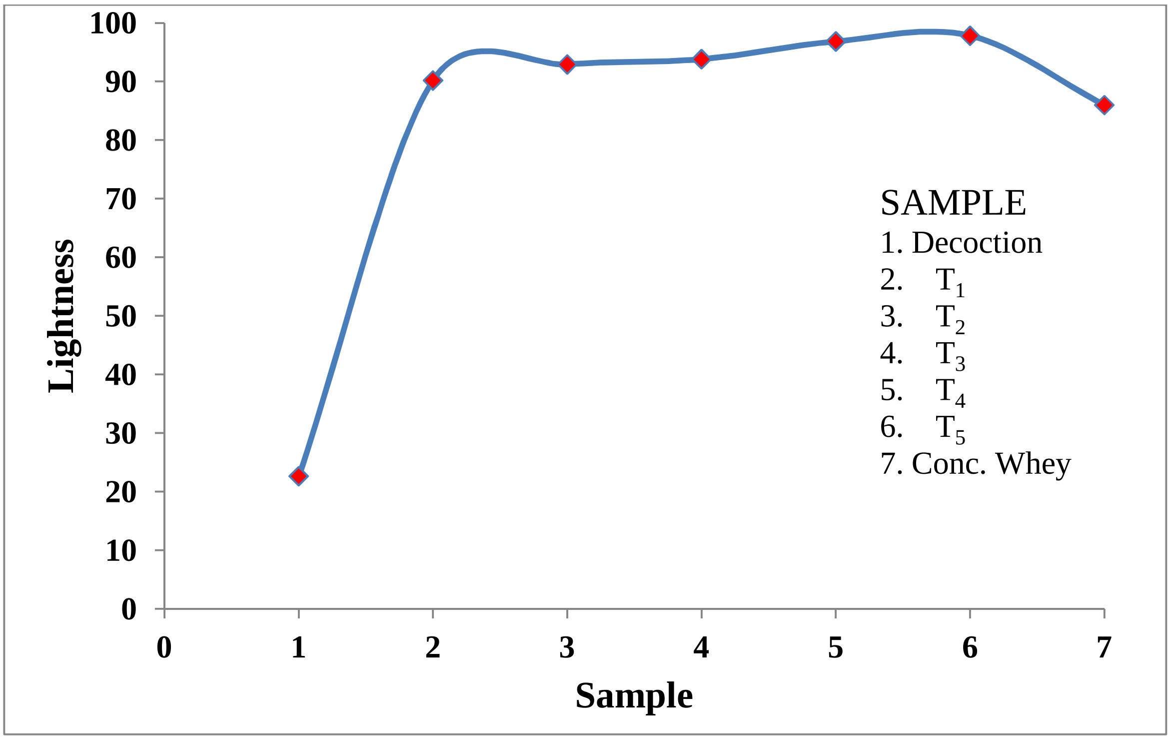

Fig 4.5 Lightness (L*) of Tea Whiteners and Decoction

The lightness values of milk were the highest followed by 25% homogenized tea whitener. The lightness values decreased with higher proportion of whey incorporation. The lower lightness value for whey incorporated sample is due to lower proportion of fat and casein in the samples. Yellowish tinge of whey and milk serum is due to the riboflavin content. The color of an opaque material is determined its ability to absorb and scatter visible light. Light scattering characteristics of milk is primarily due to fat and to a lesser extent by the casein content (Walstra *et al*., 2006). Phillips *et al.* (1995) has reported that as fat content in milk increases it becomes white, less green and less blue. Phillips and Barbano (1997) have reported addition of titanium dioxide to low fat milk based on protein substitutes improves the whiteness and sensory acceptance of the product. The higher lightness values for homogenized samples is as expected , as homogenization renders milk more opaque and densely white (Trout, 1950).

The browning index of tea samples and decoction analysed and are presented in table 4.26.

Table 4.26. Browning Index of Tea Samples and Decoction

Sample	Browning Index
Decoction	400.31
T_1	77.54
T_2	68.90
T_3	67.84
T_4	61.79
T_5	53.7

**Mean of three trials*

T_1: 50:50 Unhomogenised T_2:25:75 Unhomogenised T_3 : 50:50 Homogenised

T_4 : 25:75 Homogenized T_5: Control (toned milk)

The decoction is a deep brown solution with a browning index (BI) of 400. The ability of Tea whitener to reduce the browning index is its whitening ability. The browning index of milk tea was the lowest at 53.7. The BI of tea prepared from 25% whey incorporated homogenized whitener was 61.79 which was close to that of tea prepared from toned milk. The higher BI of tea samples prepared from whitener samples with 50% whey incorporation were higher than that of tea prepared from toned milk. The BI of tea prepared from 50% homogenized Tea whitener was close to that of tea prepared from 25% unhomogenized whitener sample. A similar trend was noted in the case of reflectance values (Table 4.22). The compositional difference of whiteners is the prime factor contributing to the variation in browning index. Earlier studies on color characteristics of coffee with respect to different levels of added milk by Macdougall (1988) based on pigment concentration (K) and light scattering (S) supports the above results

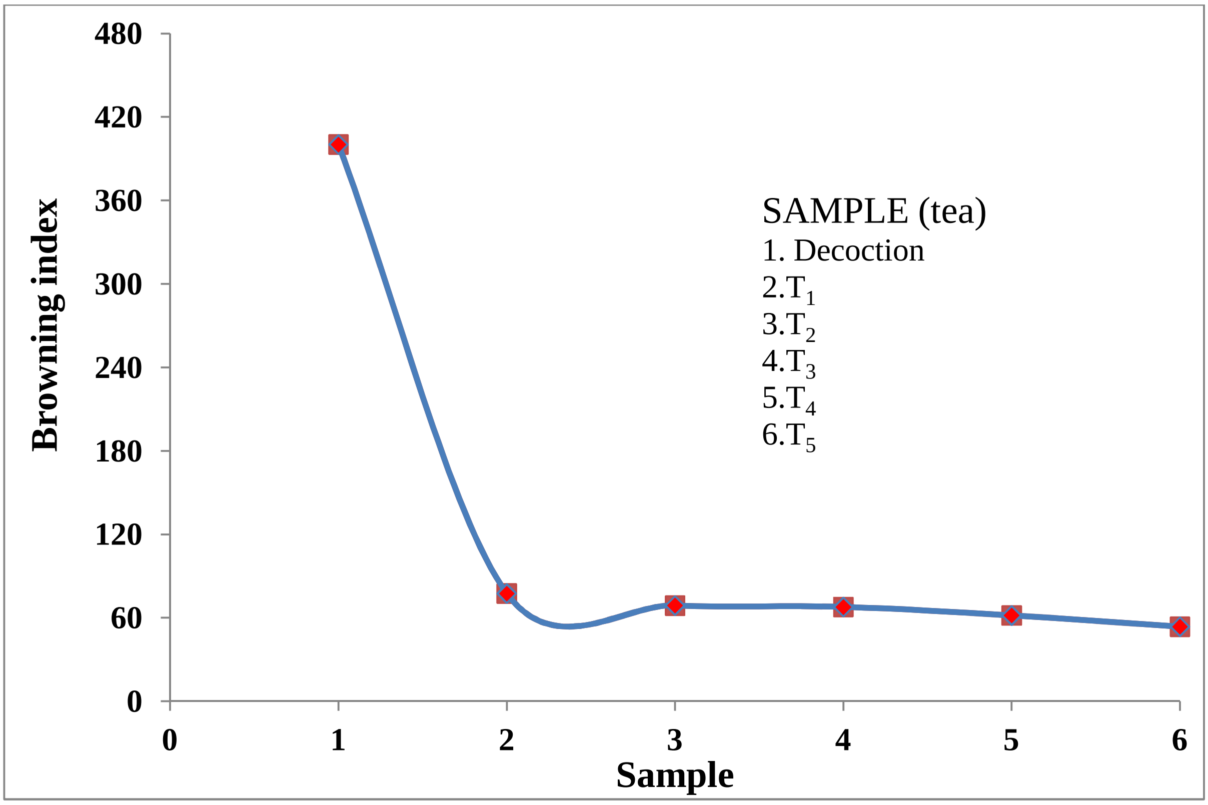

Fig 4.6. Browning Index (BI) Of Tea and Decoction

Brookfield viscometer with water bath for temperature control was used to measure the viscosity. Spindle 18 – manufacturer specified spindle for low viscosity Newtonian liquids was used in the trials. Viscosity measurement was done at 30±0.5°C at 30 rpm.

Table 4.27. Viscosity of Optimized Tea Beverage

Sample Type	Viscosity (cP)
T_1	1.95-1.98
T_2	1.65-1.70
T_3	2.04-2.10
T_4	1.86-1.92
T_5	1.62-1.65

The viscosity values of the tea beverage are tabulated in table 4.27. The viscosity of tea prepared from toned milk (control) ranged from 1.62-1.65 cP while the range was 1.95-1.98 cP for the tea prepared from 50% whey blend (T_1). The viscosity of tea produced with 25% whey blend was 1.65-1.70 cP, a bit lower than the tea from 50% blend. The results indicated that the viscosity increased with the increase in proportion of concentrated whey. However this increase in viscosity was not perceived with increase in body and texture score

(Table: 4.11). The homogenization was observed to increase the viscosity, as observed from table: 4.27. The viscosity for T_1 increased from a range of 1.95-1.98 cP to 2.04-2.10 cP upon homogenization, while for T_2 the range from 1.65-1.70 cP which increased to 1.86-1.95 cP upon homogenization (T_4). The increase in viscosity due to homogenization was reflected during the sensory evaluation with the increase in body and texture scores for the tea prepared from homogenized samples. Whitnah *et al.* (1956) have suggested the increase in viscosity due homogenization is because of particle shape changes, transfer of materials from the continuous phase to disperse phase ie, complexing of sugars with proteins, fats, enzymes etc, and electro viscous effects. Lee and Sherborn (2002) have reported binding of whey proteins to milk fat globular membrane when homogenized whole milk is heated. The viscosity of whole milk increased when milk was treated both by homogenization and heating.

The ability of whitener to resist feathering and provide the optimum whitening effect was studied using the coffee stability test. This test was used by Teehan *et al*. (1997) and Nath *et al*. (2000) to assess the coffee stability of whole milk and skim milk powders and to study the conditions in which feathering occurred.

Table 4.28. Test for Feathering Resistance (Coffee Stability Test)

Sample	Whitener qty (ml)	Sedimentation value	r.p.m	Time (min)
T_1	17	1.5	2200	5
T_2	17	1.2		
T_3	17	1.6		
T_4	17	1.2		
T_5	17	1.0		
Conc. Whey	17	2.0		

**Mean of three trials*

Legend

T_1: 50% UnHomo whey milk blend
T_2: 25% UnHomo whey milk blend
T_3: 50% Homo whey milk blend
T_4: 25% Homo whey milk blend
T_5: Control (toned milk)

A sedimentation value between 0.75 and 1.25 ml are indicative of good coffee stability (Nath *et al*., 2000). The coffee prepared from 25:75 whey-toned milk blends gave comparable sedimentation values to that of coffee prepared form toned milk. The coffee samples from 50:50 blends had higher sedimentation values which are indicative of their instability to acidic conditions (Table 4.28). The higher sedimentation values can be ascribed to higher proportion of denatured whey proteins in the blend. Pasteurization after homogenization in 50:50 blends further increased the sedimentation values. This effect was not visualized in the case of 25:75 blends as the proportion of whey incorporation is lesser. The coffee stability test is a harsh test which validates the heat and acid stability. The test results again validated that 25:75 blends of concentrated whey and toned milk can produce satisfactory Tea whitener.

4.6.2 Microbiological Quality of Tea Whitener

The microbial quality of milk and whiteners were analysed on day 1 and are tabulated in table 4.29.

4.29. Microbial Quality of Tea Whitener

Test	Sample	Average count *cfu/ml*
SPC	T_1	28000
	T_2	26500
	T_3	23500
	T_4	21500
	T_5	24000
Coliform count	All samples	Absent
PCT	All samples	Negative

**Mean of three trials*

The SPC of pasteurised milk and whiteners ranged from 21500 to 28000 cfu/ml, which are well within the prescribed standards (FSSA, 2011). The SPC of 25% blends was marginally lower than that of 50% blends and was close to the values of control toned milk. The results indicated that the total counts were almost similar in all the samples. The coliform count in all the samples were nil indicating efficient pasteurization and absence of post pasteurization contamination.

Thus it is observed from the preceding paragraphs that the physicochemical characteristics of the Tea whitener had an effect on the sensory acceptance and quality attributes of the tea beverage. Homogenization process plays an important role in the optimization process. The microbiological quality of the Tea whiteners were also studied, which indicates suitability of 25% whey blend.

4.6.3 Process Optimization

Based on the results from the chapter 4.3 to 4.6 the process optimization was finalized and the final flow diagram of the process is outlined in fig.4.7

Cheese Whey

↓

Vacuum concentration (12% TS)

↓

Pasteurization (72C°/ 15 sec)

↓

Cold storage (5 -7 C°/ 16-18 hrs)

↓

Dilution to 11.5% solids by addition of potable water

↓

Cold mixing whey and toned milk in 25: 75 ratios

↓ (left branch) **Unhomogenized blend** → **Cold Storage**

↓ (right branch) **Homogenization 1200- 1500 psi, 1 stage** → **Pasteurization** → **Cold Storage**

Cold Storage

↓

Whey-milk Tea Whitener

Fig. 4.7. Flow Diagram for Optimization of Tea Whitener

The shelf life studies of the whitener prepared as detailed in the figure was conducted and results are presented in the subsequent chapter.

4.7 Shelf Life Studies of Tea Whitener

The shelf life study of the optimised product was carried out by storing the samples packed in LDPE in a refrigerator at 7±2°C. The pasteurised blends in the whey to milk ratio 25:75 remained in good condition without any noticeable precipitation for 2 days. At the end of the second day, upon boiling curdling and precipitation was noticed in milk. During the preparation of tea, even when the whitener was cold blended with decoction, precipitation was noted in the tea beverage. Tea beverage prepared from whitener stored for 1-3 days and the sensory scores for various parameters are presented in table 4.30. The quality attributes of Tea whitener during the storage period is tabulated in table 4.31.

Table 4.30: Sensory Quality of Tea Prepared from Stored Whitener and Milk Samples.

Parameter		**Sample type**		
	Day	**T_2**	**T_4**	**T_5**
Color & Appearance	1	7.9±0.30^{a}	8.1±0.20^{d}	8.2±0.15^{l}
	2	7.8±0.20^{a}	8.05±0.15^{d}	8.1±0.20^{l}
	3	3.0±0.50^{b}	3.0±0.5.0^{e}	7.5±0.30^{l}
Body & Texture	1	7.9±0.30^{a}	8.1±0.15^{d}	8.1±0.20^{l}
	2	7.8±0.10^{a}	8±0.07^{d}	8.1±0.10^{l}
	3	3.0±0.60^{b}	4.0±0.50^{e}	7.5±0.30^{l}
Flavor	1	7.8±0.3^{a}	8.05±0.15^{d}	8.0±0.30^{l}
	2	7.7±0.20^{a}	8.0±0.10^{d}	8.0±0.20^{l}
	3	3.0±0.50^{b}	3.5±0.60^{e}	7.5±0.30^{l}
Overall Acceptability	1	7.8±0.20^{a}	8.1±0.30^{d}	8.2±0.30^{l}
	2	7.7±0.20^{a}	8.0±0.15^{d}	8.1±0.20^{l}
	3	2.0±0.50^{b}	3.0±0.50^{e}	7.5±0.30^{l}

*Similar superscripts indicate non significant difference

Table 4.31. Shelf Life Studies of Tea Whitener

Parameter	Day	Sample Type				
		T_1	T_2	T_3	T_4	T_5
Color & Appearance	1	6.9±0.15[a]	7.3±0.13[d]	7.3±0.15[l]	7.88±0.10[p]	8.03±0.15[x]
	2	6.8±0.26[a]	7.2±0.12[d]	7.2±0.14[l]	7.8±0.15[p]	7.93±0.16[x]
	3	5.25±0.18[b]	5.75±0.15[e]	5.38±0.13[m]	5.83±0.21[q]	7.8±0.19[y]
Body & Texture	1	7.2±0.11[a]	7.68±0.21[d]	7.76±0.15[l]	7.9±0.12[p]	8.00±0.14[x]
	2	6.8±0.18[b]	7.08±0.14[e]	7.18±0.11[m]	7.45±0.15[q]	7.95±0.19[x]
	3	6.75±0.18[b]	7.05±0.16[e]	7.1±0.12[m]	7.33±0.15[q]	7.92±0.09[x]
Flavor	1	6.85±0.11[a]	7.53±0.08[d]	7.33±0.08[l]	7.93±0.18[p]	8.0±0.16[x]
	2	6.3±0.12[b]	7.03±0.08[e]	6.88±0.08[m]	7.4±0.14[q]	7.8±0.19[x]
	3	5.43±0.19[c]	6.4±0.21[f]	5.45±0.18[n]	6.45±0.23[r]	7.6±0.10[y]
Overall Acceptability	1	7.4±0.19[a]	7.7±0.15[d]	7.7±0.19[l]	8±0.15[p]	8.03±0.21[x]
	2	7.35±0.11[a]	7.63±0.13[d]	7.6±0.14[l]	7.89±0.17[p]	7.98±0.13[x]
	3	5.25±0.18[b]	5.85±0.15[e]	5.45±0.23[m]	5.88±0.19[q]	7.88±0.26[x]

*Similar superscripts indicate non significant difference

The Tea whiteners and milk was subjected to sensory evaluation on a 9 point Hedonic scale, at 24 hour intervals to determine the shelf life. The sensory scores were analysed using SPSS 15.0. In studying the shelf life characteristics of the tea whitener using the sensory scores for the product Randomized Completely Block design was used. The whitener types viz: 25% and 50% whey incorporated Tea whitener -homogenized and unhomogenised and toned milk were the samples types which was blocked because there was significant difference between the samples with regard to sensory attributes particularly flavour from the time of production. The factor studied was the time period, and time gaps between subsequent sensory evaluations tests was fixed as 24 hours- i.e 1 day.

The analysis of variance of colour and appearance score of Tea Whitener and control toned milk are presented below in Table 4.30. A significant difference in scores was noted over the storage period with respect to the aspect of colour and appearance. Dunnett's two sided t test was done to assess the difference between samples. All samples were compared against the day 1 scores of the respective samples to assess the difference. The results of statistical analysis are presented in table 4.32 and 4.33.

Table 4.32. The Analysis of Variance of Color and Appearance Scores of Samples during the Storage Period.

Source	Type III Sum of Squares	df	Mean Square	F	Sig.
Corrected Model	45.915(a)	6	7.653	54.511	.000
Intercept	2903.017	1	2903.017	20679.052	.000
Block Sample Type	18.357	4	4.589	32.690	.000
Factor Day	27.559	2	13.779	98.154*	.000
Error	7.440	53	.140		
Total	2956.372	60			
Corrected Total	53.355	59			

a R Squared = .861 (Adjusted R Squared = .845)
**Significant (P<0.05)*

Table 4.33. Multiple comparisons of sample Colour and Appearance assessed using Dunnett's two sided t test

	(I) Factor_Day	(J) Factor_Day	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
						Lower Bound	Upper Bound
Dunnett t (2-sided)	day 2	day 1	-.1225	.11848	.485	-.3917	.1467
	day 3	day 1	-1.495 *	.11848	.000	-1.7642	-1.2258

Based on observed means.
*. The mean difference is significant at the .05 level.
Dunnett t-tests treat one group as a control, and compare all other groups against it.

The colour and appearance score was observed to range between 6.9±0.15 and 5.25±0.18 during storage for T_1, and the decrease was significant on day 3. The trend of colour and appearance score for T_2 was also observed to be between 7.3±0.13 and 5.75±0.15

and the score was significantly lower on day 3. Similar trend was also observed for T_3 and T_4 and the deterioration was significant on the third day for both the samples. In all the cases, scores did not show any significant difference between day 1 and day 2. In the case of control samples (T_5) also a similar trend in score was observed with significance in score noted on the third day. The fall in the score, however in the case of control sample was not marked and it ranged from 8.03±0.15 to 7.8±0.19 compared to scores below 6 for the other samples (Table: 4.31).

The analysis of variance and post hoc tests of body and texture scores of samples are presented in table 4.34 and 4.35 respectively.

Table 4.34. The Analysis Of Variance of Body and Texture Scores of Samples during the Storage Period.

Source	Type III Sum of Squares	df	Mean Square	F	Sig.
Corrected Model	9.261(a)	6	1.543	42.933	.000
Intercept	3259.014	1	3259.014	90654.868	.000
Block Sample type	6.648	4	1.662	46.229	.000
Factor Day	2.613	2	1.307	36.342*	.000
Error	1.905	53	0.036		
Total	3270.180	60			
Corrected Total	11.166	59			

a. R Squared = .829 (Adjusted R Squared = .810)

**Significant* (P<0.05)

4.35. Multiple comparisons of sample Body and Texture assessed using Dunnett's two sided t test

	(I) Factor_Day	(J) Factor_Day	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
						Lower Bound	Upper Bound
Dunnett t (2-sided)	day 2	day 1	-.45 *	.05996	.000	-.5863	-.3137
	day 3	day 1	-.435 *	.05996	.000	-.5713	-.2987

Based on observed means.

*. The mean difference is significant at the .05 level.

Dunnett t-tests treat one group as a control, and compare all other groups against it.

The body and texture score for T_1 ranged between 7.20±0.11 and 6.75±0.18 and the decrease in the score between day 1 and day 2 was observed to be significant. However, the decrease in scores between day 2 and day 3 was not observed to be significant. Similar trend was observed in the case of T_2, T_3 and T_4 samples. In the case of T_2 the body and texture scores decreased from 7.68±0.21 on day 1 to 7.08±0.14 on day 2 and subsequently to7.05±0.16 on day 3. For sample T_3 the score on day 1 was 7.76±0.15 which subsequently reduced to 7.18±0.11 and 7.10±0.12 on the second and third day respectively. In the case of T_4, the scores were observed to decrease from7.90±0.12 on day 1 to 7.45±0.15 on day 2 and subsequently to 7.33±0.15 on day 3. In the case of control samples the trend of variation observed was slightly different. The difference in the body and texture scores was found to be not significant between day 1, day 2, day 3. The results indicated that blending of whey with milk to produce the whitener affected the body and texture characteristics to a certain extent, since the scores differed significantly between day 1 and day 2.

The analysis of variance and post hoc tests of flavour scores of samples are presented in table 4.36 and 4.37 respectively.

Table 4.36. The Analysis of Variance of Flavour Scores of Samples during the Storage Period.

Source	Type III Sum of Squares	df	Mean Square	F	Sig.
Corrected Model	35.210(a)	6	5.868	72.629	.000
Intercept	2903.713	1	2903.713	35937.597	.000
Block Sample Type	18.966	4	4.741	58.682	.000
Factor Day	16.244	2	8.122	100.523*	.000
Error	4.282	53	0.081		
Total	2943.205	60			
Corrected Total	39.492	59			

a R Squared = .892 (Adjusted R Squared = .879)

**Significant* (P<0.05)

Table 4.37. Multiple comparisons of sample Flavor assessed using Dunnett's two sided t test

	(I) Factor_Day	(J) Factor_Day	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
						Lower Bound	Upper Bound
Dunnett t (2-sided)	day 2	day 1	-.435 *	.08989	.000	-.6393	-.2307
	day 3	day 1	-1.255 *	.08989	.000	-1.4593	-1.0507

Based on observed means.

*. The mean difference is significant at the .05 level.

Dunnett t-tests treat one group as a control, and compare all other groups against it.

The flavour scores during storage decreased significantly in all the samples containing whey between each day of storage. The scores for T_1 were observed to decrease from 6.85±0.11 to 6.30±0.12 on day 2 and subsequently to5.43±0.19 on day 3. In the case of T_2 the scores were 7.53±0.08**,** 7.3±0.08 and 6.40±0.21 on the 1st, 2nd and 3rd day respectively. The scores under similar storage conditions were 7.33±0.08, 6.88±0.08 and 5.45±0.18 for T_3 and 7.93±0.18**,** 7.40±0.14 and 6.45±0.23 for T_4 samples. The control had the highest score of 8.0±0.16**,** 7.80±0.19 and 7.60±0.10 on day 1, 2 and 3 respectively. There was only significant difference between scores of day 1 and day 3 in case of control sample (T_5).

The results indicated that at 7±2°C storage the sensory scores decreased gradually over the period of storage and scores of Tea whitener containing whey was lower than the control samples. The lower scores of Tea whiteners noticed in comparison to control toned milk did not cast a profound influence on the sensory characteristics of tea prepared from Tea whitener prepared by blending whey and toned in the ratio 25:75 and subsequently homogenised at 1200-1500 psi.

The analysis of variance and post hoc tests of overall acceptability scores of samples are presented in table 4.38 and 4.39 respectively.

Table 4.38. The Analysis of Variance of Overall Acceptability Scores of Samples during the Storage Period.

Source	Type III Sum of Squares	df	Mean Square	F	Sig.
Corrected Model	48.538(a)	6	8.090	42.843	.000
Intercept	3084.534	1	3084.534	16335.506	.000
Block Sample Type	11.494	4	2.874	15.218	.000
Factor Day	37.044	2	18.522	98.091*	.000
Error	10.008	53	.189		
Total	3143.080	60			
Corrected Total	58.546	59			

a R Squared = .829 (Adjusted R Squared = .810)

**Significant* (P<0.05)

4.39: Multiple comparisons of sample Overall Acceptability assessed using Dunnett's two sided t test

	(I) Factor_Day	(J) Factor_Day	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval Lower Bound	95% Confidence Interval Upper Bound
Dunnett t (2-sided)	day 2	day 1	-.0900	.13741	.738	-.4023	.2223
	day 3	day 1	-1.71 *	.13741	.000	-2.0223	-1.3977

Based on observed means.

*. The mean difference is significant at the .05 level.

Dunnett t-tests treat one group as a control, and compare all other groups against it.

The overall acceptability scores of the samples and control reflected similar trends during storage. The scores did not differ significantly between day 1 and day 2, while the difference was significant between day 2 and day 3 in all the samples containing whey. The overall acceptability score was observed to decrease from 7.4±0.19 on day 1 to 5.25±0.18 on day 3 for T_1, 7.7±0.15 to 5.85±0.15 for T_2 and 7.7±0.19 to 5.45±0.23 for T_3 on the 3rd day of storage. The overall acceptability score for stored T_4 sample was observed to decrease from 8.00±0.15 to 5.88±0.19 on the 3rd day of storage. In the case of control milk sample the overall acceptability score was observed to decrease from 8.03±0.21 to 7.88±0.26 at the end of 3rd day of storage but was not significantly different between each day of storage.

The Tea whiteners and milk sample were observed to deteriorate with storage. Tea whiteners containing whey deteriorated faster than control milk samples. There was no curdling or flaking in the Tea whitener samples which was indicative of stability. The shelf life noted in the milk samples was inferior to the earlier reports (Meunnier-Goddick and Sandra 2003). The shelf stability of the milk for 2days could be ascribed to the low microbial count observed in the milk and Tea whitener.

5. Summary and Conclusions

In India whey is produced as a byproduct during the production of channa, *paneer*, *chakka* and cheese. Whey contains about 50% of the valuable milk solids. Whey is serious pollutant and its disposal as such is not an environmental friendly option. Effluent treatment of whey to standard pollution norms warrants high inputs of cost and energy. Moreover the whey solids are a treasure of valuable milk nutrients which can be effectively used in specialized foods, cosmetics etc. Even the organized dairy industry is not utilizing whey solids effectively.

The market milk industry in India is one of the largest sectors of food processing industry and accounts for nearly 85% of the milk collected by the organized dairy sector. In recent times the cost of market milk has increased considerably causing an economic hardship for the low and middle income level consumers. A major share of milk purchased by these groups of consumers is used for whitening Tea or Coffee beverage. The present investigation was designed for effective utilization of whey through the market milk channel with the objective of developing a cost effective Tea whitener.

Experimental trials were conducted to utilize whey produced from the manufacture of cheese and *paneer* by blending with toned milk to produce an optimized product. From the preliminary trials it was observed that stability of the whitener produced by blending *paneer* whey was unsuitable for further processing, where as the cheese whey was stable to heat treatments and homogenization. In all the trials whey was concentrated and total solids were maintained at 11.5% to maintain uniform total solids in the Tea whitener.

Since the objective of the present investigation was to develop a Tea whitener, preliminary trials were conducted to optimize the procedure for preparation of tea decoction by using a standard CTC packaged tea. During the course of the study it was observed that a good decoction with 0.75-0.80% solids can be produced by boiling 2.5 g powder in 100 ml water for 2 minutes.

In the next set of trials attempts were made to optimize a standard protocol for the preparation of tea beverage by blending 60, 70 and 80 ml of toned milk each with 100 ml decoction. A uniform sugar content of 8% of the total volume was maintained in all the samples. The results indicated that the Tea produced with 80 ml milk results in the most acceptable beverage and hence throughout the course of the investigation for every 100 ml decoction 80 ml Tea whitener was added to produce a uniform quality beverage. The resultant tea had a TS content of 13.4-13.6% and every 100 ml of decoction required about 44.6ml of tea whitener.

The tea whitener was produced by blending whey and milk in the three ratios viz. 75:25, 50:50 and 25:75. The resulting whitener was used for whitening Tea beverage and the beverage was subjected to sensory evaluation on a 9 point Hedonic scale. Based on the results of sensory evaluation, blend with 75% whey content was discontinued as the tea prepared from it had the lowest ranking. The remaining two combinations were continued for further investigation

In the next set of trials the selected blends of whey: toned milk in the ratio of 50:50 and 25:75 were subjected to homogenization at 1200-1500 psi to improve the quality of the tea whitener. The sensory parameters were evaluated to assess the effect of homogenization on the quality. The sensory results indicated that homogenization resulted in a significant improvement in the various parameters especially the color and appearance and overall acceptability. Based on the results homogenization was selected as one of the process parameters in the optimization of production of tea whitener. In all the trials tea beverage prepared from toned milk was used as a control for comparative evaluation.

Whey and the whiteners were subjected to various physicochemical tests. The whey and whiteners contained about 11.4-11.6% TS and the detailed composition of the whiteners are described in the previous chapter. The pH of concentrated whey ranged between 6.2-6.25 while the pH of final tea beverage was between 6.2-6.4. The reflectance value of decoction ranged between 4.5-5 while, that of tea prepared from toned milk was 45-49. Blending of whey with toned milk lowered the reflectance values for tea beverages prepared from the same. Homogenization improved the reflectance values. The lightness value (L*) of milk and concentrated whey was 97.8 and 86.0 respectively. The lightness value (L*) of whey milk blends was lower than that of control milk. The browning index (BI) of control tea was lower than that of tea prepared from whey-milk blends implying compositional difference had a significant impact of the color characteristics of the product. The fat content influenced the whitening ability, described by the lower reflectance values, lower L* and higher BI in the case of blends and tea beverages. Viscosity of the tea beverage increased with higher levels of concentrated whey in the whitener and homogenization. The shelf life studies if the Tea whitener indicated that the product had a shelf life of 2 days at 7±2°C. The blending of whey with milk decreased the shelf life even though the overall quality of the whitener was similar to control samples. The microbial quality of the samples was well within the prescribed standards.

Conclusions

Cheese whey concentrated to 11.5% TS can effectively be used to produce an acceptable quality Tea whitener by blending with toned milk. Blending in the ratio of 25:75 was observed to be most acceptable level for optimizing the process. Homogenization had a positive impact and improved the overall acceptability of the tea beverage. The % TS of the whitener was 11.4-11.6, while the tea beverage produced from the whitener contained about 13.4-13.6% TS which included the 8% sugar. The blending of whey resulted in decreased color (reflectance and lightness) and higher browning index. Homogenization had positive influence in improving the color. Similarly viscosity values were also found to improve with homogenization. The whitener similar to milk was found to have 2 days shelf life when stored at 7±2°C. The studies indicated that cheese whey could be successfully used for the production of Tea whitener.

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APPENDIX l

Sensory Evaluation on 9-point Hedonic Scale

DAIRY TECHNOLOGY SECTION

Name of Judge: - Date:

Time: Batch no:

--- is /are served to you for organoleptic evaluation. Please judge the product on 9- point hedonic scale and write your valuable comments below.

Attributes	**T_1**	**T_2**	**T_3**	**T_4**	**T_5**
Appearance & colour					
Flavour					
Body & Texture					
Overall acceptability					

Comments on T_1:

Comments on T_2:

Comments on T_3:

Comments on T_4:

Comments on T_5:

Signature:-

9-Like extremely; 8-like very much; 7-like moderately; 6-like slightly; 5 – neither like nor dislike; 4-dislike slightly; 3-dislike moderately; 2-dislike very much; 1-dislike extremely