COMPARATIVE STUDY OF IMMUNO-THERAPEUTIC PROPERTIES OF BOVINE AND HUMAN MILK

THESIS

By

SUJATA PANDIT

Submitted to

CHAUDHARY SARWAN KUMAR HIMACHAL PRADESH KRISHI VISHVAVIDYALAYA PALAMPUR-176 062 (H.P.) INDIA

IN

Partial fulfilment of the requirements for the degree

OF

DOCTOR OF PHILOSOPHY IN HOME SCIENCE
(FOOD SCIENCE AND NUTRITION)

2007

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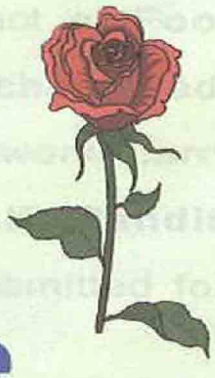
Dedicated to Sai Baba & To My Loveable Parents



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CERTIFICATE - I

This is to certify that the thesis entitled **"Comparative study of immuno-therapeutic properties of bovine and human milk"**, submitted in partial fulfilment of the requirements for the award of the degree of **Doctor of Philosophy (Home Science)** in the subject of **Food Science and Nutrition** of Chaudhary Sarwan Kumar Himachal Pradesh Krishi Vishvavidyalaya, Palampur, is a bonafide research work carried out by **Ms Sujata Pandit (H-2003-40-02)** daughter of **Sh. S.K. Pandit** under my supervision and that no part of this thesis has been submitted for any other degree or diploma.

The assistance and help received during the course of this investigation have been fully acknowledged.

(Manoranjan Kalia)
Chairman,
Advisory Committee

Place : Palampur
Dated: the 21 September, 2007.

CERTIFICATE - II

This is to certify that the thesis entitled **"Comparative study of immuno-therapeutic properties of bovine and human milk"** submitted by **Ms Sujata Pandit (H-2003-40-02)** daughter of **Sh. S.K. Pandit** to the Chaudhary Sarwan Kumar Himachal Pradesh Krishi Vishvavidyalaya, Palampur, in partial fulfilment of the requirements for the degree of **Doctor of Philosophy (Home Science)** in the subject of **Food Science and Nutrition,** has been approved by the Advisory Committee after an oral examination of the student in collaboration with an External Examiner.

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Needless to say, errors and omissions are mine.

Sujata Pandit

(Sujata Pandit)

Place : Palampur

Dated : the September, 2007.

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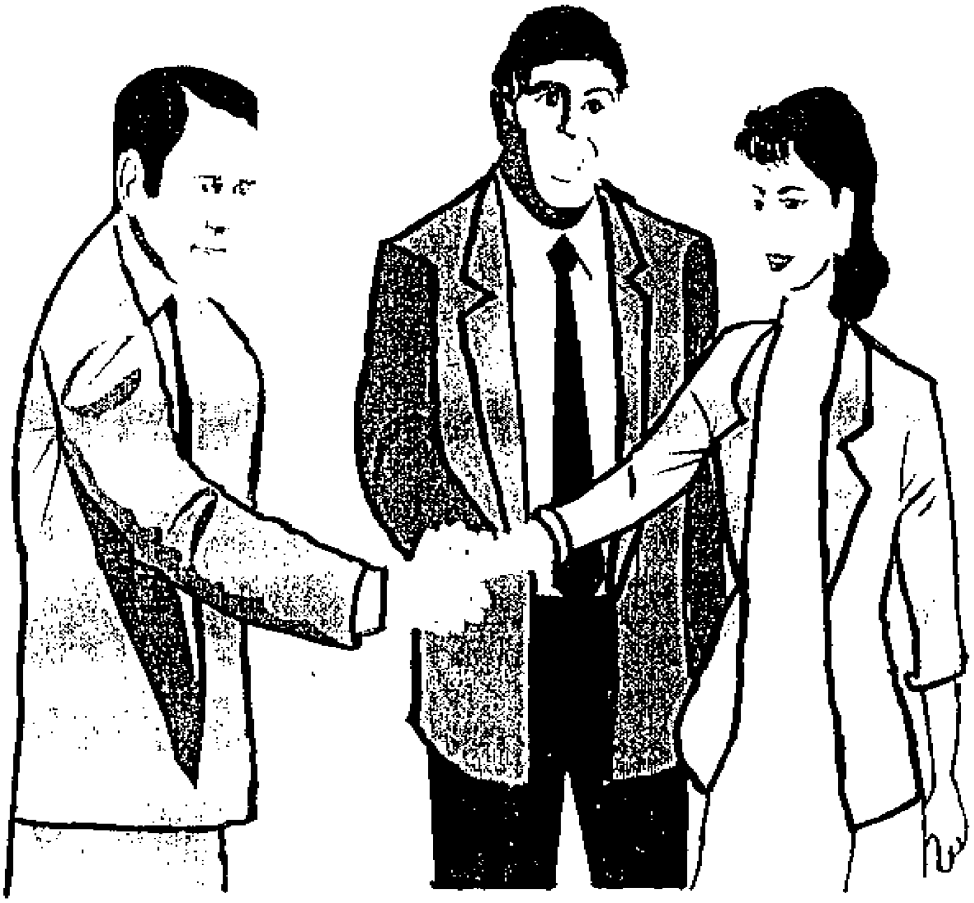


INTRODUCTION

Chapter-I

INTRODUCTION

Human milk is regarded as the optimal source of nutrients for the young infant, provided the maternal diet is nutritionally adequate and a sufficient quantity is consumed. (Anonymous, 1980). Milk is a "biological package" of nutrients harmonized together in adequate proportion due to the high biological values of proteins, presence of number of short chain unsaturated fatty acids and the unique carbohydrate lactose in milk. Milk is a source of infant's food occupies a pivotal position in the nutritional market. Every biological species synthesizing milk, tailors at the genetic level, the amount and nature of milk constituents, to suit the nutritional requirements of their off spring. Likewise, human milk possesses additional virtues compared to cow and buffalo milk as an infant's food. A possible re-organization of nutrients in cow/buffalo milk has been proposed to humanize such milk for its nutritional consonance with human milk (Anonymous, 1995).

The theological slogan 'human milk is for baby and cow's milk is for the calf' needs some deliberations. The composition of milk in different species is always 'bio-tailored' to meet the nutritional demands of the offsprings (Ganguli , 1980). Milk is the sole food of the off-springs at the time of birth. It is immaculately tailored to the nutritional requirements of the baby. The nutrients are so well designed that no other food can substitute for it. The bio-specificity of the mammary gland is responsible primarily for the nutritional excellence of milk. Milk comprises proteins, fats, lactose, minerals and vitamins of these the first three are the dominant members; whereas, the other two fall under a minority group. (Talwar, 1985)

Cattle (both cow and buffalo) are main sources of milk believed to have been domesticated 6,000 to 10,000 years ago. The oldest written records of the human race are found in Sanskrit. They are believed to date back about 6,000 years. But by then use of bovine milk by human beings had already been practiced. Knowledge of bacteriology has laid a foundation of on which are built the methods to be followed during producing, processing and handling of milk and its products for human consumption.

Composition of the milk from three different species namely cow, buffalo and human shows that human milk is quite different from cow milk in composition and even more so from buffalo milk. Being a bio-fluid, its control in chemical composition is at site of synthesis. With parturition, the mammary glands start synthesis and secretion of milk. In the first few days milk varies significantly in composition from the ultimate stable fluid milk. It is

distinguished by the name colostrums. Colostrum contains more solids than the regular milk. It is richer in protein and fat, but not in lactose.

While preparing baby food one has to identify the nutrients specific to mother's milk which are more or less in market milk. High levels of mucopolysaccharides, lysozyme, unsaturated fatty acid, lactose and low levels of proteins, fat and minerals as in mother's milk should be the objectives in humanized cow/buffalo milk. Hence, the problem for a more satisfying degree of conversion is not merely a standardization of fat and solid but a constitutional change of cow/buffalo milk. For substitution a baby food has to be formulated from cow/buffalo milk so as to be almost a duplication of human milk. This can be accomplished by enzymatic and chemical processing. Cow/buffalo milk is fortified with mucopolysaccharides, vegetable oil, selective degradation of casein particles and withdrawal of minerals by electro dialysis. In other words humanization of milk by the above approach would provide a better nutritional food for the infants (Mathur and Sarkar, 1997).

Newly born mammals including humans get first mamillary secretion milk as food, which scientifically is the dispersion of fat in water with other constituents. Breast milk is child's first immunization (Yeg, et al. 2003). Milk quality is determined by the proportions and properties of individual constituents together with the physical, chemical, organoleptic, microbiological and hygienic characteristics and its impact from the physiological point of view.

Breast milk is considered superior for infants over other modified milk formulae owing to the products of dietary intake and morbidities suffered by the child, as to its various inherent properties and advantages. However, breast milk is deficient in vitamins, iron and calcium. A progressive decline in the incidence of breast feeding has been observed around the world. The main cause for mothers to stop breast feeding are lack of education, industrialization and urbanization, lack of sufficient production of breast milk, inconvenience and social status (Sarkar and Mishra, 1996).

Nutrition problems among children are common in India and one of the reasons is poor feeding practices among the young children. The best way of ensuring child survival is to initiate steps to improve nutritional status of children as well as nutrition and health of child (Pandey and Tripathi 1998) AIDS was found to get transmitted through breast feeding (Anon, 1992). Under these circumstances, cows and buffalo milk could be humanized compositionally for infants but could not be adapted fully to the composition of human milk due to structural differences in proteins and fat (Schmidt, 1975).

During early infancy, human infants should ideally be nursed on mother's milk, which constitutes nature's best food. However socio-economic condition, the plan of mother's nutrition, rare The physiological disturbance and fear of transmittable diseases etc. have greatly influenced the infants feeding pattern. In the early 20th century, use of dried milk for infants feeding became a prominent feature of infantile dietetics. With the advent of ready to

use infant's formulas there has been a tremendous growth in the dried milk industry for the manufacture of infant's milk foods which are available in Indian market today (Thompkinson and Mathur, 1995). In view of the easy availability, most of the breast milk substitutes, either prepared industrially or at homes, utilize mainly bovine milk. Efforts have long been made on circumvent some problems with limited success, as the weaning formulations are available in the market. In the proposed study, an effort would be made to achieve the objectives cited below and to find out answers to some of the problems still confronted.

OBJECTIVES

- To study the immuno-therapeutic, nutritional and physico-chemical properties of bovine milk with human milk.
- To analyze the proximate composition of humanized milk.
- Based on the information collected, to develop weaning formulation(s) for infants and lactose intolerant subjects.
- Feasibility studies for commercialization.

It is hoped that the results of the study would prove to be of significant value to the students, researchers, food scientists, human nutritionists, policy-makers, social workers, industrial entrepreneurs and all those interested in the humanization of bovine milk.

REVIEW OF LITERATURE

Chapter-II

REVIEW OF LITERATURE

A great deal of effort has been made since long to humanize of the bovine milk to be adapted for human infants because these formulations are the only sources of nutrients for infants that are not breast-fed during the first months of life. The relevant information pertaining to comparative study of immuno-therapeutic properties of bovine and human milk are reviewed under the following heads:

2.1 Human milk and humanized milk.

2.2 Weaning and supplementary feeding.

2.3 Recommendation for the formulations.

2.4 Preparation of weaning foods.

2.5 Immunology and therapeutic properties.

2.6 *In-vitro* protein digestibility of weaning foods.

2.1 Human milk and humanized milk

Breast milk is considered superior over other modified milk formulae due to its inherent properties and advantages notwithstanding breast milk is deficient in vitamins, iron and calcium (Baggot, 1975).

Pawlik *et al.* (1975) worked on the use of whey in production of humanized infant formulae III. Chemical and biological characteristics of whey concentrates and evaluation of their suitability as components of humanized formulae they worked on (I) dried rennet whey, (ii) dried acid whey, (iii) heat-precipitated whey protein concentrate dried as for whey, (iv) dried ultra filtration concentrate and (v) dried reverse osmosis concentrate prepared by methods based on those described in parts I & II. The salient point of preparation were (I) dried rennet whey (ii) dried acid whey (iii) heating of whey to 92-95°C for 10 minutes, cooling to 50°C, allowing coagulum to sediment for 2 hours, decantation, and drying of residue; (iv) dried ultra filtration concentrate and (v) dried reverse osmosis concentrate as described. Mean composition of humanized infant formulas I, II, III, IV and V (%) was moisture 3.5, 7.5, 4.7, 2.8 and 2.6; protein 13.0, 11.9, 32.7, 24.5 and 11.8; Lactose 73.8, 64.3, 47.8, 66.6 and 79.6; and ash, 8.7, 12.0, 7.4, 5.1 and 6.2, respectively.

Ganguli (1976) reported the ∝-casein content of buffalo's milk (BM) was reduced by fractionating whole casein with urea, and reconstituting only the beta-kappa-casein fraction in diluted skim-milk. Ca-content of the

beta-kappa-casein-fortified BM was reduced by 50 per cent by electro dialysis. Since human milk contains higher levels of unsaturated fatty acids than buffalo milk, the buffalo milk fat was replaced by maize oil. The electrodialysed product was homogenized with maize oil to give a final fat content of 2.5 –3.0 per cent and lactose was added to increase its contents to 6.6-6.8 per cent. The curd tension and chemical composition of the final product were similar to those of human milk.

Ganguli *et al.* (1978) approached new technology with certain modification to buffaloes skim milk, including electro dialysis to reduce calcium, regulated proteolysis with trypsin to modify proteins, incorporation of vegetable oils and milk fat, and addition of lactose and vitamins. A spray-dried product was obtained that had a chemical composition that satisfied the FAO/WHO draft standards for infant formulae.

Romond *et al.* (1980) studied the in-vitro influence of human milk cow's milk and infant formulae on the growth of *bifidus* bacteria they observed in raw milk, pasteurized human milk and cow's milk and commercial humanized infant formulae for growth promoting activity for 34 *bifidus* bacterium strains. They found the growth promoting effects of the humanized infant formulae on the *bifidus* bacteria were similar to those of cow's milk, indicating that these formulae do not contains the *bifidus* factor present in human milk.

Kisza and Ozimek (1981) studied the humanization of the fat fraction of dried milk and infant foods. Changes in the fat fraction of dried milk in relation to the method of humanization. He reported the milk with increased content of unsaturated fatty acids and tocopherols produced by cow feeding or by addition of soybean oil (7.4:2.6 ratio of milk fat to oil) was standardized to 3.10-3.15 per cent fat, homogenized, pasteurized, concentrated and spray-dried.

Talwar (1985) defined milk is the sole food of the off spring on its birth. It is immaculately tailored to the nutritional requirements of the baby. The nutrients are so well designed that no other food can substitute for it. The bio-specificity of the mammary gland is responsible primarily for nutritional excellence of milk. Milk comprises proteins, fats, lactose, minerals and vitamins of these, the first three are the dominant members where as the other two fall under a minority group. Composition of the milk from three different species namely cow, buffalo and human shows that human milk is quite different from cow milk in composition and even more so from buffalo milk. Being a bio-fluid, its control in chemical composition is at the site of synthesis.

Renner *et al.* (1991) reported that milk is very complex secretion, consisting of cells (leucocytes, macrophages and epithelial cells), lipids (triacylglycerols, free fatty acids, The phospholipids, sterols, hydrocarbons and fat-soluble vitamins), carbohydrates (lactose, dioligosaccharides,

galactose, glucose and glycoproteins), proteins (caseins, α-lactalbumin, lactoferrin, secretory IgA and other immunoglobulins, lysozyme, enzymes, hormones and growth factors), non-protein nitrogenous compounds (urea, creatine, creatinine, uric acid, amino acids including glutamine, nucleic acids, nucleotides and polyamines), water-soluble vitamins, macronutrient elements, and trace elements.

Gallagher (1992) observed that in the first few days' milk varies significantly in composition from the ultimate stable fluid milk. Lactogenesis development has its unique synthetic capacity during pregnancy, so that the initiation of an adequate supply of milk accompanies the birth of the infants. The name colostrum distinguishes it. Colostrum contains more solids than the regular milk. It is richer in proteins and fat but not in lactose. Newly born mammals including human get first mamillary secretion. Milk is determined by the proportions and properties of individual constituents together with the physical, chemical organoleptic, microbiological and hygienic characteristics and its Impact for the physiological point of view.

Parker (1993) reported that breast feeding is an integral component of the complex psychological and metabolic dependencies of the infant on its mother, with single physical functions, such as nursing, providing the stimuli of touch, balance, smell, hearing and vision and each having specific effects on the infant.

Breast milk is considered superior for infants over other modified milk formulae owing to its various inherent properties and advantages. However, breast milk is deficient in vitamins, iron and calcium. A progressive decline in the incidence of breast-feeding has been observed around the world. The main causes for mothers to stop breast-feeding are lack of education, industrialization and urbanization, lack of sufficient production of breast milk, inconvenience and social status (Sarkar and Misra 1996).

Nutrition problems among children are common in India and one of the reasons is poor feeding practices among the young children. The best way of ensuring child survival is to initiate steps to improve nutritional status of children. Nutrition and health of child is the products of dietary intake and the morbidities suffered by the child (Pandey and Tripathi, 1998).

Milk contains both fat-soluble and waters soluble vitamins. It is a remarkable source of high quality and easily absorbed proteins the like of which is found in muscles, tissues and blood of humans (Rahman, 1999).

Milk is polyphasic emulsion having a range of physical, chemical and biological properties, its complicated make up of different systems comprise on oil-in-water emulsion with fat globules dispersed in continuous serum phase, a colloidal suspension of casein micelles globular proteins and lipoprotein particles and also a solution of lactose, soluble proteins and lipoproteins, minerals. Calcium, The phosphorous, magnesium, sodium and zinc vitamins and other components (Povey, 2001)

Dahiya and Sehgal, (2002) reported the feeding and rearing practices significantly influence the physical and mental development of the child. Good nutrition in the early months of life is more usually determined by the feeding practices whether the optimum/complete food is given at the right time and in the right way and the right frequency. Breast milk is child's first immunization.

The nutrition and health of child is the product of dietary intake and morbidity. Several studies demonstrated that during major diseases via diarrhea and acute respiratory infection. Feeding practices are jeopardized If there is repeated morbidity's and if the adverse feeding practices during morbidity's continue. Then the nutritional status of the child is likely to deteriorate (Pandey, 2002)

Pushpa *et al.* (2002) studied cow's milk proteins induce wide spectrum of allergic disorders in infants and young children causing undesirable effects such as irritation in the respiratory and gastro intestinal track, dermatitis, diarrhea and nausea. The incidence of sensitivity to cow's milk is approximately 1-2 per cent during the first two years of infancy.

Yeg. *et al.* (2003) defined that milk as food, scientifically is the dispersion of fat in water with other constituents. Breast milk is child's first immunization Milk quality is determined by the proportions and properties of individual constituents together with the physical, chemical, organoleptic, microbiological and hygienic characteristics and its impact from the physiological point of view.

Huria (2004) reported that the milk is a universal food perhaps the most versatile fluid available naturally from the secretion of female mammary glands for the purpose of nourishing young ones. Since humans also nurse their young, the idea of using milk of animals by humans was able to domesticate animals. At the present time the milk of the cow, buffalo, camel, goats, sheep, reindeer, ewe and mare is used in the various parts of the world. Milk is not only nutritious for human; it is also a substrate for various micro-organisms.

Kalia (2006) reported milk as an article of food dates back to pre-historical era when mammals arrived on the planet earth as a sequel to revolutionary evolution and suggested a recipe intended particularly for infants is nutritionally meaningless unless the food is actually consumed by the individual.

2.2 Weaning and supplementary feeding

Weaning is not sudden withdrawal of child from the breast. It is mother's milk alone is not sufficient to sustain growth beyond 4-5 months. It should be supplemented by suitable foods rich in protein and other nutrients. These are called "Supplementary foods." These are usually cow's milk, fruit juice, soft cooked *seera*, *dhal* and vegetables.

Hartmann *et al.* (1985) studied the village located women and the few remaining hunter-gathers societies and suggested that the normal duration of lactation in women is three to four years. In these societies, and

more recently in a number of developed countries, weaning is prolonged and gradual i.e. child-led weaning. The cessation of sucking results in distension of the glands with milk and atrophy of the epithelial structures.

Feeding of infants during the first six months of life are based on the assumption that breast milk from healthy, well nourished mother should be able to provide adequate energy intake for normal infants until they reach the age of four to six months. Human milk is regarded as the most suitable food for the newborn infants. It satisfies all the nutrient requirements and in addition, provides various factors, which protect the infant against attack from pathogens. Supplementary food is required when the energy requirement of the infant exceeds the amount provided by maternal milk. The age at which this point is reached depends on milk quantity and quality, size and requirement of the infant and other factors such as health of the mother and baby (Walker, 1990). Among most rural area in Thailand, chewed rice paste, gruel or banana are usually introduced to infants diet as early as few days after birth as reviewed by Dhanamitta and Vong-ek (1978). The weaning is a process in which a baby is offered small quantities of family diet along with breast milk In view of the present uncertainty, the approach of by the DHSS (1988) would seem the best: "the age at which individual infants should be offered solid foods varies. Very few infants will require solid foods before the age of three months but the majority should be offered a mixed diet not later than the age of six months." Walker (1990) recommended the introduction of

weaning foods at the age of 4 months. When an extra source of energy and other nutrients such as additional iron is required in human milk. The meaning of the term weaning is to be taken off the breasts", " to accustom to food other than mother's milk", to coax away from morbidity" or " introduction of drop feeding". Weaning usually means introduction of solids and semisolids (Gupta and Sehgal, 1991).

Weaning, if not done properly, is often followed by diarrhea and months of growth failure leading to kwashiorkor, marasmus and immune deficiency marked by recurrent and persistent infections which may be fatal (Anand, 1999).

2.3 Recommendation for formulations

The Indian standard specification for infant food was first published in 1960 (1S;1547;1960) having values - moisture 2.8 per cent, proteins 20.0 per cent, fat 18.0-28.0 per cent carbohydrate 35.0 per cent, total ash 8.5 per cent, vitamin A 1500 IU /100g, vitamin D 400-800 IU/100g, iron 40mg /100g, bacterial count 50.000 /g, coliform count 10/g and was later revised in 1968 and 1985. The protein content is 12.0 per cent and vitamin A 105.0/u /100g, iron content 5.0mg/100g, bacterial count 40,000/g.

The ministry of health and family welfare had amended the PFA rules of 1989 revised in 1991-1992. In the recent amendment the minimum requirement of 12 per cent milk fat has been deleted and the new value varies from 10.0-16.0 per cent. It is also emphasized that the important micro-

nutrients are compulsory for infant milk food or infant formula as these micro-nutrients are very essential for the normal growth and development of the infants in addition to maintain normal physiological functions.

Hambraeus and Lonnerdal (1994) studied the bio-availability of iron and suggested that iron is relatively higher in milk containing lactoferrin and its usefulness for supplementing baby foods. Lack of some commercial incentives seems to be the major impediment, undermining technological advancement to provide superior nutrition to the infants. Revision of compositional standards of infant formulas under the auspices of CCFS and BIS has recently paved way for the introduction technological innovations to meet critical nutritional requirements of infant. (Thompkinson and Mathur, 1995).

The Indian standard infant milk substitutes specification (IS 14433:1997) indicates that the infants milk substitutes should have value as moisture 4.5g/100g, milk protein 12.00g/100g, fat 12.0-18.0g/100g, ash 6.0-8.5g/100g, vitamin A 35uµg/100g, vitamin D 180µg./100g, vitamin C 35mg/100g, calcium 230mg/100g, magnesium 22mg/100g, potassium 370mg/100g, sodium 90mg/100gm, The phosphorus 115mg/100g.

2.4 Preparation of weaning foods through humanized milk

Ivanov *et al.* (1971) prepared two humanized milk formulas (i) 'Bebe o' and (ii) 'Bebe I', from high quality milk (Total bacterial count <500,000/ml), Supplemented with high quality whey from white pickled

cheese, lactose, sunflower seed oil, vitamin preparation and ferrous salt and was spray dried on a laboratory Anhydrous drier. Gopaldas *et al.* (1982) reported that the protein content varied from 12.80 to 15.10g and the energy supplied by the mixes varied from 343 to 389 Kcal/100g. Sanchez-Marroquin *et al,* (1986) prepared infant formulas with nutritional characteristics, protein 17-23 per cent, fat 7-12 per cent, crude fibres 1.9–3.7 per cent and energy value 380 Kcal/100g.

Catericheo *et al.* (1989) reported that the infant food had moisture 4.6, protein 18.7 ether extract 12.5, crude fibre 1.0 and ash 3.3 per cent with a calculated energy value of 427 Kcal/100g. The moisture, protein, energy, ash, iron and calcium content of the mixtures ranged from 5.90 to 6.03 per cent, 9.84 to 9.95g, 416.40 to 440.99 Kcal, 3.77 to 4.32 g, 17.75 to 18.42 mg and 150 to 190 mg/100g, respectively as reported by Gupta and Sehgal (1991).

Chierici and Vihi (1994) reported the modern infant formulae try to be as close as possible to human milk and to approximate its macro-nutrient composition. Current formulae are generally categorized into two broad groups: Casein – dominant and whey-dominant, of which the whey-dominant formulae should theoretically offer a protein composition closer to that of human milk.

Gahlawat and Sehgal (1994) reported the moisture, protein, ash, fat iron, crude fibre and calorie content of the weaning foods ranging from

5.45 to 6.15 per cent, 13.82 to 14.17 g, 4.20 to 4.61 g, 1.27 to1.60 g, 13.87 to 16.01 mg, 1.33 to 1.89 g and 347.5 to 364.0 Kcal/100g, respectively. Almost similar values for protein (13g) and energy (351 Kcal) were reported from weaning foods formulated by using malted *ragi*, malted horse gram and roasted groundnut (Chandershakhar *et al.*, 1988). However, higher protein (19.4g) had been reported form a weaning food (Soylac) prepared by blending soybean, wheat, rice flour, skimmed milk, minerals and water (Shulk *et al.*, 1986).

Sarkar and Mishra (1996) studied the cultured milk products as feeding substitute and developed a number of cultured milk products. Rowan and Anderson (1997) examined the bacteriological quality of infant milk formulae under a Variety of preparation and storage conditions. Pendli and Vali (1997) studied ,the development of wheat germ based weaning food formulations. They develop infant mixes based on wheat germ a by-product of flour milling industry in combination with ragi and green gram.

Berger *et al.* (2000) reported the nutritional implications of replacing bovine milk fat with vegetable oil in infant formulae and their impact on commercialization. Nair *et al.* (2003) studied the fortification methods, emerging technologies and role of nutritionally enriched milk food products and their impact on health. Etcheverry *et al.* (2004) studied the bi-availabilities of calcium and zinc in bovine milk formulas adapted for human because infant formulas can also supply a significant portion of macro and

micronutrients for children up to 3 years of age. Kvistgaard et al (2004) with team of pioneer scientists proved the inhibitory effects of human and bovine milk constituent's on Rotavirus infections. As rotravirus is the major cause of severe dehydration, diarrhea in infants, which comes through milk.

2.5 Immunology and therapeutics regarding humanized milk

RDA recommended that all newborns must receive a single intra-muscular dose of 0.5 to 1.0 mg of phylloquinone as prophylaxis against hemorrhagic disease of the newborn. Low birth weight infants may require a second injection at about one week of age. Because the vitamin D content of human milk is extremely low (about 22 IU/liter), breast-fed infants may need supplemental vitamin D (400IU/d). If they have limited exposure to sunlight, Breast-fed infants whose mother's are strict vegetarians require supplemental vitamin B_{12} and infants on cow's milk formulas require iron supplementation for the first three months.

Janas and Picciano (1996) reported that the major nucleotide of cow's milk is present in significant quantities in cow's milk based infants formulae but not in human milk based infant formulae. High level of dietary erotic acid cause hepatic Lipid accumulation, which is unique in the rats. Robert Eolson (1990) observed that the normal breast-fed infant of a well nourished mother receives sufficient quantities of all vitamins except vitamin K and D. The newborns have sterile intestine and cannot initially synthesize menaquinones. Because human milk contains only 1-2 µg/litre phylloquinone

as compared to 10-15 µg/litre in Cow's milk that meets the estimated daily allowances.

Jyothirmayi *et al.* (1999) studied the wide variations in total bacterial counts, coliforms, yeast and molds. Bacillus cereus and staphylococcal counts found in infant food samples and the differences in counts were found statistically significant ($P<0.01$) than by existing standard methods of enumeration. Resuscitation had improved the recovery of sub lethally injured or stressed cells of all types of microbes that may invariably be found in infant foods.

Kunz (1992) observed human milk contains less casein than most species do: only 20 per cent of total protein in early milk is casein, while mature milk contains ~ 40 per cent casein. The major whey proteins i.e. soluble proteins are lactoferrin α-lactalbumin and secretary 1gA. Other protein components of whey are lysozyme serum albumin bile-salt stimulated lipase and amylase. There is a multitude of enzymes and binding proteins present in human milk at low concentrations. The physiological significance of these components is still largely unknown.

Molenaar *et al.* (1992) studied the lactoferrin. They used ^{35}S-labelled cRNA probes to localize the sites of α -lactalbumin, α-SI-casein and lactoferrin mRNA synthesis in sheep. Early in lactation, mammary gland expression of α -lactalbumin and α-SI-casein was high in some alveoli but not in others. Those alveoli with expression of α-lactalbumin and α-SI-casein

contained few fat globules in their cells and lumina, whereas those ions, which expression of these proteins were absent contained abundant fat globules. These latter alveoli also almost exclusively expressed lactoferrin. Their findings suggested that milk secretion either is heterogeneous across lobules or occurs sequentially with time in the alveolus as newly secreted milk accumulates.

Patel (1992) studied the importance of dietary calcium in human nutrition in the different dairy products. The concentration of calcium varies from 60 mg/100g for cottage to 500-700mg/100g for various cheeses. The absorption of milk calcium in the human body is more compared to other sources of calcium due to the presence of co-nutrients like lactose fat, protein and phosphorus in milk. Also there is structural similarity between the calcium phosphate of milk, casein micelles and the amorphous calcium phosphate which accumulates in the mitochondria of human cell.

Sarkar and Mishra (1992) studied the final keeping quality of any milk product depends upon the initial quality of the raw milk. Paul (1999) reported the influence of lactose intolerance on the absorption of protein, Fats, Carbohydrates and minerals.

Menon (2000) studied the role of milk in human diets/ nutrition and he reported that milk is nearly perfect food for all ages of man. There is no substitute for milk in infancy and it contains a compendium of nutrients as well as a readily assimilable protein of high biological value and lipids with

special characteristics. Deshpandey *et al.* (1993) studied the correlation between different immunological activities namely, trypsin inhibitor activity (TIA), immunoconglutinin (1k), total protein (TP) and globulin (GLB) in cow serum, colostral/ milk whey and calf serum. All the immunological activities were positively correlated with each other irrespective of whether in cow serum, whey and calf serum and suggested that human milk contains significantly higher levels of nucleotide and nucleotide derivatives compared with infant formulae, however their metallic fate and the role they play in the health of the breast fed infant is not known.

Mathur (1993) examines the technical feasibility of incorporating certain factors viz., immunoglobulin, lactoferrin, lactoperoxide and lysozyme in the infant formulation with the objective of enhancing the bio-protective factors so as to make the practice of bottle feeding safer. Bhatia et al. (1994) reported the level of lactoferrin differs considerably with in species, milk and colostrums. Human milk is the richest source of lactoferrin with level as reported 1.0 mg/ml in comparison to Murrah buffalo milk level that is 0.32 mg/ml. Marijênand Harerkar(1994)suggested that the nucleotide salvage pathway may not be capable of providing sufficient purine nucleotide for proliferating lymphocytes. Chadha *et al.* (1995) observed the influence of infant feeding practices on the growth and morbidity profile of infants in the urban slums of Delhi.

Cohen and Silva (1995) demonstrated that de novo purine biosynthetic activity is present in S-phase thymic lymphocytes. G_1 phase lymphocytes, however may only salvage pathways to maintain there purine nucleotide pools. Perignon *et al.* (1996) found limited capacity of lymphocytes to salvage pyridines. Jannes(1996) studied human milk nucleotides and nucleotide derivatives compared with conventional infants formulas. He reported nucleotide can be synthesized endogenously and thus are not essential nutrients; however they affect the immune system, small intestinal growth and development, lipid metabolism and hepatic function.

Thompkinson and Mathur (1999) reported that world over food manufactures have developed wide range of infant formulations after the advent of first commercial formulae in 1866. However, the commercial formulations manufactured are mostly bovine milk based with the technological advancement and know how, the authors emphasize on the need to provide nutritionally superior infant formulations to meet the requirements of millions of infants in our country. Khedkar *et al.* (2000) reviewed the various scientific literatures to assess the validity of the published experimental evidence and some of their own experimental findings on therapeutic properties of acidophilus Milk. It has however, been recognized that lactobacillus acidophilus is the only lactobacillus species which can grow well and get established under the environmental conditions in the intestine and

accordingly considerable attention has been devoted to the use of this organism in therapeutic practice.

Sood and Slattery (2001) studied the light scattering measurements through (Nicomp 370; submicron Particle Analyzer) to determine the average size and the size distribution of the casein micelles from skimmed human milk. A great size variation has been observed in the naturally occurring casein micelles, both in milk from one donor at different times of lactation and between milks from different donors. Lesman and Gilboa (2003) observed the *Pseudomonas acruginosa* lectin – a powerful problem for human and bovine milk analysis.

Lonnerdal and Lyers (2004) stated that lactoferrin is a major component of breast milk protein constituting 10-15 per cent of the total content. Each molecule of lactoferrin is capable of binding two atoms of iron and it has been proposed that lactoferrin is involved in iron absorption in the new born. It has been hypothesized that lactoferrin could aid in the delivery of iron to specific sites on the brush border membrane of the small intestine. It is also possible that lactoferrin in milk may have some other biological effects in the infants, such as inhibiting bacterial growth, stimulating mucosal growth and proliferation as well as modulating immune function. Bovine lactoferrin can now be obtained commercially and be used In infants formulas but clinical evaluations of its effects have so far been disappointing.

2.6 *In-Vitro* protein digestibility

Livia (1982) studied *in vitro* digestibility of fermented milk products as well as size of curds at the stages of digestion and determined the amount of nitrogen in regular milk, low fat milk, butter milk and yogurt. Better digestibility of roasted weaning foods may be attributed to the reason that globulin's which are the larger protein of legume seed protein are resistant to denaturation and in the native state are not broken down by digestive enzyme (Walker and Kocher, 1983)

Singh (1984) reported that the lowering the concentration of antinutrients by roasting increases protein digestibility. An increase in protein digestibility after heat treatment to *Bengal gram*, maize and soybean has been reported (Srivastav *et al.*, 1990). Catricheo *et al.* (1989) reported that the protein digestibility of an infant food is 85.6 per cent. Gupta and Sehgal (1991) reported that the in vitro protein digestibility of mixtures varied from 80.22 to 84.43 per cent. Processing of cereals and pulses brought about 24.93 to 28.29 per cent increase in protein digestibility (*in vitro*) of the weaning mixtures.

The Protein digestibility of the weaning foods varied from 53.48 to 86.07 per cent as reported by Ashturkar (1992). Daniel *et al.* (1992) reported that the in vitro digestibility of protein in case of uncooked weaning foods varied from 60.6 to 87.1 per cent and in case of cooked 67.6 to 83.5 per cent. Pawar *et al.* (1994) reviewed that the protein digestibility of the weaning

foods varied from 60.89 to 70.98 per cent. The higher protein digestibility (70.98 %) was observed in the malted weaning foods.

Gahlawat & Sehgal (1994) reported that the protein digestibility of roasted weaning foods ranged from 75.06 to 76.29 per cent, where as malted weaning foods had value ranging from 84.70 to 85.14 per cent. Protein digestibility of processed weaning foods was significantly higher than the raw ones. Malting resulted increase in protein digestibility (32%) as compared to roasting (17-18%). Etchewerry *et al.* (2004) studied the comparative calcium, zinc and iron bio-availabilities from a commercial human milk fortifier with human milk. The bio-availability of each mineral was assessed using and *in vitro* digestion/Caca-2 cell culture model. They revealed that calcium uptake from human milk (HM+S-26/SMA) was not different from any of the human milk fortified with the bovine milk proteins, except for unfortified human milk (HM) and HM + colostrums in which calcium uptake was significantly lower (-89 and –38%), respectively. Uptake of zinc and iron were significantly higher for HM + S-26/SMA than for the other HM + fortifiers. Charanjit *et al.* (2006) attempted the preparation of flavoured milk of cow and buffalo milk incorporation with carrot juice. Three products were produced, one was with 10 per cent carrot juice and rest flavoured either 20 or 30 per cent carrot juice. The acceptability of products in terms of texture taste, flavour and overall acceptability were evaluated by a panel of tasters using a standard score sheet. The most acceptable product was subjected to chemical analysis

for the determination of its nutritive contents. Product was found significant in storage for four days at 4±1°C temperature.

From the literature reviewed as above, it can be concluded that some information on the infant milk formulation and weaning food is available. However, the information pertaining to humanized milk formulation needs to be generated by undertaking systematic studies on the development and evaluation of weaning infant formulation products as emphasized in the objectives of this study.

MATERIALS AND METHODS

Chapter-III

MATERIALS AND METHODS

The present studies have been conducted in the Department of Food Science and Nutrition; College of Home Science, Livestock Products Technology Laboratory, College of Veterinary and Animal Sciences of the CSK Himachal Pradesh Agricultural University, and the Laboratories of the Indian Veterinary Research Institute, Palampur campus during the years from 2004 to 2007.The supplies of milk required for the successful completion of the research have been made available by the university dairy farm sources, local market and from Government hospital by the help of concerned staff, Sub-Division Civil Hospital Palampur, who were also helpful in securing required human milk by the breast-fed mothers.

3.1 Experimental layout

The experiment was laid out according to the completely randomized block design (CRBD) and replicated thrice. An effort has been made to keep

the various variables at minimum and constant to reduce any experimental error. The present study conducted on the following three groups of milk.

Group I- Cows milk (Local hill Cows)

Group II- Buffalo's milk (Local hill Buffalo's)

Group III-Human milk

3.2 Preparation of samples

Fresh cow milk and buffalo's milk non-pasteurized, non-fortified and non-homogenized was received from the university dairy farm as well as from local area dairy farm. Milk samples were collected in sterile bottles and transported to the laboratories. Human milk was collected from breast fed mothers on request individually and collected by the breast pump and collected in sterile bottles.

3.2.1 Carrot reconstitution

Carrots was procured form the market for the preparation of carrot juice. Carrots were cleaned, washed chopped and processed to their juice and packed in sterile bottles.

3.2.2 Process of making *seera* (wheat germ) reconstitution

Seera was procured from the local market. Then added sterile water in 3:1 ration in to it where 3 was the part of water and 1 was the part of *seera*. Boiled this dilution under 60°C temperature for 10 min and packed it in sterile bottles.

3.2.3 Process of making banana reconstitution

Bananas procured from the market. Peeled and mashed in sterile water in ratio 2:1, where 2 were the parts of banana and 1 was the part of water. Boiled the dilutions less than 60°C temperature for 10 min. and packed in the sterile bottles.

3.2.4 Addition of humanized cow milk with carrot, banana and *Seera* reconstitution

Preparation of humanized milk formulations; the cow's milk was selected. The humanized milk was blended in different ratio with carrot, banana and *seera* reconstitutions as shown in Table 4.5 to get an appropriate level of total solids and nutrients as well as energy levels. Milk with different concentrations of dilution was sterilized in 250ml sterile bottles at a pressure of 0.7 kg/cm^2 (10 psi) for 10 min. Humanized milk was standardized for the preparation of final product. The cow's milk and carrot juice, banana dilution and *seera* dilution were mixed in the ratio of 82:10, 72:20, 62:30 (by weight basis). While addition of 8 per cent sugar and 0.2 per cent Lactose were kept constant in all the formulations. After stirring, the mix was heated at 72°C for 15 seconds. The samples were hot filled in the glass bottles (200ml) and capped. The storage study was done at refrigerated temperature i.e. 4±1°C. The humanized milk formulations were analyzed for pH, acidity by following standard methods (A.O.A.C., 1980). Ostward viscometer was used to determine the comparative viscosity of different the samples at 20°C. The

sediments were determined accordingly to the standard procedure based on centrifugation (ISI 1960). Developed humanized milk formulations were stored at 4^0C temperature. Humanized milk was analyzed further for the physical and bio-chemical characters.

The milk samples were analyzed chemically in following parameters:-

1. Specific gravity
2. The pH of milk
3. Titratable acidity
4. Total solids
5. Solids-Not-Fat (SNF)
6. Fat Percentage
7. Protein Content
8. Lactose Content
9. Ash Content
10. Moisture content
11. Immunological content (Lactalbumin and Lactglobullin content)
12. Development of infant food milk formulations
13. Standardization of the prepared humanized milk formulations
14. In-Vitro protein digestibility of infant food milk formulations.

3.3 Determination of Specific Gravity

The specific gravity of all the milk samples was determined by Indian standards Methods IS: 1183 (1965) using a zeal lactometer calibrated at

29°C. Each sample was mixed by shaking the bottle gently 4-5 times. The sample was then poured in to the lactometer jar, taking care that no air bubble were formed. The lactometer was allowed to move freely without touching any side and the reading was recorded corresponding to the top of the meniscus on the stem without the error of parallax with in 30 seconds after the lactometer came to rest. The reading was repeated and the mean of three observations was taken. All the readings were recorded with in the range of 17°C to 21°C temperature. Corrected lactometer reading were computed at 17°C by adding 0.1° for every of below 20°C and subtracting 0.1 for every of below 20°C.

Specific gravity was calculated as:-

Specific gravity =corrected lactometer reading +1/1000

3.4 Determination of titratable acidity

For determination of titratable acidity the method was adopted from AOAC (1990). Twenty ml well mixed sample was pipetted out into suitable dish and diluted with 40 ml carbon dioxide free distilled water. Two ml of phenolphthalein (0.5% alcoholic) was added and the same was titrated with N/10 sodium hydroxide to first persistent pink colour. Triplicate readings were recorded and average value was taken as volume of alkali used. Acidity was reported as per cent lactic acid by weight (1ml 0.1 NaOH = 0.009 g lactic acid), which was calculated as follows:

$$\text{Per cent titratable acidity} = \frac{\text{No of cc of N/10 NaOH used} \times 0.009}{\text{Weight of milk in gram measure}} \times N$$

3.5 Determination of pH

Standardization of pH meter

i. Depressed the STAND BY PUSH – button

ii. Accurately measured the temperature of the buffer solution and set the manual control to this temperature.

iii. Set the function control to pH

iv. Standardized the instrument with a buffer solution that had a pH as close to the pH of the sample as possible.

v. Poured buffer solution into a beaker and immersed the tip of the electrodes into the solution.

vi. Depress the READ push – button. Allowed meter reading to stabilize.

vii. Adjusted the standardize control until the meter read pH of the buffer. Made sure to reload the control in place.

viii. Depressed the STAND BY PUSH – button and removed the buffer solution. Washed any remaining solution from the electrodes with distilled water from a wash bottle and dried them with soft paper.

The pH determination

1. Depressed STAND BY PUSH - button.

2. If the sample temperature differed from that of the buffer solution, measure the sample temperature and adjust the temperature control accordingly.

3. Immersed the tips of the electrodes in the prepared sample, turned on the magnetic stirrer (be sure the rod does not hit the electrodes), depress the READ push button, and allowed the meter reading to stabilize.
4. Read pH value of the sample.
5. Depressed the STAND BY push button.
6. Rinsed the electrodes with distilled water and immersed in a beaker of distilled water.

3.6 Determination of Total Solids

Total solids were estimated by the oven drying method IS:1479 (Part II 1961). The clean dry empty dish (made up of aluminum alloy, 7 to 8 cm diameter and about 1.5 cm in height) with lid was weighed accurately with the help of electronic balance. Five ml of prepared sample was pipette in to the dish and weighed accurately with the lid on. The dish was kept horizontal to promote uniform drying and protected from direct contact of the water bath. After at least 30 min the dish was removed, the bottom wiped and transferred to the oven maintained at 98-100°C placing the lid by the side of dish. The dish was taken out from oven after 3 hours, covered with lid, allowed to cool for about 30 min and weighed. The dish was uncovered and returned to the oven along with lid and heated for one hour more. It was then removed to the desiccators, cooled and weighed as before. The process was repeated till the

loss in weight between successive weightings did not 0.5 mg. The lowest weight was noted.

$$\text{Total Solids (\% by weight)} = \frac{100}{W_1} \times W$$

Where W= Weight in gram of the milk after drying

W_1= Weight in gram of the prepared sample taken for the test.

3.7 Estimation of solids-not-fat (SNF)

Solids not fat was obtained by subtracting the fat content from the total solids determined by gravimetric method

SNF (%) = Total Solids (%) - fat (%)

3.8 Determination of Fat

The fat content was determined according to the standard Garber method as prescribed under IS:1224 (Part I 1977). 10.76 ml milk was pipetted out from well mixed sample into the calibrated butyrometer (Calibrated with 10%scale) containing 10ml of sulphuric acid of 1.18 g/ml strength, without mixing the contents. Then 1 ml of 1SO amyl alcohol was added with the help of automatic till measure. The butyrometer was stopper and the contents were mixed.

3.9 Determination of total protein

Total proteins were determined using total nitrogen method of Kjeldahl with slight modifications. Five gram of milk was taken into 500 ml

Kjeldahl flash and 25 ml of concentrated H_2SO_4 and 4 g of digestion mixture (sodium sulphate, copper sulphate, mercuric oxide and selenium powder in the ratio of 76:20:3:1) was added. The milk was digested over a digestion set to a clean greenish blue colour. After cooling 100 times dilution was done with distilled water and read on automatic nitrogen analyzer technico-II taking 100 ppm standard buffer solution for base line.

Technico –II works on colourimetric principles that an emerald green colour is formed by the reaction of ammonia, sodium salicylate, sodium nitroprusside and sodium hypochlorite (chlorine source) in a buffer alkaline medium at a The pH of 12.8 - 13.0. The ammonia salicylate complex was read at 660 nm well prepared samples were fed into Technico-II, Automatic samples, placing 100 ppm standard solution side by side for avoiding technical error. Distilled water was put after each 10 samples. Samples were analysed for nitrogen automatically through technico-II and values were recorded with the help of Automatic recorder on graph paper.

Nitrogen percentage was calculated from graph taking 100 ppm standard solution graph as 1 per cent nitrogen.

Total Protein (%) = Nitrogen (%) x 6.38

3.10 Determination of Lactose

Lactose was determined according to the method described by follin, W. (1980).

Reagents:

1. 10 Per cent sodium Tungstate
2. 2/3 N sulphuric acid.
3. Standard Lactose Solution
4. Follin-W_u Alkaline copper solution.
5. The phosphomolybidic acid solution
6. 1:4 dilute acid molybidate solution

Procedure:

One ml of thoroughly mixed sample was taken into a 100ml volumetric flask to which were added 2ml of 10 per cent sodium tungstate and 2ml of 2/3 N sulphuric acid. The contents are mixed well, allowed to stand for 5 minutes, then diluted to the mark with water and filtered. In a follin and W_4 sugar tube, 1ml of the filtrate and 1ml of water and into another tube 2 ml of standard lactose solution was taken. An aliquot of 2.0 ml of follin Wu alkaline copper solution was added into each tube and heated in boiling water for 8 minutes. After cooling 4 ml of concentrated molybolic acid reagent was added to each tube. After one minute the volume was made up to the 25 ml mark by adding 1:4 diluted acid molybdate solution. It was then mixed well and compared on spectro- photometer at 420 nm range.

$$\text{Lactose (\%) in milk} = \frac{\text{Density of unknown}}{\text{Density of known}} \times 0.6 \times \frac{100}{0.01} \times \frac{1}{1000}$$

3.11 Mineral contents

The organic matter in the sample (0.5 g) was wet digested with diacid mixture (25ml) (Piper, 1966). The volume was made to 50 ml. The digested sample were analysed for iron using atomic absorption spectrophotometer. Calcium and phosphorus were determined by using Flame photometer.

3.12 Moisture content

The moisture content was determined on the basis of AOAC (1990). Weighed samples 5 g in triplicate were partially dried on steam bath and then in a hot air oven at 80°C in pre-weighed crucibles. The crucibles were transferred immediately to a desiccators cooled and weighed. The loss in weight represented the moisture content of the samples.

Calculations

Weight of crucible =W (g)

Weight of sample =W_1 (g)

Weight of crucible + sample = x(g) before drying

Weight of crucible + sample = y(g)after drying

$$\text{Per cent moisture content} = \frac{\text{loss in weight (g)}}{\text{weight of sample}} \times 100$$

$$\% \text{ moisture content} = \frac{(x-w) - (y-w)}{w} \times 100$$

3.13 Ash content

The ash content was obtained as laid down in AOAC (1990). After determining the moisture the sample in the crucibles were burnt on a hot plate far some time and were then placed in a muffle-furnace at 55°C for 4 hours from. The weight of residue left in the total ash content was calculated.

Calculations

Weight of empty crucible = W (g) weight of empty crucible

Sample before ashing = W_1 (g) weight of empty crucible.

Sample after ashing = W_2 (g)

$$\text{Per cent ash content} = \frac{\text{Weight after ashing (g)} \times 100}{\text{Weight of sample (g)}}$$

$$= \frac{W_2 - W}{W_1 - W} \times 100$$

3.14 Immunological contents quantification

Many of the proteins found in milk fat globule membranes (MFGM) have been isolated and characterized in an attempt to determine their biological functions by the method suggested by Mather *et al.* (1980).

3.14.1 Whey fractionation

The raw milk obtained from various sources in one litre quantity was skimmed by centrifuging the 4,080xg for 30 minutes at 30°C. The casein was coagulated with 4 units of chymosin/100 ml of skim milk and removed by centrifuging at 16,300xg for 45 minutes at 30°C.

3.14.2 Differential centrifugation / size –exclusion

Ammonium sulfate was added to the whey to 50 per cent saturation and allowed to stand overnight at 4°C. The solution was then centrifuged at 16,300xg for 60 minutes at 40°C. The precipitate was dissolved in phosphate buffer to give a protein concentration of 3 per cent (fraction C). The sample was subsequently dialyzed and diluted to 0.15 per cent protein. This sample stood overnight at 4°C, after which it was centrifuged at 2000xg for 30 minutes. The supernatant was concentrated once again to 3 per cent protein (fraction E). The preceding protocol was based on the work of Janolino and Swaisgood (1975).

3.15 Developed humanized cows milk Formulations for the infant

Three formulation A, B, and C were prepared using carrot, Banana and *Seera* (Cereal Product) reconstitutions as per the code given in Table 4.5 (composition and coding of the formulation)

3.16 Standardization of the developed humanized cow's milk formulations

All the humanized milk formulations were subjected to sensory evaluation by a panel of 10 trained judges who were the teaching staff of Post Graduate Teaching Department of Home Science College, CSKHPKV Palampur. Subjective evaluations were done using descriptive-cum-numerical scoring system. Repeated trials were standardized. Human milk was used as the control for conducting palatability and acceptability trials of the formulated humanized milk. Score cards (Appendix-I) were designed to include the

desirable characteristics to assess the quality of all the formulations. The description range from superior to inferior in descending order. The results of palatability trials were compiled and classified 't' test was applied to find out the significance of difference between the mean scores of the sensory properties of the control sample and the experimental mixes. The formulae was followed:

$$t = \frac{[M_1 - M_2]}{\sqrt{\frac{\Sigma(X_1-M_2)^2 + \Sigma(X_2-M_2)^2}{N1 + N_2 - 2} \quad \frac{(N_1 + N_2)}{N_1N_2}}}$$

Where,

X_1-M_1 = Sum of squares of deviations and

X_2-M_2 = From the mean

N_1N_2 = Number of scores of the two samples;

Σ = Summation

To calculate the degrees of freedom the formula used was; df = (N_1+N_2-2). The hypothesis is to be rejected or accepted according to t_1,t_2 where t_1 and t_2 are calculated and the table value of 't' for (N_1+N_2-2) degrees of freedom.

If the calculated value of 't' is lesser than the table value of 't' at any of the levels of significance indicates the difference is insignificant and vice-versa.

3.17 In vitro protein digestibility of humanized milk formulations

In vitro protein digestibility of humanized milk formulations was determined by the method of Akeson and Stahmann (1964).

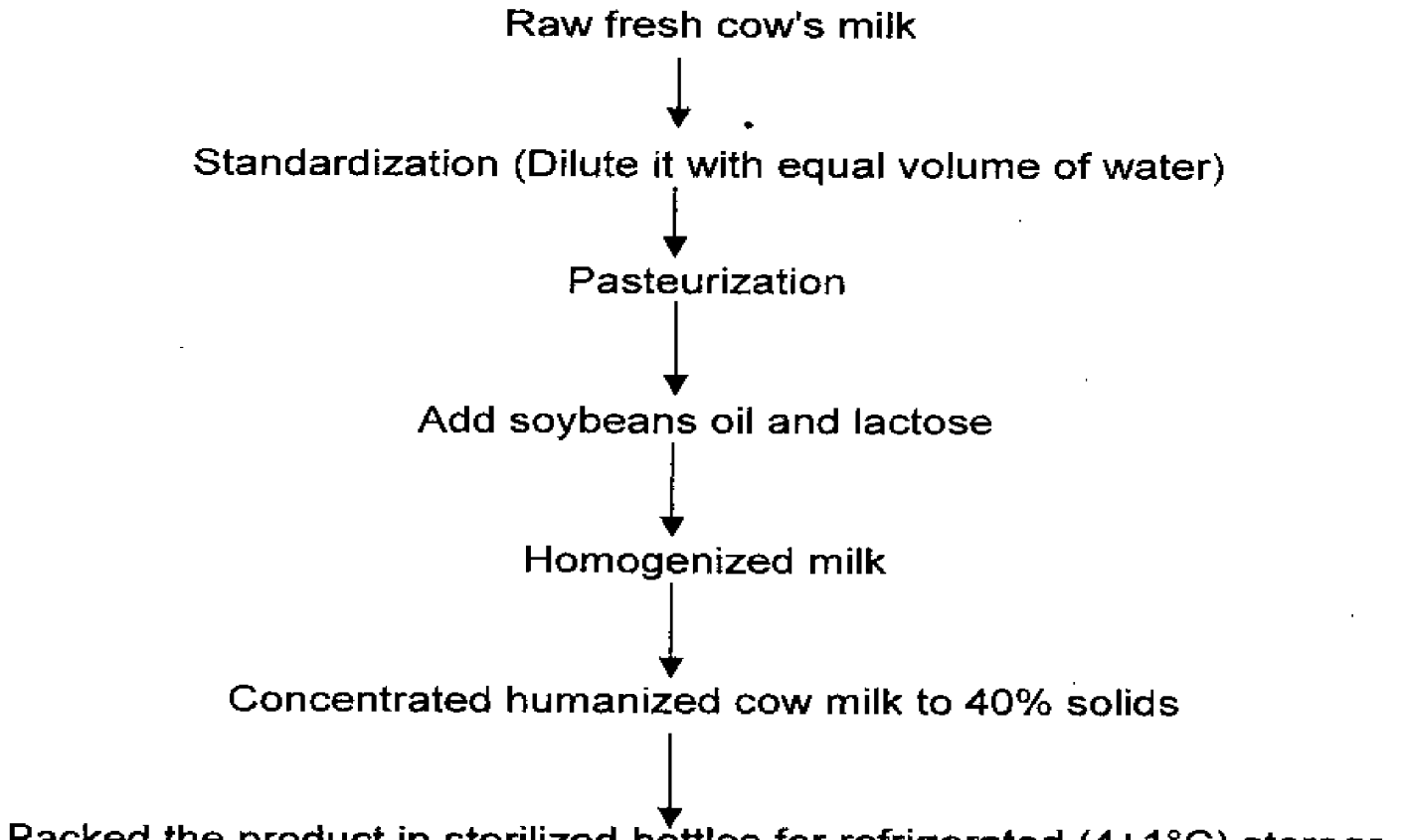
Regents

1. Pepsin Solution: Dissolved 5g Pepsin in 1 Litre of 0.1N HCl
2. Pancreatin solution: 4g of pancreatin dissolved in 1L Borate buffer.
3. 0.2 N Sodium hydroxide
4. 0.1 N HCl
5. 0.2 M Boric acid –Dissolved 27.32g in distilled water and made the volume 1 litre.
6. Borate buffer (pH 6.8): To 40 ml of 0.2 M Borate acid added 50ml Boric acid and diluted to 200 ml.
7. Toluene

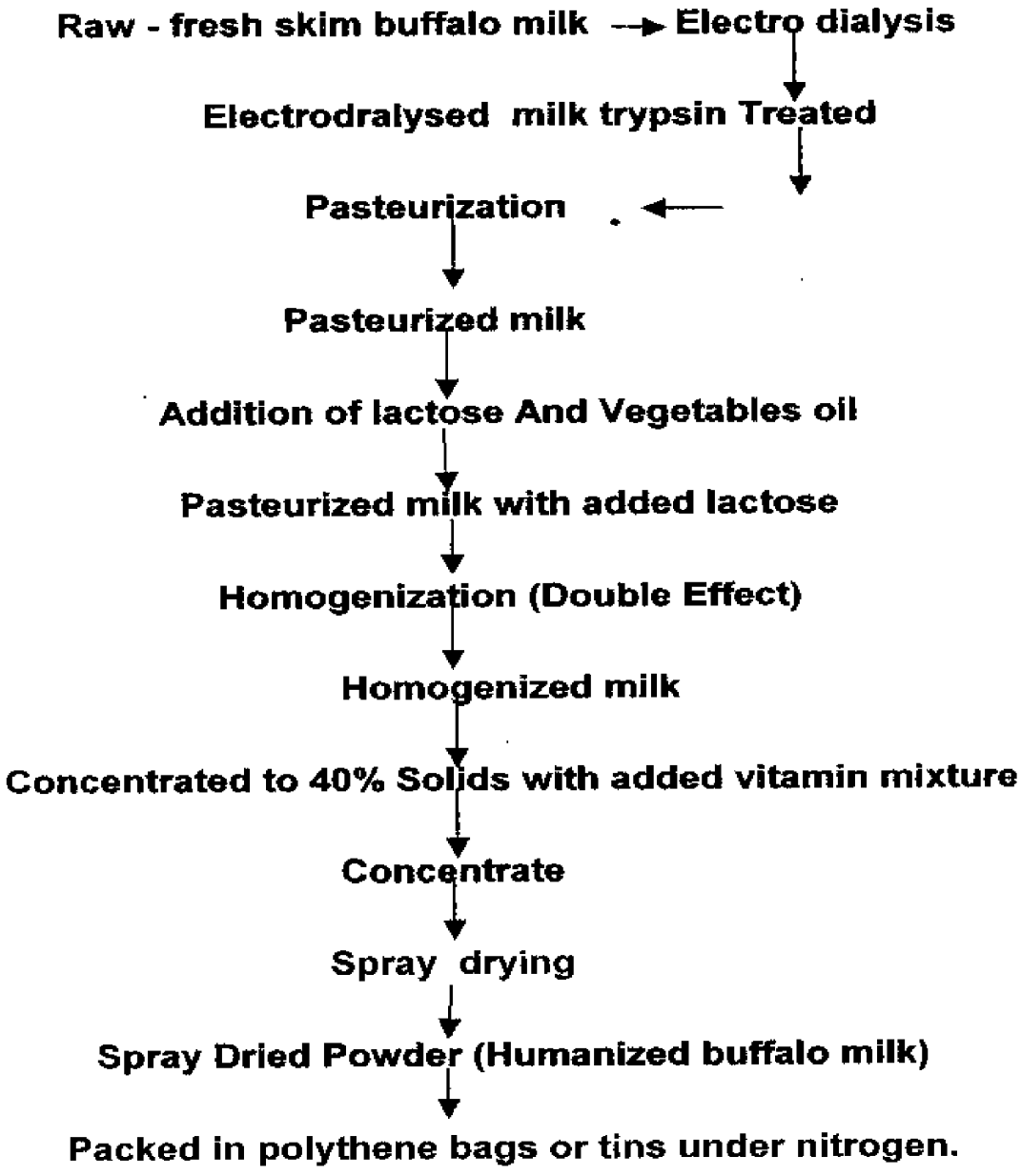
Procedure

Took a sample of 0.2 g/ml milk in 250 ml conical flask. Added 50ml of pepsin solution and few drops of toluene in that sample and incubated at 37^0 C for 16 hours in water bath shaker. After that samples were neutralized with 0.2N NaOH for the setting pH 7.0. AT the same time enzyme blank was run under same conditions omitting the protein sample. A few drops of toluene were used to maintain aseptic environment in the system. Treated the samples with 10% TCA solution to collect the supernatant. The contents were centrifuged at high speed and filtered through Whatman No. 4 filter paper. The residues were analyzed for nitrogen content by macro Kjeldahl method. The digestibility co-efficient was determined by subtracting the residual protein from the initial protein on the basis of 100 g of sample.

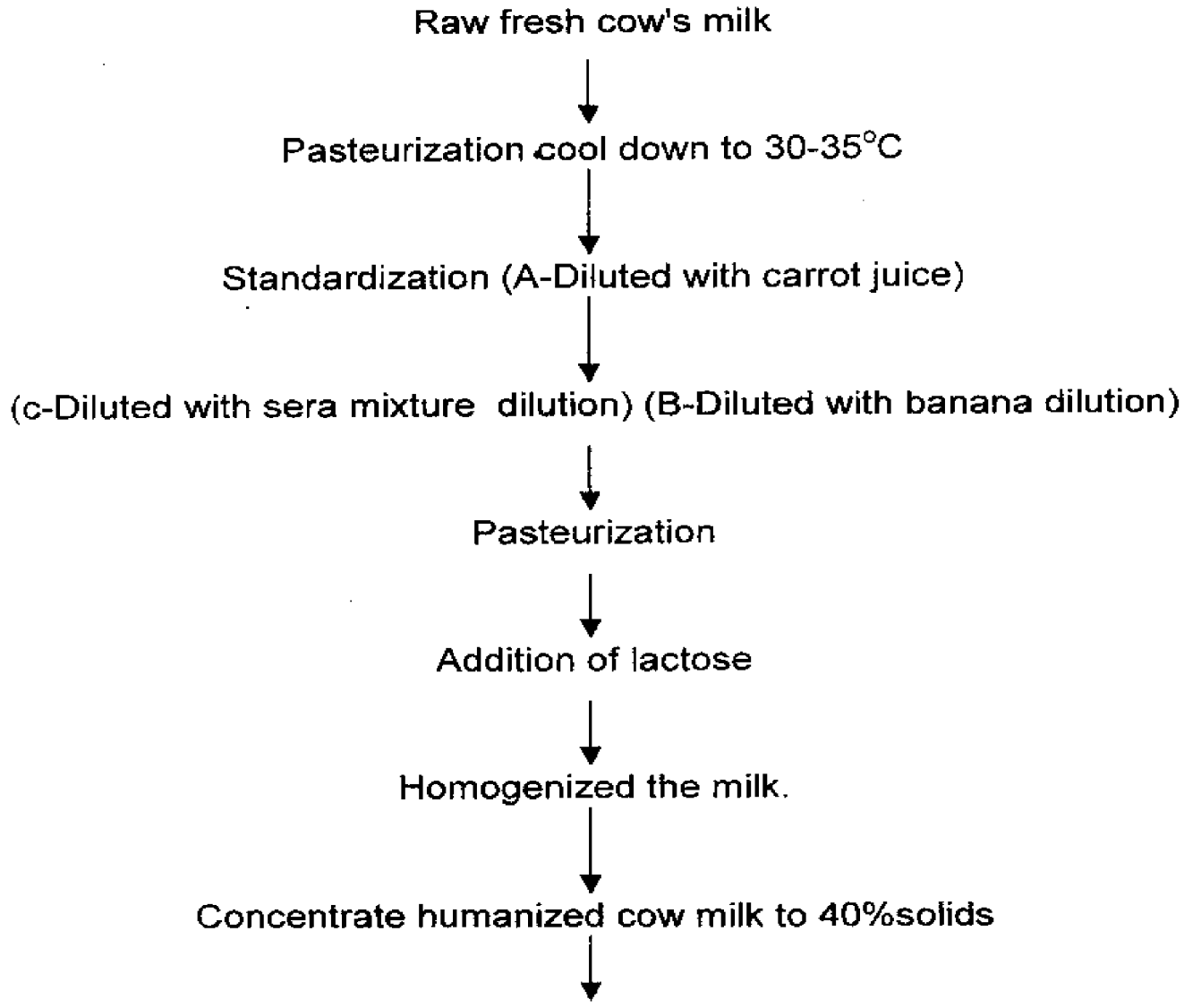
Flow Sheet depicting process for humanization of cow's milk.



The steps made for the manufacture of humanized buffalo milk (HBM)



Process for making Humanized milk formulations with cow's milk.



3.18 Microbiological evaluation

Total bacterial count was determined initially at 0 day and then after different storage intervals (fresh, 3 days after 7 days). Standard plate count procedure was used to determine the total bacterial count.

Standard plate count

One gram of the weaning food sample was transferred to 9 ml sterilized dilution blank. After shaking well, further dilutions were made and plated out in the following ready made media. Lactobacillus MRS broth agar and Tryptone Glucose Beef Extract Agar (Himedia). The composition is used as dehydrated medium given in Appendix-V.

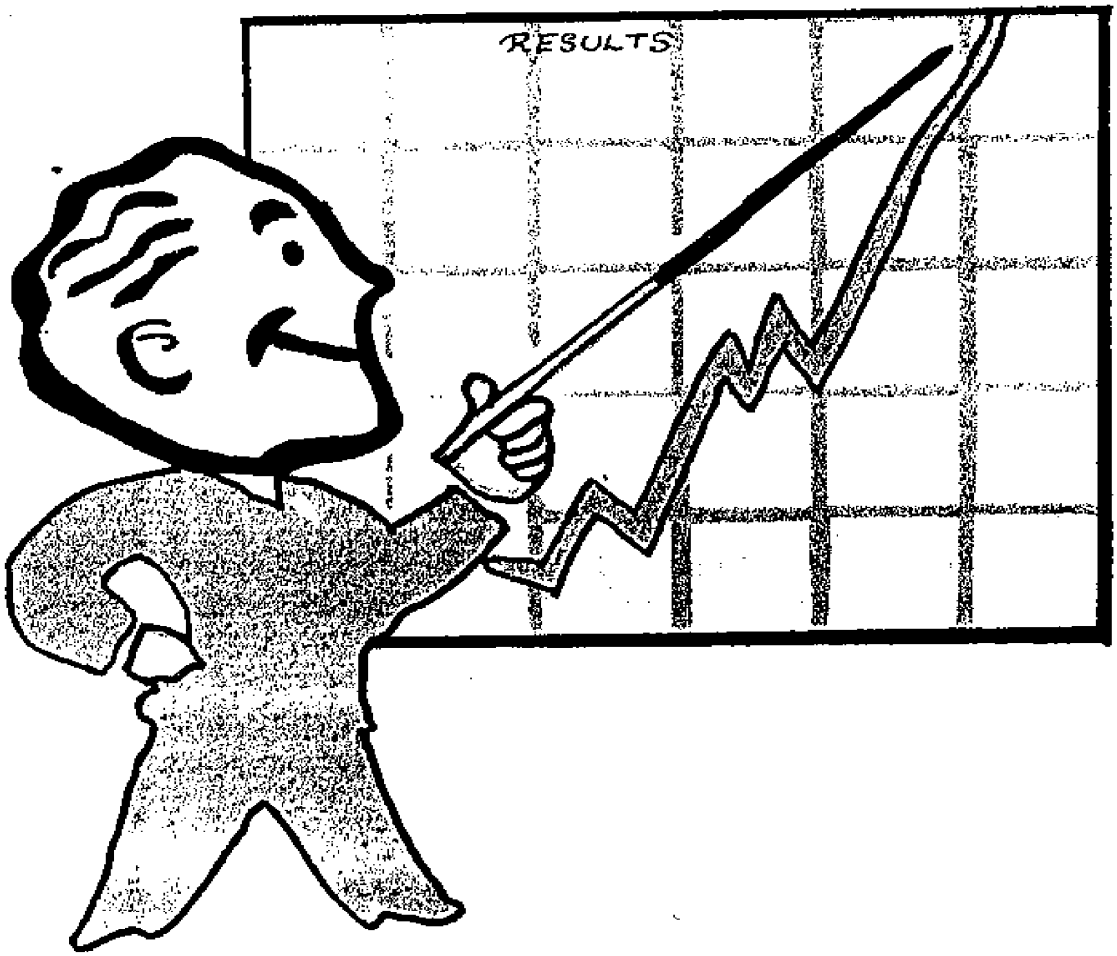
The plates were incubated at 37°C for 48 hours. The colony forming units (C.F.U.) were counted according to the standard procedure using magnifying glass in colony counter.

3.19 Economics

Per unit cost of the preparations was calculated on a standard bulk basis. The per unit cost determined reflected the costs of ingredients at the local retail price.

3.20 Statistical analysis

The data obtained throughout the investigations were suitably tabulated and statistically analysed by the method of Analysis of Variance (ANOVA) as proposed by Gould (1978). The results have been compared as least significant difference (LSD) at 5 per cent level of significance.



EXPERIMENTAL RESULTS

EXPERIMENTAL RESULTS

The present investigation entitled "Comparative study of immuno-therapeutic properties of bovine and human milk" was carried out in two different stages. In the first stage three different groups of milk i.e. cow, buffalo and human milk were subjected to different physical and chemical analysis. In second stage, Humanized cow milk was used in different infant feed formulations. The results thus obtained are presented hereunder:

4.1 The physico-chemical characteristics

4.1.1 The physical parameters

i) Specific gravity (20°C)

ii) The pH of milk (20°C)

iii) Titratable acidity

iv) Curd tension

v) Freezing point

4.1.2 Chemical parameters

i) Total solid

ii) Solids not fat

4.1.3 Proximate composition

i) Fat percentage

iii) Protein content

iv) Lactose content

v) Mineral content

vi) Ash content

vii) Moisture content

4.2 Proximate compositional characteristics of develop infant food formulations

4.3 Immunological characteristics

4.3.1 Immunological content

4.4 Organoleptic evaluation of the developed humanized milk formulations

4.4.1 Sensory acceptability

4.5 *In-vitro* protein digestibility of the developed humanized milk formulation

4.6 Microbiological evaluation of the humanized milk formulations

4.7 Economic feasibility of developed infant formulations

4.1 The physico-chemical characteristics

4.1.1 The physical parameters of milk.

A. The physical characteristics of buffalo, cow and human milk

The average values for physical parameters viz. specific gravity, pH, titratable acidity (per cent lactic acid), curd tension (g), freezing point (°C) are given in table 4.1 and in figure 1 to 2. The mean value for specific

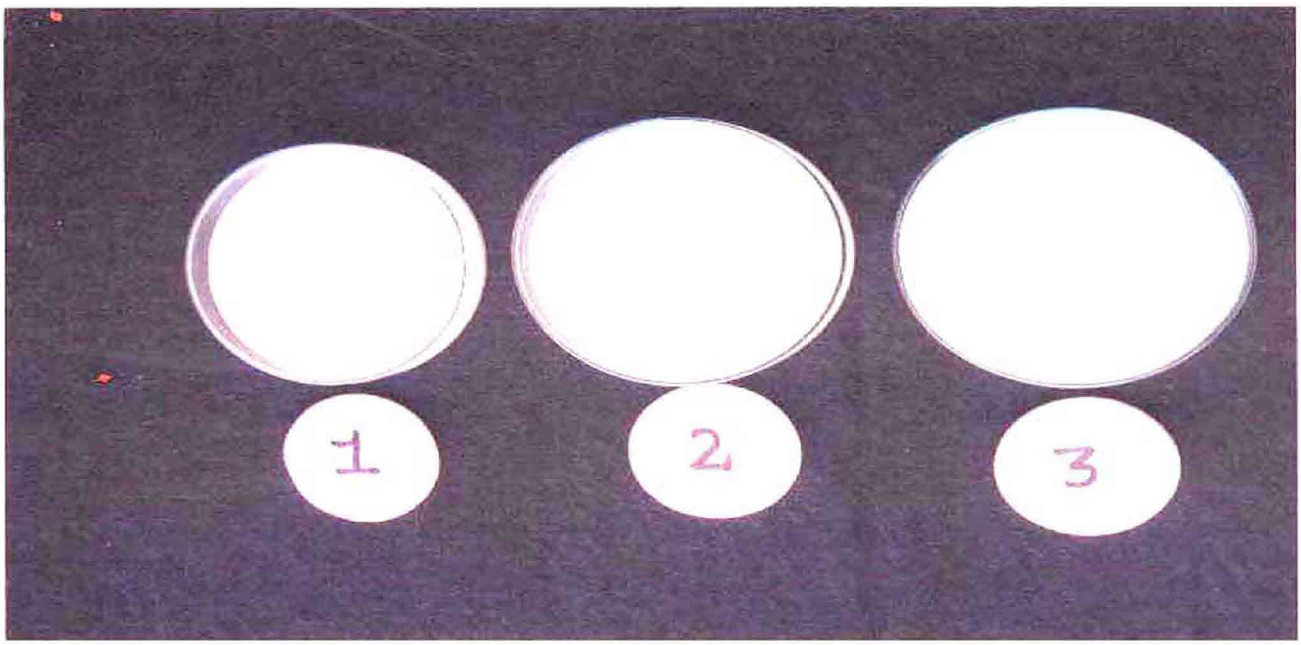


Plate 1: **1. Human milk** **2. Cow milk** **3. Buffalo milk**

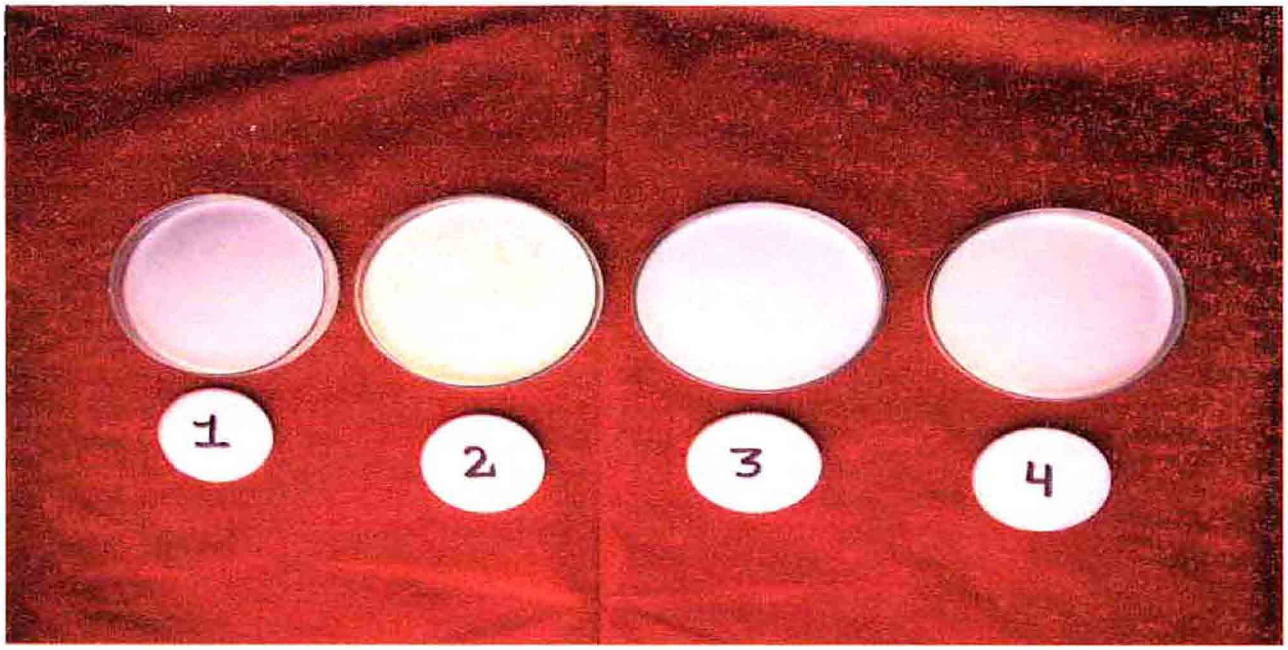


Plate 2: **1. Human milk** **2. Cow milk**
3. Buffalo milk **4. Humanized milk**

gravity of buffalo, cow and human milk were 0.0323±0.012, 0.0317±0.0011 and 0.0310±0.0011 respectively. The specific gravity found in the range of 1.0304 to 1.0415; 1.0288 to 1.0337 and 1.0244 to 1.0330 respectively. The 100 ml buffalo milk has higher specific gravity that is 1.0323±0.012 followed by cow milk 1.0317±0.0011 than human milk 1.0310±0.0011.pH mean values of buffalo milk was 6.74±0.087 and in the range between 6.38-6.88, cow milk pH was 6.56±0.046 and in the range of 6.51-6.56 as well as human milk pH was 6.41±0.02, which was in the range of 6.48-6.57. Titratable acidity of buffalo 0.165±0.005, cow 0.150±0.103 and human milk was 0.155±0.105 respectively. Acidity range of buffalo milk was found 0.162-0.188, cow milk 0.135-0.170 and human milk 0.133-0.170. The titratable acidity was found similar in cow and human milk i.e. 0.150 and 0.155 respectively. It was different in buffalo milk i.e. 0.165 per cent. The curd tension per unit volume mass was found in buffalo milk 32.85±0.024, in cow milk 28.54±0.020 and in human milk 20.50±0.018. It was found in the range of 30.80-33.20 in buffalo milk, 28.58-29.02 in cow milk and 17.49-21.40 in human milk. Freezing point found higher in buffalo milk i.e. -0.5454°C followed by cow milk –0.5301°C than human milk –0.520°C. Freezing point of buffalo, cow and human milk was in the range of –0.5437 to –0.5459°C; -0.5190 to –0.5305°C and –0.519 to – 0.521°C respectively. The physical appearance of buffalo milk was tincher blue in colour. Cow's milk was yellowish in colour, whereas human milk was in white colour but in thin consistency.

Table 4.1 The mean values of The physical parameters of buffalo, cow and human milk

Sr. No.	Physical parameters	Buffalo milk	Cow milk	Human milk
1	Specific gravity (at 20°C)	0.0323±0.012 (1.0304-1.0415)	0.0317±0.0011 (1.0288-1.0337)	1.0310±0.0011 (1.0244-1.0330)
2	The pH (at 20°C)	6.74±0.087 (6.38-6.88)	6.56±0.046 (6.48-6.56)	6.41±0.020 (6.38-6.57)
3	Titratable acidity (as % lactic acid)	0.165±0.005 (0.162-0.188)	0.150±0.103 (0.135-0.170)	0.155±0.105 (0.133-0.170)
4	Curd tension (g)	32.85±0.024* (30.80-33.20)	28.54±0.020* (24.58-29.02)	20.50±0.018* (17.49-21.40)
5	Freezing point (°C)	-0.5454±0.034 (-0.5437 to -0.5459)	-0.530±0.026 (-0.5190 to-0.5305)	-0.520 ±0.020 (-0.5190 to 0.5210)

**Value of range is given in parenthesis.

Group mean ± SD value.

* Significant at 1% level ($P \leq 0.01$)

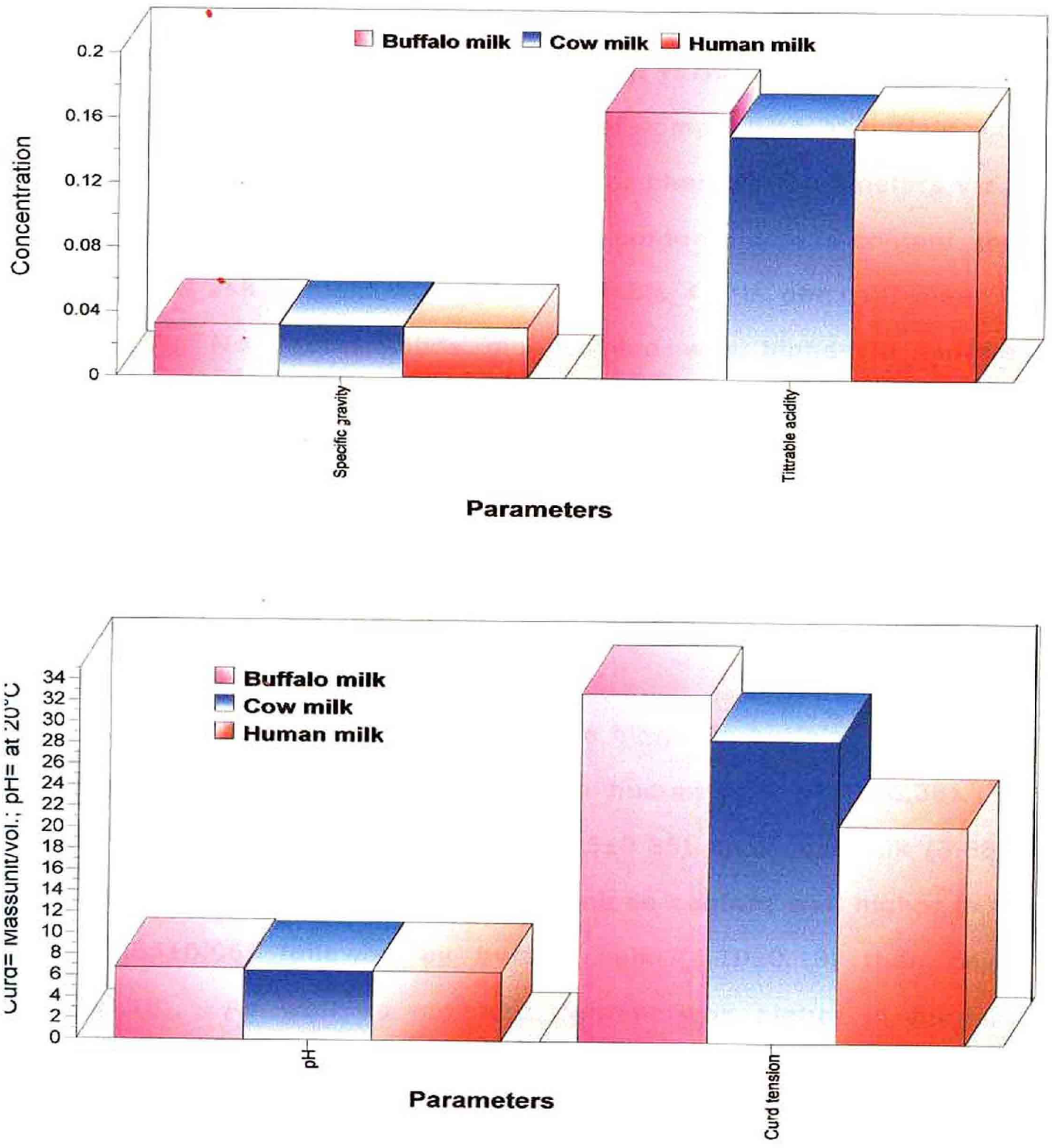


Fig. 1. Comparison of physical parameters of milk (Bovine and Human)

4.1.2 Chemical composition of milk from various sources

Table 4.2 depicts the chemical composition of buffalo, cow and human milk. The average mean values for chemical parameters viz., Total solids, solids not fat, fat content, protein content, lactose content, moisture content and ash content were given in table 4.2 in per cent mean values and in Fig No.3.Total solids mean value was higher in buffalo milk (18.40±1.33) followed by cow milk (17.93±0.92) than human milk (13.28±0.31) per cent, respectively. Total solids found in the range of (18.93 –20.65) in buffalo milk, (17.93-19.05) in cow milk and (11.20-12.50) in human milk. Solid-not-fat percentage mean values were almost similar in buffalo and cow milk that is (11.50±0.51) and (11.08±0.21) and lower in human milk that is (9.09±0.24).

Fat content mean values were higher in buffalo milk (7.32±1.70) followed by cow milk (6.45±0.53) than human milk (4.17±0.36). Protein content was higher in buffalo milk (5.35±0.35) than cow milk (3.68±0.05) and lower in human milk (1.9±0.19). Lactose content was higher in human milk (6.65±0.05) followed by buffalo milk (4.70±0.18) than cow milk (3.68±0.18). As well as moisture content was higher in human milk 88.00±0.89 followed by cow milk 87.50±0.86 as buffalo milk 81.10±0.79 per cent. Ash content average mean values were 0.866±0.0439 in buffalo milk,0.848±0.0522 in cow milk and 0.686±0.0234 in human milk . Ash content was in the range 0.812-1.040 of buffalo 0.746-0.968 of cow and 0.65-0.760 of human milk.

4.2 Proximate composition

Table 4.3 depicted the proximate composition of milk from various sources cow, buffalo and human. Cow milk contained moisture content, protein, fat, carbohydrates and casein mean values 87.5○± 0.86, 3.21±0.02, 4.13±0.03, 4.40±0.33 and 2.82±0.01 (g) respectively, Buffalo milk contained 81.17±0.69, 4.37±0.03, 8.81±0.07, 5.01±0.07 and 3.05±0.02 (g) respectively, whereas human milk contained moisture content, protein, fat, carbohydrates and casein mean values 88.01±0.87, 1.17±0.005, 3.42±0.01, 7.43±0.01 and 0.46±0.03 g/100ml respectively of milk samples. Moisture content was found in the range 3ł.50±0.86 to 88.00±0.89 of all the milk i.e. cow, buffalo and human. Protein and fat contents were found in the range of 1.89 – 5.35; 3.10 – 8.9 in all the milk i.e. cow, buffalo and human. Carbohydrates mainly lactose was found in range of 3.3 – 7.4 in all the milk and casein in range of 0.42 – 3.10 g/100 ml of all milk samples.

Table 4.4 depicts the proximate composition of cow milk, carrot, banana and *seera* used as ingredients to developed infant food formulations. The cow milk contained moisture content 87.50±0.86, protein 3.2±0.02, fat 4.1±0.03, carbohydrate 4.4±0.04 and provided energy 67 Kcal respectively. Carrot contained moisture content 90.80±0.89, protein content 0.60±0.05, fat 0.20±0.15, carbohydrates 10.60±0.09 and provided energy 48 Kcal/100g respectively.

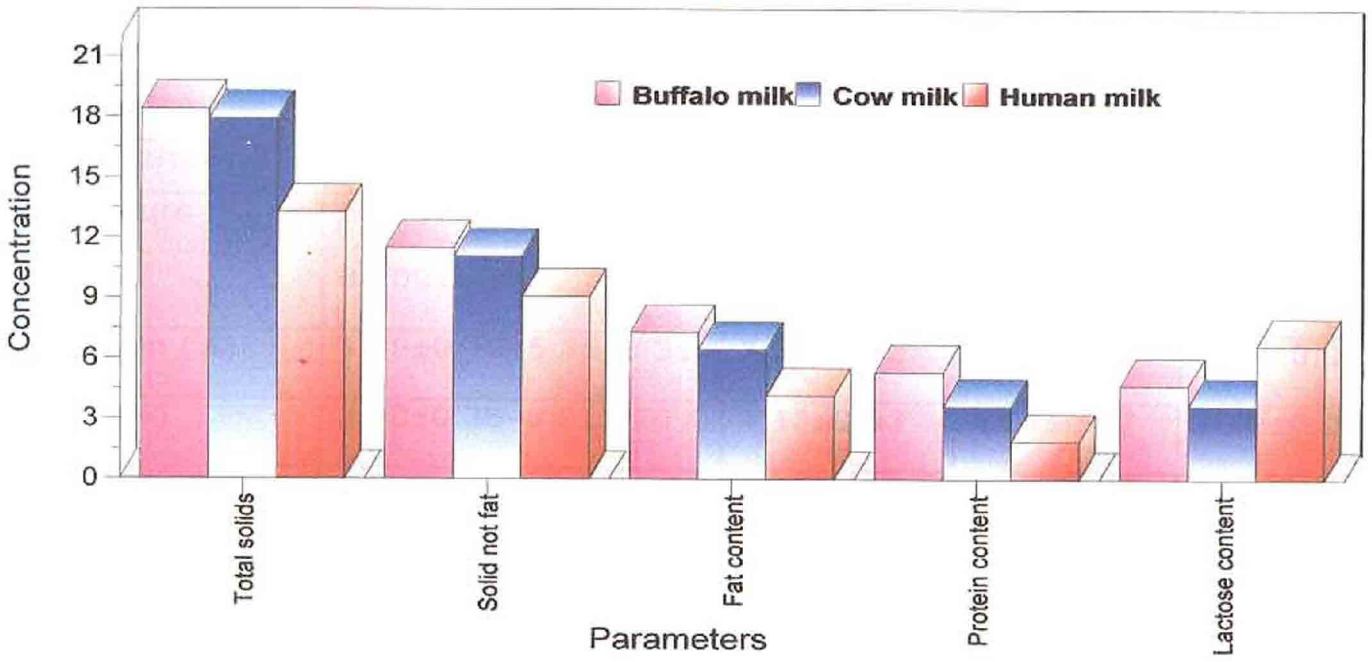


Fig. 2. Comparison of chemical parameters of milk (Bovine and Human)

Table 4.3. Proximate composition of milk of various sources cow, buffalo and human (Values per 100 g of milk).

Constituents	Range	Cow	Buffalo	Human
Moisture (%)	87.50±0.86–88.00±0.89*	87.57±0.86*	81.17±0.69*	88.01±0.87*
Protein (%)	1.89±0.19–5.35±0.35	3.21±0.02	4.37±0.03	1.17±0.05
Fat (%)	3.10±0.36–8.9±1.70	4.13±0.03	8.81±0.07	3.42±0.01
Carbohydrates (lactose %)	3.3±0.18–7.4±0.05 *	4.40±0.03	5.01±0.04	7.432±0.04
Casein (g)	0.42 – 3.10	2.82±0.01	3.05±0.02	0.46±0.03

Group mean ± SD value.

* Significant at 1% level ($P \leq 0.01$)

Table 4.4 Proximate composition of carrot, banana, *seera* and cow milk (values per 100g)

S.N	Nutrient	Cow milk	Carrot	Banana	*Seera*
1	Moisture content (%)	87.80±0.86*	90.80±0.89	70.10±0.69	80.38±0.69
2	Protein content (g)	3.20±0.02	0.60±0.04	1.20±0.002	27.13±0.16
3	Fat (g)	4.10±0.03	0.20±0.15	0.30±0.17	7.30±0.13
4	Carbohydrate (g)	4.40±0.03	10.60±0.09	27.20±0.16	53.30±0.02
5	Energy (Kcal)	67	48	116	386.34

Group mean ± SD value.

* Significant at 1% level ($P \leq 0.01$)

Banana provides moisture content 70.10±0.69, protein 1.20±0.002, fat 0.30±0.17, carbohydrate 27.20±0.16 and provides energy 116 Kcal respectively. *Seera* provides 80.38±0.69, protein 27.13±0.16, fat 7.30±0.13, carbohydrate 53.30±0.26 and provides energy 386.34 Kcal respectively. Table 4.5 showed the proximate composition of developed humanized milk formulation. One set of developed humanized infant food milk formulations coded as (C_1, C_2 and C_3). In which formulated cow milk standardized according to palatability and carbohydrates, protein and fat contents tends to close to human milk composition and can be named as humanized milk formulation. C_1 and C_2 had values of moisture content, protein, fat, carbohydrates 87.54, 4.38, 3.30, 45.0; 86.54, 4.32, 3.39, 49.1 and energy 249.12, 265.79 Kcal respectively. C_3 provides moisture content, protein, fat, carbohydrate, 85.54, 4.41, 3.60, 50.1 and energy 272.49 Kcal respectively. Whereas, formulations developed with carrot reconstitution coded as CA_1, CA_2 and CA_3 having values of moisture content, protein, fat, carbohydrates, 84.40, 8.36, 4.38, 61.90; 87.20, 13.58, 4.46, 62.58 and 84.10, 19.96, 4.88, 65.60 and energy values of 266.90, 362.44 and 512.50 Kcal respectively. Infant food formulation developed with banana reconstitution coded as CB_1, CB_2, CB_3 had moisture content, protein, fat, carbohydrate mean values 83.0, 11.91, 5.50, 69.23; 82.10, 20.12, 6.38, 71.07; 87.10, 22.10, 7.31, 75.43 and energy value of these were 412.78, 455.96 and 497.61 Kcal respectively. Likewise, infant food formulations developed with *seera* reconstitution and coded as CS_1, CS_2,

CS_3 and having mean values of moisture content, protein, fat, carbohydrate values 86.50, 22.17, 7.39, 53.03; 87.30, 23.13, 7.61, 55.04; 87.40, 17.13, 7.82, 59.01 and energy values of these were 423.61, 497.1 and 505.50 Kcal respectively. Table 4.6 revealed the mineral content in milk of cow milk contained calcium 120.29 mg and phosphorus 90.30 mg. Buffalos milk contained 210.73 mg calcium and phosphorus 130.12 mg. Whereas, human milk contained 28.33 mg calcium and 11.45 mg phosphorus as per the mean values. Table 4.7 revealed the mineral content of developed infant formulations as per the mean values. Humanized cow milk coded as C_1, C_2 and C_3 contained calcium 100.87±0.031, 120.97±0.030, 110.95±0.040 mg/100g respectively and phosphorus 112.0±0.041, 161.74±0.070, 154.62±0.069 mg/100g respectively. Carrot reconstituted developed infant food formulations CA_1, CA_2, CA_3 contained calcium 202.85±0.120, 210.80±0.0140, 220.89±0.220 mg/100g respectively and phosphorus 200.07±0.011, 274.70±0.147, 280.20±0.150 mg/100g respectively. Banana reconstituted developed infant food formulations CB_1. CB_2, CB_3 contained calcium 269.05±0.144, 254.03±0.150, 234.06±0.112 mg/100g respectively and phosphorus 196.05±0.072, 162.02±0.07, 166.05±0.071 mg/100g respectively. *Seera* reconstituted developed infant food formulations CS_1, CS_2, and CS3 contained calcium 137.58±0.065, 151.33±0.069, 166.05±0.071 mg/100g respectively and phosphorus 140.02±0.050, 130.00±0.040, 152.00±0.068 mg/100g respectively. These values found significant at 1% level of significance i.e. ($P \leq 0.01$).

Table 4.5. Proximate composition of developed infant food formulations (values per 100g)

Nutrients / Product	Product developed with Humanized milk			Product developed with Humanized milk with carrot			Product developed with Humanized milk with banana			Product developed with Humanized milk with *seera*		
	C_1	C_2	C_3	CA_1	CA_2	CA_3	CB_1	CB_2	CB_3	CS_1	CS_2	CS_3
Moisture content (%)	87.54	86.54	85.54	84.40	87.20	84.10	83.00	82.10	87.10	86.50	87.30	87.40
Protein content (g)	4.28	4.32	4.41	8.35	13.58	19.06	11.91	20.12	22.10	22.17	23.13	17.13
Fat (g)	3.30	3.39	3.60	4.38	4.46	4.88	5.50	6.38	7.31	7.39	7.61	7.82
Carbohydrate (g)	45.0	49.1	50.1	61.90	62.58	65.60	69.23	71.07	75.43	53.03	55.04	59.01
Energy (Kcal)	249.12	265.79	272.49	266.90	362.44	512.50	412.78	455.96	497.61	423.61	497.10	505.50

Formulations of developed infant food:-

C_1:- Cow's milk/sugar/soyabean oil/ Water	Ratio (%):-70/10/10/10
C_2:- Cow's milk/sugar/soyabean oil/ Water	Ratio (%):-60/20/10/10
C_3:-Cow's milk/sugar/soyabean oil/Water	Ratio (%):-50/30/10/10
CA_1:- Cow's milk/sugar/soyabean oil/Carrot juice	Ratio (%):-70/10/10/10
CA_2:-Cow's milk/sugar/soyabean oil/Carrot juice	Ratio (%):-60/20/10/10
CA_3:- Cow's milk/sugar/soyabean oil/Carrot juice	Ratio (%):-50/30/10/10
CB_1:-Cow's milk/sugar/soyabean oil/Banana	Ratio (%):-70/10/10/10
CB_2:-Cow's milk/sugar/soyabean oil/Banana	Ratio (%):-60/20/10/10
CB_3:-Cow's milk/sugar/soyabean oil/ Banana	Ratio (%):-50/30/10/10
CS_1:- Cow's milk/sugar/soyabean oil/*Seera*	Ratio (%):-70/10/10/10
CS_2:-Cow's milk/sugar/soyabean oil/*Seera*	Ratio (%).-60/20/10/10
CS_3:-Cow's milk/sugar/soyabean oil/ *Seera*	Ratio (%):-50/30/10/10

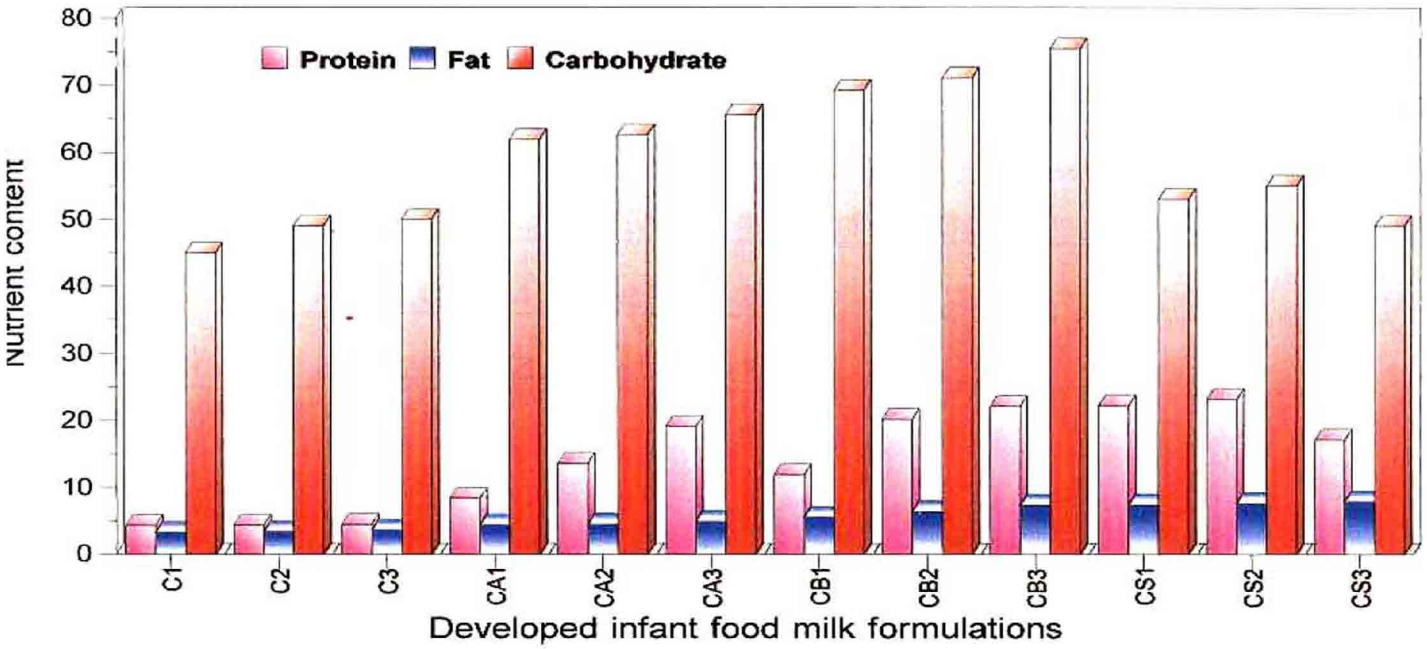


Fig. 3. Proximate composition of developed infant food milk formulations

Table 4.6. Mineral content of milk of various sources cow, buffalo and human

Sr. No.	Milk	Range		Calcium (mg/100g)	Phosphorus (mg/100g)
		Calcium	Phosphorus		
1	Buffalo	210.70 – 210.75**	130.01 – 130.15**	210.73	130.12
2	Cow	120.01 – 120.32**	90.00 – 90.35**	120.29	90.30
3	Human	28.29 – 28.35**	11.30 – 11.50**	28.33	11.45

**Mean values of range

Table 4.7. Mineral content of different humanized milk formulations

Sr.No.	Product	Calcium (mg/100g)	Phosphorous (mg/100g)
1	C_1	100.87±0.031	112.00±0.041
2	C_2	120.97±0.030	161.74±0.070
3	C_3	110.95±0.040	154.62±0.069
4	CA_1	202.85±0.120	200.07±0.011
5	CA_2	210.80±0.140	274.70±0.147
6	CA_3	220.89±0.220	280.20±0.150
7	CB_1	269.05±0.144	196.05±0.072
8	CB_2	254.03±0.150	162.02±0.070
9	CB_3	234.06±0.112	166.05±0.071
10	CS_1	137.58±0.065	140.02±0.050
11	CS_2	151.33±0.069	130.00±0.040
12	CS_3	166.05±0.071	152.00±0.68
	CD (5%)	56.78**	50.97**

Average mean value ± SD value

** Significant at 1% level ($P \leq 0.01$)

4.3 Immunological Characteristics

The data in Table 4.8 showed the specific and non-specific antimicrobial factors in milk and the immunoglobulin with their functional site molecules.

4.3.1 Immunological Content of Milk

Table 4.9 reflected the immunoglobulin (IgA, IgG, IgM) as designated by WHO of colostrum and milk of various species i.e. cow, buffalo and human. Table 4.9 showed colostrum of cow has IgA (3.9g), IgG (47.0 g) and IgM (4.2g) respectively. Buffalo colostrum have IgA (2.4g), IgG (57.2 g) and IgM (2.7 g) and human colostrum have IgA (17.4 g), IgG (0.4 g) and IgM (1.6 g) respectively.

Table 4.10 reflected the immunoglobulin (IgA, IgG, IgM) as designated, by WHO in milk various species i.e. cow, buffalo and human. Table 4.10 showed immunoglobulin levels in cow milk i.e. IgA (0.14g g), IgG (0.60 g) and IgM (0.05 g). In buffalo milk IgA (0.12 g), IgG (0.62 g) and IgM (0.10 g) and human milk contained immunogloblin levels of IgA (1.0g), IgG (0.04 g) and IgM (0.10 g)respectively.

Table 4.8 Specific and non-specific antimicrobial factors in milk

Specific	
SigA, IgA, IgA_2	Macrophages PMN (phagoiytosis)
IgG	Lamphocytes
IgM	Lymphocytes
Complement $C_1 - C_9$	MHC (Major Histocompatibility Complex)
Non-specific	
Lactoperoxidase	Keratinizing epithelium
Lactoferrin	Bile salt stimulated lipase
Lysosymes	Lipid-antiviral factor
Xanthine oxidase	Properdin
Superoxidase Dismutase	Conglutinin
N-acetyl-β-D-glucosaminidase	β-lysine
Oilgosaccaridès	Ubiquitin
Glycopeptides	Free fatty acids
Folate binding protein	α-Lactalbumin

Source (Ekstrand, 1989).

Table 4.9. Immunoglobulin in colostrum (g/lt) of cow, buffalo and human milk

Sr.No.	Immunoglobulin class	Cow colostrum	Buffalo colostrum	Human colostrum
1	IgA	3.9	2.4	17.4
2	IgG	47.6	57.2	0.4
3	IgM	4.2	2.7	1.6

Table 4.10. Immunoglobulin in milk (g/lt) of cow, buffalo and human

Sr.No.	Immunoglobulin class	Cow Milk	Buffalo Milk	Human Milk
1	IgA	0.14	0.12	1.0
2	IgG	0.60	0.62	0.04
3	IgM	0.05	0.10	0.10

4.4 Organoleptic evaluation of the developed infant food formulation

4.4.1 Sensory acceptability of developed infant food formulations

The formulation from standardized method were prepared according to the sensory acceptability as explained in Chapter III and subject to sensory evaluation by a panel of 10 judges for evaluation. The Scores are presented in Table 4.11. The overall acceptability of developed infant food formulations was higher (7.82) and did not change much with the storage of 15 days at 4°C±1. Other developed infant food formulations were in range of 5.75 to 7.82. Moderately liked were (6.50) to much liked (7.82). A consistent decline in the over all acceptability ratings of the formulations were reported by the judges as the level of *seera* reconstitution increased in the infant food formulations. However, the *seera* reconstitution containing infant food formulations up to 30 per cent and carrot, banana and *seera* reconstitutions up to 20 per cent level were rated quite acceptable liked moderately to liked very much. Pure cow milk samples were rated as lowest (5.80) in acceptability rating because of the absence of any pigmented colour and flavour. For colour minimum score was attributed to sample C_1 and maximum to the samples of C_2 followed by samples CA_1, Sensory score for best flavour (7.99) was given to samples C_3 and samples of CS_3 got the minimum score of 5.20. Similarly, for taste again samples of C_2 were rated above the remaining samples with samples of C_1 having rated for poor taste. The texture parameters got almost the same scores except for the C_1 and CS_3.

Table 4.11. Sensory acceptability of developed infant food formulation

Sr. No.	Products	Colour(*)	Flavour(*)	Taste(*)	Texture(*)	Overall acceptability(*)
1	C_1	5.07	6.18	5.21	6.75	5.80
2	C_2	7.38	8.12	8.05	7.74	7.82
3	C_3	7.23	7.99	7.85	7.27	7.58
4	CA_1	7.35	7.10	7.05	7.25	7.19
5	CA_2	7.06	6.28	6.24	7.36	6.73
6	CA_3	4.56	7.36	7.49	7.05	7.36
7	CB_1	7.46	7.26	7.42	6.99	7.28
8	CB_2	7.05	6.26	6.28	7.30	6.72
9	CB_3	7.10	6.86	6.88	7.18	7.00
10	CS_1	7.13	6.78	6.90	7.28	7.02
11	CS_2	6.85	6.85	6.68	7.25	6.90
12	CS_3	5.58	5.20	7.08	5.16	5.75
	CD (5%)	0.42	0.19	0.54	0.18	0.28

* Data reported are the average mean values of ten judges.

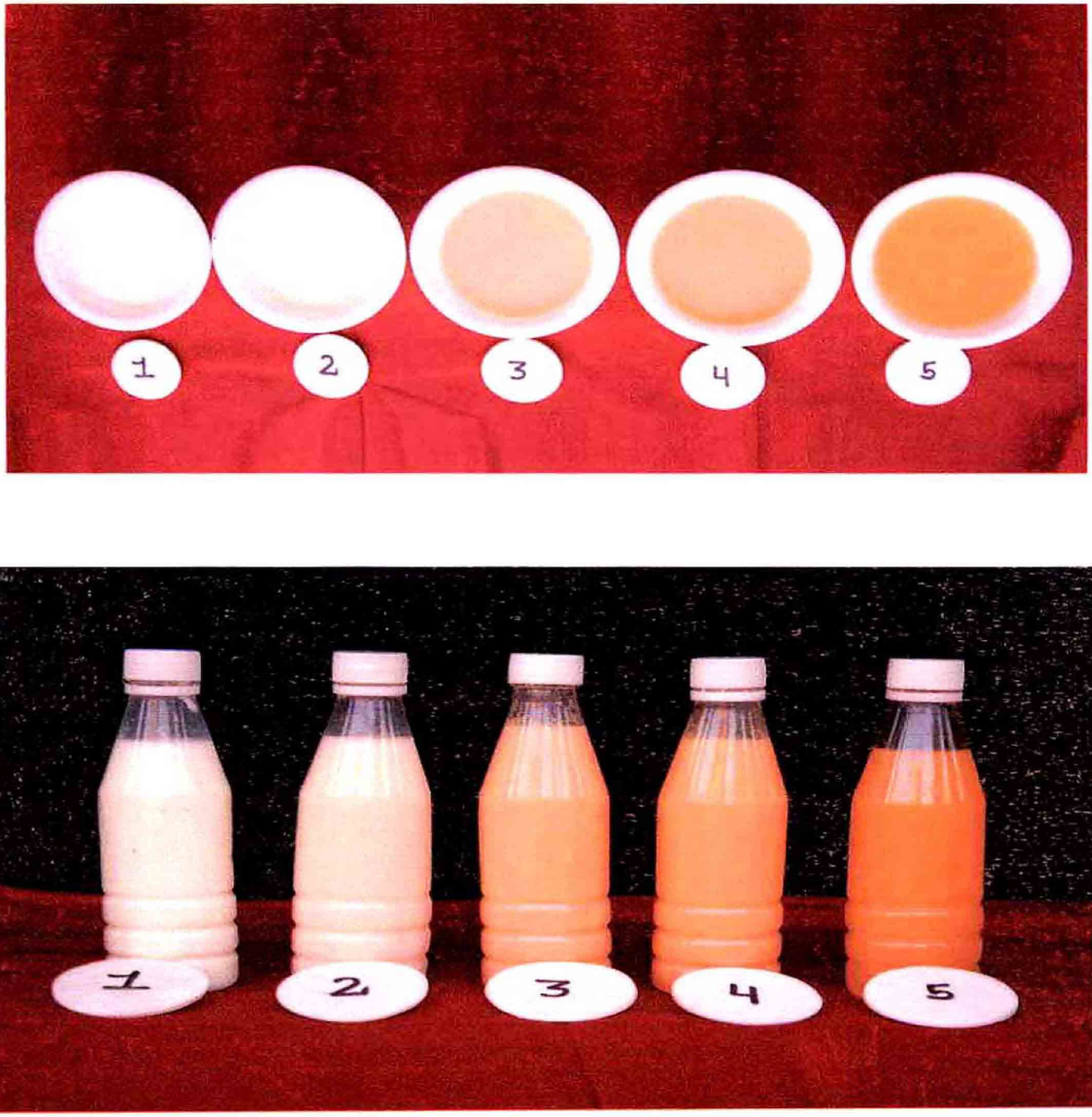


Plate 3. Developed infant food milk formulations with carrot reconstitution

4.4.2 Colour scores of developed humanized formulations at different levels of storage

The results in Table 4.12 depicted the scored point of C_1,C_2, C_3 infant food formulations were 6.80, 6.70, 6.30 at fresh level respectively, 6.60, 6.50, 6.00 after 7 days of storage level respectively, 6.40, 6.30, 5.80 were after 15 days storage at 4±1°C refrigerated temperature respectively.

The infant food formulation prepared with carrot reconstitution CA_1, CA_2, CA_3 scored 6.10, 8.20, 7.50 at fresh level respectively, 6.90, 8.00, 7.30 at 7 days of storage level respectively, 6.60, 7.70, 7.10 after 15 days of storage level respectively. Infant food formulations prepared with banana reconstitution CB_1, CB_2, CB_3 scored 6.30, 6.50, 6.90 at fresh level respectively 6.20, 6.30, and 5.80 at 7 days storage level respectively, 6.10, 6.10, 5.40 after 15 days storage level respectively. Infant food formulations prepared with *seera* reconstitution coded as CS_1, CS_2, CS_3 scored 6.80, 6.60, 6.10 at fresh storage level respectively, 6.70, 6.50, 6.00 at 7 days storage level respectively and 6.40, 6.30, 5.90 at 15 days of storage level respectively.

4.4.3 Texture scores of developed infant food formulations at different storage intervals

The results of Table 4.13 depicted the texture score. The infant food formulation C_1, C_2, C_3 scored 7.40, 47.20, 7.20 respectively points at fresh level; 7.20, 7.10, 7.10 after 7 days storage level respectively, 7.10, 7.00, 6.70 after 15 days storage level respectively.

Table 4.12. Colour scores of humanized milk formulation at different levels storage.

Sr.No.	Product	0 day at 4°C±1	7 days at 4°C±1	15days at 4°C±1
1	C_1	6.80	6.60	6.40
2	C_2	6.70	6.50	6.30
3	C_3	6.30	6.00	5.80
4	CA_1	6.10	6.90	6.60
5	CA_2	8.20	8.00	7.70
6	CA_3	7.50	7.30	7.10
7	CB_1	6.30	6.20	6.10
8	CB_2	6.50	6.30	6.00
9	CB_3	6.90	5.80	5.40
10	CS_1	6.80	6.70	6.40
11	CS_2	6.60	6.50	6.30
12	CS_3	6.10	6.00	5.90
	CD (5%)	NS	NS	NS

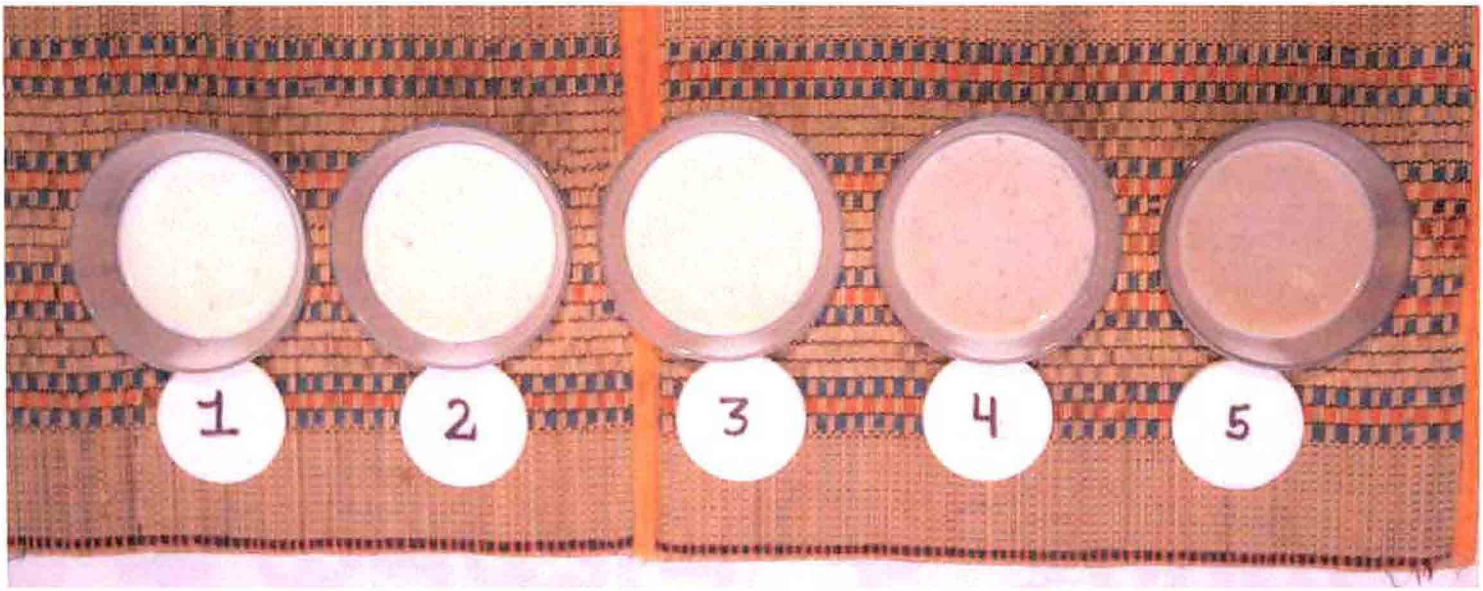


Plate 4. Developed infant food milk formulations with banana reconstitution

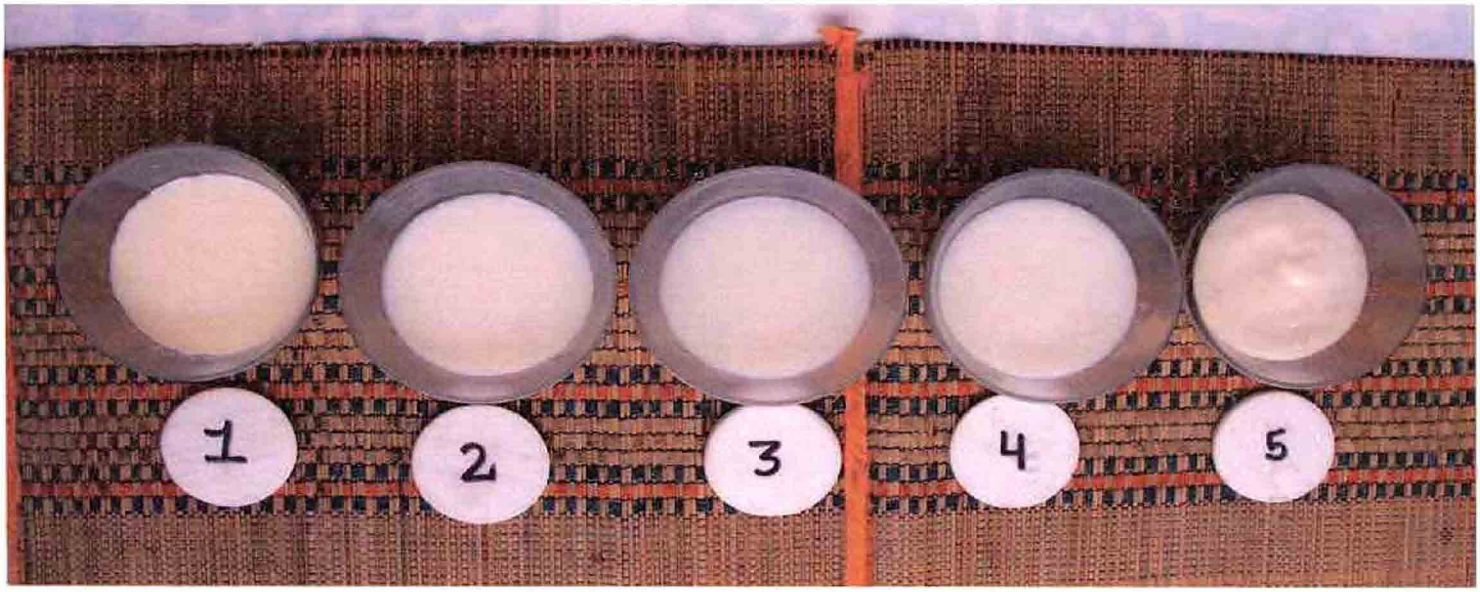


Plate 5. Developed infant food milk formulations with Cera reconstitution

Table 4.13. Texture scores of humanized cow's milk formulation at different storage intervals.

Sr.No.	Product	0 day at 4°C±1	7 days at 4°C±1	15 days at 4°C±1
1	C_1	7.40	7.20	7.10
2	C_2	7.20	7.10	7.00
3	C_3	7.20	7.10	6.70
4	CA_1	7.00	6.90	6.50
5	CA_2	7.20	7.00	6.90
6	CA_3	6.80	6.80	6.70
7	CB_1	6.80	6.50	6.30
8	CB_2	6.90	6.80	6.50
9	CB_3	6.60	6.50	6.40
10	CS_1	6.70	6.40	6.20
11	CS_2	7.10	7.00	6.60
12	CS_3	6.70	6.50	6.40
	CD (5%)	0.70	NS	NS

Infant weaning food reconstitution with carrot coded as CA_1, CA_2, CA_3 scored 7.00, 7.20, 6.80 at fresh levels respectively, 6.90, 7.00, 6.80 at 7 days storage level, respectively 6.50, 6.90, 6.70 at 15 days storage level respectively. Infant weaning food reconstitution with banana coded as CB_1. CB_2, CB_3 scored 6.80, 6.90, 6.60 at fresh level respectively, 6.50, 6.80, 6.50 at 7 days storage level respectively and 6.30, 6.50, 6.40 at 15 days of storage level respectively. Infant weaning food formulation reconstituted with *seera* coded as CS_1, CS_2 CS_3 scored 6.70, 7.10, 6.70 at fresh level respectively, 6.40, 7.00, 6.50 at 7 days storage level respectively and 6.20, 6.60, 6.40 at 15 days storage level respectively. The results were non-significant except fresh storage level it was 0.70 per cent significant.

4.4.4 Taste score of developed infant food formulations at different storage intervals

Predicting the results of (Table 4.14) taste score of 12 different prepared infant food formulations when analysed at 0, 7 and 15 days. The C_1 scored 7.60, 7.60, and 7.00 points, whereas, in case of formulation (C_2) there was decrease in score 7.40, 7.30 and 7.00 respectively. The C_3 samples scored 7.10, 7.20 and 6.90 points for 0, 7, and 15 days of storage level respectively. In case of carrot reconstituted formulations CA_1, CA_2, CA_3 scored 7.80 7.30,7.70 at fresh level respectively,7.50,7.10,7.50 at 7 days storage level respectively,6.80,6.906.40 at 15 days storage level respectively. Infant formulations prepared with banana reconstitution CB_1,

CB_2, CB_3 scored 7.60,7.20,6.80 at fresh level respectively,7.60,7.00,6.80 at 7 days storage level respectively and 6.50, 6.80, 6.50 15 days storage level respectively. Infant formulation prepared with *seera* reconstitution CS_1, CS_2, CS_3 scored 7.20,6.60,6.20 at fresh level,7.00, 6.50, 5.90 at 7 days storage level and 6.30. 6.20, 5.30 at 15 days storage level respectively. The results were significant (P>0.75) at fresh, 0.60 at 7 day and 0.55 at 15 days storage at refrigerated temperature i.e. 4±1°C.

4.4.5 Flavour scores of developed infant food formulations at different level of storage

Table 4.15 showed the flavour score of developed infant food formulations in case of formulation C_1, C_2, C_3 the score were 7.70,7.50,7.30 fresh level respectively,7.50, 7.40, 7.10 at 7 days storage level respectively and 7.40,7.10,7.00 at 15 days storage levels respectively, in CA_1, CA_2,CA_3 score points were 7.80,6.90,7.00 at fresh storage level respectively, 7.20,6.40,6.80 at 7 days storage level and 6.80,6.30,6.60 at 15 days storage level respectively, likewise in CB_1, CB_2, CB_3 score were5.90,6.00,5.90 at fresh storage level respectively,5.40,5.90,5.40 at 7 days storage level and 5.30,5.50,5.30 at 15 days storage level respectively. whereas, in CS_1, CS_2, CS_3 the seera reconstituted infant food formulations scored points were 6.40,6.60,6.30 at fresh storage level respectively, 5.80,6.20,6.10 at 7 days storage level and 5.40, 5.90 , 5.80 storage level of 15 days respectively.

Table 4.14. Taste score of infant food milk formulations at different level of storage.

Sr.No.	Product	0-day at 4°C±1	7 days at 4°C±1	15days at 4°C±1
1	C_1	7.60	7.60	7.00
2	C_2	7.40	7.30	7.00
3	C_3	7.10	7.20	6.90
4	CA_1	7.80	7.50	6.80
5	CA_2	7.30	7.10	6.90
6	CA_3	7.70	7.50	6.40
7	CB_1	7.60	7.60	6.50
8	CB_2	7.20	7.00	6.80
9	CB_3	6.80	6.80	6.50
10	CS_1	7.20	7.00	6.30
11	CS_2	6.60	6.50	6.20
12	CS_3	6.20	5.90	5.30
	CD (5%)	0.75	0.60	0.55

Table 4.15. Flavour score of developed infant food milk formulation at different storage time.

Sr.No.	Product	0 day at 4°C±1	7 days at 4°C±1	15days at 4°C±1
1	C_1	7.70	7.50	7.40
2	C_2	7.50	7.40	7.1[illegible]
3	C_3	7.30	7.10	7.0[illegible]
4	CA_1	[illegible].20	7.00	6.80
5	CA_2	6.90	6.40	6.30
6	CA_3	7.0[illegible]	6.80	6.60
7	CB_1	5.90	5.40	5.50
8	CB_2	6.00	5.90	5.30
9	CB_3	5.90	5.40	5.30
10	CS_1	6.40	5.80	5.40
11	CS_2	6.60	6.20	5.90
12	CS_3	6.30	6.10	6.80
	CD (5%)	0.65	0.70	NS

The results were non-significant in case of 15 days storage. However, results found significant at fresh level 0.65 points and 6.70 points after 7 days storage level.

4.5 *In-vitro* protein digestibility of developed infant food formulations

Table 4.16 depicted the in-vitro protein digestibility of developed infant food formulations. It was in C_1,C_2 C_3 , 69.56, 70.29, 68.52 per cent respectively. In case of carrot reconstituted formulations CA_1, CA_2, CA_3, 70.77, 74.50, 73.60 and in banana reconstituted formulations CB_1, CB_2, CB_3, 65.41, 64.87, 66.14 per cent respectively. *Seera* reconstituted infant food formulations CS_1, CS_2, CS_3 have 70.50, 65.80 ,64.68 per cent in-vitro protein digestibility respectively. These values found significant at 1% level of significance i.e. ($P \leq 0.01$).

4.6 Microbiological evaluation of the developed infant food formulation

In order to study the keeping quality of developed infant food formulations stored in glass bottles. As the proteolysis in the product increases, but the lactic acid content remained constant during 15days of storage at 4±1°C temperature.

Table 4.16. In-vitro protein digestibility of the developed humanized milk formulation.

Sr.No.	Product	Protein digestibility (%)
1	C_1	69.56
2	C_2	70.29
3	C_3	68.52
4	CA_1	70.77
5	CA_2	74.50
6	CA_3	73.60
7	CB_1	65.41
8	CB_2	64.87
9	CB_3	66.14
10	CS_1	70.50
11	CS_2	65.80
12	CS_3	64.68
	CD (5%)	0.68

The mean values of the bacterial count at different dilution levels and at different storage days i.e. fresh, after 7 days and after 15 days are presented in Table 4.17. There was colony form unit (at 10^{-2}) gram in range of 4.09×10^2 to 5.0×10^2 respectively at 7 days storage and 15×10^2 to uncountable after 15 days storage. At (10^{-4}) colony from unit/gram in the range of 1×10^{-4} to 4×10^{-4} at 7 days storage and 2×10^{-4} to 209×10^4 after 15 days storage respectively. All the values were mean of three replicate values.

4.7 Economic feasibility of commercialization of developed infant food formulations

4.7.1 Cost of products

The product cost as calculated from the cost of ingredients used in the preparation of 1 liter of finished product including one third of this cost added as processing cost; and the market prices (including taxes and profits) such products available in market are shown in Table 4.18. The initial cost of developed infant food formulation were 16.25, 17.50 and 19.25 rupees/liter that were C_1, C_2 and C_3. Carrot reconstituted product CA_1, CA_2 and CA_3 having cost of Rs 21.85, 22.00, 22.50 per liter. Banana reconstituted product CB_1, CB_2 and CB_3 having cost of Rs 20.00, 21.25, 21.50 and *seera* reconstituted product CS_1, CS_2, CS_3 having cost of Rs 17.00, 18.25 and 19.50 /liter.

Table4.17. Standard plate count of different milk formulations

S.No.	Product	Storage days	cfu/g (at 10^{-2})*	cfu/g (at 10^{-4})*
1	C_1	7	4.09×10^2	1×10^4
		15	43×10^2	2×10^4
2	C_2	7	4.15×10^2	1×10^4
		15	15×10^2	3×10^4
3	C_3	7	4.20×10^2	1×10^4
		15	23×10^2	7×10^4
4	CA_1	7	4.21×10^2	1×10^4
		15	UC	54×10^4
5	CA_2	7	4.25×10^2	1×10^4
		15	UC	209×10^4
6	CA_3	7	4.28×10^2	2×10^4
		15	67×10^2	40×10^4
7	CB_1	7	4.48×10^2	1×10^4
		15	51×10^2	42×10^4
8	CB_2	7	4.50×10^2	1×10^4
		15	20×10^2	10×10^4
9	CB_3	7	4.10×10^2	1×10^4
		15	12×10^2	0×10^4
10	CS_1	7	4.67×10^2	1×10^4
		15	23×10^2	2×10^4
11	CS_2	7	5.0×10^2	1×10^4
		15	40×10^2	11×10^4
12	CS_3	7	5.0×10^2	3×10^4
		15	35×10^2	2×10^4

* Mean of three replicate values.

Table 4.18. Cost of different humanized milk formulations.

S.No.	Product	Cost of the Product(Rs/lt)
1	C_1	16.25
2	C_2	17.50
3	C_3	19.25
4	CA_1	21.85
5	CA_2	22.00
6	CA_3	22.50
7	CB_1	20.00
8	CB_2	21.25
9	CB_3	21.50
10	CS_1	17.00
11	CS_2	18.25
12	CS_3	19.50

Plate 6. Developed infant food milk formulations packed in bottles

The costs of some market brand formulated infant formula milk (Amulaya) having quite high cost that Rs 88/500 ml net weight. Although the product costs in the market include spray drying, packaging, taxes, handling, transportation and profit but still the products made at home will be cheaper than the commercial products.

DISCUSSION

Chapter-V

DISCUSSION

The results of present investigation entitled "Comparative study of immuno-therapeutic properties of bovine and human milk" presented in chapter IV (Experimental Results) are discussed under the following sub-heads:

5.1 The physico-chemical characteristics

5.1.1 The physical parameters

Perusal of the data presented in Table 4.1 showed that the average mean values of specific gravity of buffalo milk was higher, i.e. 1.0323 followed by cow milk 0.0317 and then human milk i.e. 0.0310. The range of specific gravity of buffalo, cow and human milk was 1.0304 – 1.0415; 1.0288 – 1.0337 and 1.0244 – 1.0330 respectively, thereby meaning that there is high fat percentage in buffalo milk than in cow and

human milk. As the high fat percentage and proteins of buffalo milk particularly the whey proteins were more resistant to heat denaturation as compared to the cow milk proteins and human milk proteins. The pH was all most similar in all the milk samples. The pH of buffalo, cow and human milk was in the range of 6.3 – 6.8, 6.4 – 6.8 and 6.3 – 6.5 (Table 4.1). The pH of normal cow milk ordinarily falls between 6.5 and 6.7 as also reported by Jennes and Patton, (1996) and Singh (1984). Therefore, in general, the reconstitution behaviour of dried milk products made from buffalo milk was indistinguishable from those made from cow milk. Titratable acidity was found similar in cow and human milk i.e. 0.150 and 0.155 per cent respectively. It was higher in buffalo milk (0.16%). Titratable acidity of buffalo, cow and human milk was found in the range of 0.162 – 0.188; 0.135 – 0.170 and 0.133 – 0.170; respectively. So it can be concluded that the diacetyl and acetion contents along with volatile acids contribute to the characteristic aroma and flavour of the product. Volatile acidity remained almost unchanged but the diacetyl and acetion contents increased significantly during 15days storage. The curd tension per unit mass volume was lower in human milk. Curd tension found higher in buffalo milk followed by cow milk than human milk 32.85, 28.54 and 20.50 respectively. Curd tension of buffalo, cow and human milk was in the range of 30.80 – 33.20, 24.58 – 29.02 and17.49 – 21.40. Curd tension showed the quantitative differences between bovine and human milk. Buffalo milk is richer in calcium as compared to cow and human milk. As a result, buffalo milk

exhibited higher curd tension and therefore is not preferred for infant feeding without proper alteration of its composition. Freezing point was higher in buffalo milk i.e. –0.5454°C followed by cow milk i.e. –0.5301°C than human milk i.e.-0.5201°C. Freezing point of buffalo, cow and human milk was in range-0.5437°C to -0.5459°C; -0.5190°C to –0.5305°C and –0.519°C to -0.521°C. It showed the concentrations of both the casein and whey proteins were higher in bovine milk than human milk. The values of treated and untreated samples were in close agreement with those reported by Sindhu (1995). Infant requires three more amino-acids besides the eight essential amino acids. These three additional amino-acids have been identified as cystine, histidine and tyrosine and can be introduced during manufacture process of humanized milk formulations. Higher levels of beta-lactoglobulin are also responsible for allergic reaction to bovine milk among hypersensitive infants. The physical appearance of buffalo milk had a tinge of tincher blue in white colour. Cow milk was in yellowish colour, whereas human milk was also in white colour but in thin consistency. It may be because of certain differential phenomena exist in the appearance of vitamin A in cow and buffalo milk β-carotene - the precursor for vitamin A is not solely transformed to the latter in case of cow milk. As a result, the unprocessed β-carotene appears in cow milk and imparts a yellow colour. This is not the case with buffalo milk where a total conversion of β-carotene to vitamin –A takes place and there by β-carotene cannot leave the mammary glands. That is why buffalo and human milk has

more vitamin–A, no β-carotene and is tincher blue or whiter in look unlike cow milk. Considering the relatively high energy needs of infants and in relation of the limited capacity of child's stomach to accommodate large quantities. The proper alteration therefore had to be sought and the most common was to dilute cow or buffalo milk for infant feeding. As well as to increase the density and lactose content, the sugar was added 8 per cent per ratio and lactose 2 per cent per ratio in standardized manner.

5.1.2 Chemical parameters

Perusal of the data presented in Table 4.2 shows the chemical composition of buffalo, cow and human milk. Moisture content was found 81.19 in buffalo milk, in cow milk 87.57 and in human milk 88.00; it was higher in human milk than cow milk and lower in buffalo milk. The data revealed that total solids are in almost similar amount in buffalo and cow milk (18.40, 17.93) than human milk (13.28), as likewise in solid-not-fat (SNF) percentage, it was 11.50 and 11.08 in buffalo and cow milk than human milk i.e. 9.09. But there is a difference in fat, protein and lactose content. Fat and protein contents were higher in buffalo milk i.e. 7.32; 5.35 followed by the cow milk 6.45; 3.64 than in human milk 4.17; 1.9. Human milk has lower content of protein casein.

Cow's milk protein concentration is close to the human milk protein concentration. So this can be the easy digestion factor in human among the other milks as also reported by Mathur (1993). Cow milk on this

basis then selected for the development of other humanized infant food formulations. Lactose constituents were higher in human milk 6.55 followed by buffalo milk 4.71 than cow milk 3.68. The solution to compensate lactose content in humanized milk formulations was the addition of lactose as per standardized amount reported by Krishnamurthy (1997), Huria and Acharaya, (1997).

5.2 Proximate composition

The results of Table 4.3 revealed the comparison of milk content according to the species. Buffalo milk has higher, protein content as shown in the average mean value (4.3) followed by cow milk (3.2) than human milk (1.1) per cent. Moisture content in all the three groups of milk was in range of (81.17 – 88.20), fat content was higher in buffalo milk (8.8%) followed by cow milk (4.1%) than human milk (3.4%). Milk carbohydrate - lactose is unique for its nutritive value. For the infant, lactose is indispensable because galactose can be made available only from lactose. Furthermore, intake of lactose causes the proliferation of benevolent micro-organisms in the intestinal tract due to its selective fermentation, giving rise to lactic acid. These micro-organisms in turn synthesize certain vitamins of the B group, which are ultimately observed by the body. Hence the consumption of bovine milk as a source of lactose is of significant importance for infants and at the same time helps digestion in the young and old. Carbohydrates mainly lactose was higher in human milk (7.4%)

followed by buffalo milk (5.0%) than cow milk (4.4%). Casein content was higher in buffalo milk (3.0g) followed by cow milk (2.8g) than human milk (0.4g), respectively. Thus, broadly these values are in conformity with those reported by Nayale, (2000); Javeel, (2005) and Gopalan *et al* (1996). Slight variation between the values of proximate constituents observed in the present investigation and those reported earlier by some authors as above may be attributed to the variations in the climatic conditions where they lived. Dilution is the best method to lower the casein content of milk during humanization of milk process as reported by Talwar (1985).

Table 4.4 showed the proximate composition of used ingredients carrot, banana, *seera* and cow milk the average mean values showed that banana and *seera* provides higher values of carbohydrates i.e. 27.20 and 53.30g per 100 g respectively. The protein content was found higher in *seera* i.e. 27.13 followed by cow milk i.e. 3.20 per cent. Fat content was higher in *seera* i.e. 7.30 followed by cow milk i.e. 4.10. *Seera* provides higher calorie content i.e. 386.34 Kcal that is why it is selected in the development of infant food milk formulations. Seera is the fermented product of the wheat grain. Fermentation of lactose to lactic acid by bacteria in milk enables the transformation of milk to curd, which is another popular milk product and better digestible.

5.2.1 Proximate composition of developed infant food formulations

The results of Table 4.5 revealed the proximate composition of developed humanized milk formulations. The moisture content of all the formulation were in the range of 82.10 to 87.54 per cent; protein content 4.28 to 27.13 per cent; fat content 3.30 to 7.82 per cent; carbohydrate content 45.0 to 75.43 per cent and energy provided by these developed formulations were in the range of 249.12 to 505.50 Kcal, respectively. Infant food formulation developed with *seera* reconstitution coded as CS_1, CS_2 and CS_3 provided higher energy mean value i.e. 423.61, 497.10, 505.50 Kcal followed by the infant food formulation developed with banana reconstitution CB_1, CB_2 and CB_3 and provided energy mean values 412.78, 455.96 and 497.61 Kcal than infant food formulation developed with carrot reconstitution coded as CA_1, CA_2 and CA_3 which provided energy mean values 266.90, 362.44 and 502.50 Kcal respectively. Milk also has a symbiotic relationship with other food products when ingested in combination. The biological value of crude protein rated at 52 increases to 67 when supplemented with seera. Similarly, banana and carrot protein with the biological values of 71 is to 86 and 58 boosted to 81 when supplemented with bovine milk (having a biological value of 89).It has also been established that the nutritive value of milk as a whole is higher than the values for each constituent separately. In this respect the proteins from cow and buffalo milk are fairly similar. However, the In-vitro protein digestibility of proteins from buffalo

milk observed to be slightly slower rate compared to the corresponding proteins from cow milk and human milk.

The difference in quantitative and qualitative aspects of various milk constituents between all milks in turn leads to the difference in various physico-chemical and functional properties of all milks (Table 4.3). These differences in physico-chemical and functional properties make the milk behave differently when they are processed for the manufacturing of different products.

5.2.2 Mineral content

Results of Table 4.6 revealed the average mean values of mineral content of milk of various sources i.e. cow, buffalo and human. Buffalo milk contained higher values of calcium and phosphorus i.e. 210.73 mg calcium and 130.12 mg phosphorus followed by cow milk containing calcium 120.29 mg and phosphorus 90.30 mg per 100 g sample. Human milk contained lower content of calcium i.e. 28.33 mg and phosphorus 11.45 mg. Bovine milk contains about 2 dozen minerals and has high contents of calcium, phosphorus and potassium. It is however, deficient in iron, copper and magnesium. Buffalo milk is richer in calcium followed by cow milk than human milk.

Table 4.7 results revealed the average mean values of developed weaning foods, which had 0.80 to 120.06 mg calcium and 0.69 to 102.00 mg phosphorus per 100 g, the calcium and phosphorus content

were maximum in case of infant food formulations developed with banana reconstitution. All the other infant food formulations developed with carrot and *seera* reconstitution's calcium and The phosphorus values ranged with the range of prescribed by ISI (1997).

According to the recommendations given by ISI (1997) for infants weaning foods, the minimum requirement for calcium, the phosphorus and iron were 230, 115 and 5.0 mg/100g. The calcium content of developed infant food formulation was above the recommended level and for the iron content there is need of iron fortification with milk. When CA_1, CA_2, CB_1, CB_2 and CS_1 compared with other formulations, these were well with all the mineral requirement of calcium and phosphorus in infants. The results obtained as the mean values of (CA_1) 202.85, 200.07; (CA_2) 210.80, 274.70; (CB_1) 220.89, 280.20; (CB_2) 269.05, 196.05; (CB_3) 254.03, 162.02; (CS_1) 234.06 and 166.05 mg/100g, respectively. A high content of calcium (128.9 mg/100g) was also reported in weaning foods by Dodd *et al* (1997). As like this high content of calcium (290 mg) and iron content (7.63 g) was reported in waning food formulations by Chandrashakhar *et al* (1988). In these infant milk formulations no addition of minerals mix was added. The mineral content showed significant differences ($P \leq 0.05$) among the different developed infant food formulations.

The bio-availability of trace elements from infant formulas has been demonstrated to by much lower than human milk. Based on this fact it was recommended by the American Academy of Pediatrics (1980) that

infant foods be supplemented in order to provide the infant with adequate supplies of these.

5.3 Immunological characteristics of milk

Table 4.8 showed the specific and non-specific characteristics as reported by the Ekstrand (1989) immunoglobulin were classified as IgA, IgG and IgM by WHO. SigA is the major immunoglobulin class of human milk and colostrums unlike bovine milk. In addition of milk SigA also occurs in intestinal tract. These molecules gives protection against invading pathogens by binding to their appearance.

5.3.1 Immunological contents

Table 4.9 reflected the immunoglobulin (IgA,IgG,IgM) as designated by WHO of colostrum and milk's of various species i.e. Cow, Buffalo and Human. Table 4.9 showed colostrums of human have higher value i.e. 17.4 g/l followed by Cow's colostrum (3.9 g/l) than buffalo's 2.4 in IgA. IgG was higher in buffalo colostrum (57.2 g/l) followed by Cow's colostrum (47.6 g/l). IgM was higher in Cow's colostrum (4.2 g/l) followed by buffalo's colostrums (2.7 g/l) than human colostrum (1.6 g/l).

Data in Table 4.10 showed that milk of humans have higher value 1.0 in IgA followed by Cow's milk 0.14 than buffalo milk 0.12. IgG values found higher in buffalo milk (0.62 g/l) followed by Cow's milk (0.60

g/l) than human milk (0.04 g/l). IgM values found higher in buffalo's milk as well as human milk i.e. (0.10 g/l) followed by cow's milk i.e. (0.05 g/l).

SIgA is the major immunoglobulin class of human milk and colostrums unlike bovine milk. In addition of milk SIgA also occurs in intestinal tract. The molecule gives protection against invading pathogens by binding to their antigenic sites so as to block their attachment to epithelial (outer surface). It protects nourishing infants from gastro-intestinal invasion by pathogens especially *E. Coli* causing diarrhea. IgM is most effective against invading microorganisms. Studies showed that it is the first immunoglobulin to be secreted in response to an antigen. Its production begins 2 to 3 days antigen is first encountered. IgM is an extremely efficient agglytinizing and cytolysis antibody. Maternal IgG confers a high degree of immunity to neonates. In Man, IgG molecules of all its subclasses can pass the placental barrier and confer high level of passive immunity to the new born. However, in bovine, placenta acts as a barrier and immunity is nearly absent. So, there is a requirement of external support of antibodies. The normal breast-fed infant of a well nourished mother receives sufficient quantities of all vitamins except vitamins K and D. Concerning vitamin K, new borne have sterile intestine and can not initially synthesize menaquinones as reported. Because human milk contains only 1-2 mg/liter of phylloquinone, as compared to 10-15 mg/liter in cow's milk. which needs the estimated daily allowances. Trace

mineral supplements can be used in therapeutic doses only to treat bonafide deficiencies (e.g., iron, copper and zinc).

5.4 Organoleptic evaluation of developed infant food formulations

The sensory acceptability scores of developed different infant formulations presented in Table 4.11 revealed that formulation contained carrot reconstitution up to 30 per cent juice addition do not differ much with respect to overall acceptability as per judgment indicated by the taste panel on a nine point hedonic scale. Formulations prepared with banana containing 40 per cent are less acceptable due to poor organoleptic attributes and higher tendency to disintegration during storage. The use of carrot, juice (10.30%) and banana (10.30%) was also technologically feasible, as these combinations have not affected the overall acceptability. The formulations prepared with addition of carrot (10.30%) were more advantageous as these contain high contents of β-carotene, protein and other nutritionally relevant constituents, and are fairly acceptable. Additionally, prompting use of banana and *seera* in such formulations can enhance the energy values. By addition of mineral mixture dietetic characteristics can be enhance (Livia, 1982; Mathur, 1991; Sarkar and Mehra, 1992).

The data of Table 4.12 revealed that infant food formulation reconstituted with carrot coded as CA_1, CA_2 and CA_3. CA_2 scored the highest points i.e. 8.20, 8.00, 7.70 followed by CA_3 scored 7.50, 7.30 and

7.10 than CA_1 scored 6.10, 6.90, 6.60 respectively during fresh storage of refrigerated at 4±1°C temperature. Colour in all the samples was best when stored fresh, less after 7 days followed by the 15 days. Infant food formulation reconstituted with banana (CB_1, CB_2, and CB_3). CB_1 scored 6.30, 6.20, 6.10 and CB_2 scored6.50, 6.30 and 6.00 respectively; whereas, CB_3 scored 5.90, 5.80 and 5.40 points at the storage level of fresh, 7 and 15 days. They scored fewer points as compared to creamish white colour of banana when compared with the pigmented colour of carrot. The scores did not vary much when the mixes contained yellow colour milk and white colour *seera*. As this *seera* reconstitutions (CS_1, CS_2, CS_3) scored CS_1 6.80, 6.70, 6.40; CS_2 6.60, 6.50, 6.30; CS_3 6.10, 6.00, 5.90 respectively at different storage level of fresh, after 7 and 15 days. The results were non-significant during the storage levels.

Table 4.13 revealed that the texture scores for the fresh sample varied form the minimum 6.20 to the maximum 7.40 points at fresh storage, at 7 day storage the range of scores was 6.40 to 7.20 and after 15 days storage the texture range was 6.20 to 7.10. The scoring trend was same as observed in case of colour. There was decrease in score in case of two of the formulations as the storage time increased. In case of others there was a decrease in the texture score after 15 days storage. The values showed significant difference ($P \leq 0.05$), when samples were fresh but showed non-significant difference after 7 days and 15 days storage.

The results of 4.14 revealed that the taste score of the 12 different developed infant foods formulations varied when analyzed at 0, 7 and 15 days as per the observations made by the panelists. The infant food formulations of carrot reconstitution proved to be more acceptable than others. As the storage time increased up to 15 days, the acceptability remained the same. The level of significance was 0.75 at fresh storage, 0.60 at 7 days storage and 0.55 at 15 days storage.

The results of 4.15 revealed the flavour of the 12 different developed food formulations analyzed at 0, 7 and 15 days of the storage level. The flavour scores of the humanized milk formulations showed a decrease in the scores as the storage time increased. In case of formulations (C_1, C_2, C_3, CA_1, CA_2 and CA_3) the score was more than others i.e. CB_1, CB_2, CB_3, CS_1, CS_2 and CS_3. Only in one case the value was less i.e. CB_3. The result showed C_1 scored 7.70, 7.50 and 7.40 score points respectively followed by C_2 scored 7.50, 7.40 and 7.10 than C_3 i.e. 7.30, 7.10 and 7.00 respectively. The formulations CA_1 scored 7.20, 7.00 and 6.80 values respectively followed by CA_3 7.00, 6.80 and 6.60 than CA_2 i.e. 6.90, 6.40 and 6.30. CB_1 formulation scored 5.90, 5.40 and 5.30 points respectively followed by CB_2 formulation i.e. 5.90, 5.40 and 5.50 than CB_3 i.e. 5.90, 5.40 and 5.30 points respectively. The score points obtained by formulations CS_1 and CS_2 were 6.40, 5.80 and 5.40 and 6.60, 6.20 and 5.90 respectively. There was a similar trend in the scores, when the samples

were fresh and after 15 days of storage, the values showed significant difference ($P \leq 0.05$) at different levels of storage.

The similar results were reported by Dahiya and Kapoor (1994) that acceptance for taste and aroma of bajra based supplements, all the supplements containing whole wheat, pearl, millet, Bengal gram, green gram, grain and amaranth levels were found to be acceptable. Reddy *et al* (1990) also reported that weaning mixes remained acceptable even after being stored for a period of one month.

5.5 In-vitro protein digestibility of the developed humanized milk formulations

In-vitro protein digestibility in table 4.16 results reveals the developed food formulations ranged from 64.87 to 76.56 per cent as presented in Table 4.16. Humanized cow milk incorporating with carrot have better digestibility as compared to the mixes having banana and seera. The relatively low digestibility of the milk formulations may be attributed to the reason that globulins, which make up the larger casein cells of protein and not digested early (Walker and Kochar, 1983). The increase in protein digestibility may be due to destruction of trypsin inhibitor and by opening of protein structure through denaturation. Heat treatments of proteineous foods result in the destruction of the protein

which explains the improvement in their protein digestibility (Boonvisut and Whitaker, 1976).

5.6 Microbiological evaluation of developed infant and milk formulations

Microbiological studies were done to detect the bacterial contamination during process and storage. For detection standard plate count method was used. In the present investigation, the standard plate count technique was used to detect the bacterial contamination in developed formulation, if any. The mean values of the bacterial count at different duration levels and at different storage days i.e. after 7 days and after 15 days are presented in Table 4.17. There was no growth when the samples were fresh but as the storage time increased there was the maximum growth in samples after 15 days of storage. Samples after these days of storage can adhered to the ISI specification. As the dilution of the sample increases the number of organisms or colonies developed decreases. It was more in case of second dilution than that of fourth dilution.

5.7 Economic feasibility of commercialization of developed infant food milk formulations

5.7.1 Cost of product

Milk and milk products are the second largest food expenditure groups, next to cereals, accounting for 18 per cent of the total food expenditure (McKinsey,1998) and around 9 to 10 per cent of the total consumption expenditure (Sexena, 1997). The present level of per capita availability of milk is 228 g per day (83.2 kg/year) as reported by Huria (2004).

The product costs which have been calculated from the cost of ingredients used in the preparation of one liter of finished products are depicted in Table 4.18. The preparation cost of various food products varies with the cost of ingredients used in the preparation of these products. The preparation cost of seera based prepared formulations were less than those prepared with carrot and banana. Similarly, the cost of carrot reconstitution increase with addition of carrot than banana reconstitution. No doubt that blending of carrot and banana will result in enhanced costs but not at the cost of quality, which is important in marketing of the products in the form of increased sale. The calculated cost prices are much less than the market prices of various brands of similar product available in the market.

In addition, it can be said that commercially valuable products can be prepared with carrot, banana and *seera* at a much lower cost as compared to the market prices of such like products available in the market. Although, in marketing the manufactures has to include the cost of processing, packaging, handling, transportation, taxes and profit even then the products are likely to be cheaper than the market prices if intended to be prepared at the household levels. On the commercial scale however, the prices will have to be kept competitive and quality control kept as the top priority.

SUMMARY

Chapter –VI

SUMMARY AND CONCLUSION

The present study "Comparative study of immuno-therapeutic properties of bovine and human milk" was undertaken to assess the physico-chemical studies, immunological studies, therapeutic studies, development of infant food formulations their organoleptic evaluation studies, storage studies, microbiological studies and economic feasibility of commercialization of developed infant food formulations. In first part cow, buffalo and human milk were selected. In second part cow milk was selected for the purpose of supplementation and development of infant food formulations in the form of different humanized milk formulations with carrot, banana and seera reconstitution. The conclusions drawn from this investigation are summarized below:

1. Humanization process of bovine milk is significant, because through it milk composition can be equaled to human milk composition.

2. Considering the relatively high energy needs of infants and in relation of the limited capacity of child's stomach to accommodate and digest larger quantities, it was necessary to increase the energy density of the weaning food; therefore, sugar may be added in 8.0 per cent per ratio to improve lactose content. It was added 2.0 per cent as ratio of additive to standardize the milk.

3. The difference in quantitative and qualitative aspects of various milk constituents between all milks in turn leads to the differences in various physico-chemical and functional properties.

4. These differences in physico-chemical and functional properties make the milk behave differently when processed for the manufacture of different products. Humanized cow milk was significant because its composition was found close to natural human milk.

5. Developed infant milk formulations with carrot i.e. (CA_3) had significant increase in all the nutritional contents i.e. protein (2.01%), fat (3.90%) and carbohydrates (5.60%).

6. Bovine milk contains about 3 to 5 times higher amounts of proteins compared to human milk. These metabolic products of protein in

excess of the nutritional requirements of infant would, therefore need to be excreted because these impose a higher renal osmolar load on the under-developed kidney of the infant and excessive amounts of neurological damage, specially among pre-term (premature) infants.

7. Higher levels of casein in bovine milk lead to formation of tough curd in the digestive tract, which tends to increase the emptying time in comparison with human milk. As the data showed in table 4.2.Therefore it is imperative that casein content in the weaning food formulations may be kept under control by reducing the casein through dilution. That is one reason buffalo milk can not digested by the infants easily.

8. Infant requires three more amino-acids besides the eight essential amino acids. These three additional amino-acids have been identified as cystine, histidine and tyrosine and can be introduced during manufacture processes of humanization of bovine milk and weaning food milk formulations.

9. Higher levels of beta-lactoglobulin are also responsible for allergic reaction to bovine milk among hypersensitive infants.

10. Mean scores of twelve different developed infant food milk formulations for the organoleptic characteristics showed non-

significant differences among themselves. All the formulations were rated 'good' by the panelist (average score of 6 on a 9 point hedonic scale).

11. Developed infant food milk formulations were prepared by mixing all the different ingredients i.e. sucrose, carrot, banana, *seera*, lactose and cow's milk etc. in different proportions. The developed infant formulations had 82.10 to 87.54 per cent moisture content, 4.28 to 23.13 per cent protein content, 3.30 to 7.82 per cent fat content. Carbohydrate ranged 45.50 to 75.43per cent (lactose) and energy as 249.12 to 505.50 Kcal per 100g.

12. Among twelve formulations developed, the formulation coded as CA_2 containing ingredients of carrot juice, cow's milk, lactose, sucrose was considered as the best.

13. Calcium content of developed formulations was in range 100.87 – 269.05 mg/100g and phosphorus content in range 112.00 – 280. 20mg /100g. Formulation prepared with banana reconstitution had higher calcium content i.e. 269.05 mg and phosphorus content 280.20 mg/100g. The bio-availability of trace elements from infant formulas has been demonstrated to by much lower than human milk. Based on this fact it was recommended by the American

Academy of Pediatrics (1980) that infant foods be supplemented in order to provide the infant with adequate supplies of these.

14. In-vitro protein digestibility of the developed formulations varied from 64.68 to 78.50 per cent. Weaning mixes incorporating carrot juice had better protein digestibility (in-vitro) as compared to the others.

15. Table 4.12 revealed that infant food formulation reconstituted with carrot coded as CA_1, CA_2 and CA_3. CA_2 scored the highest points i.e. 8.20, 8.00, 7.70 followed by CA_3 scored 7.50, 7.30 and 7.10 than CA_1 scored 6.10, 6.90, 6.60 during fresh storage of refrigerated at 4±1°C temperature. Colour in all the samples was best when stored fresh, less after 7 days followed by the 15 days.

16. The developed formulations were stored in glass bottles as well as plastic bottles for a period of 15 days at 4°C±1 temperature. The storage related changes in the products developed were monitored at 4°C±1. The marginal change in the reflectance spectra was noticed when stored beyond 15 days. Slight discoloration of the products was noticed but the reconstitution characteristics remained in the acceptable limits. There was no pathogenic microbial growth when the samples were fresh, a few organisms were present after 7 days and the number increased after 15 days of

storage in local and household conditions. For commercial preparations the shelf-life will need to be enhanced significantly for allowing marketing and transportation of the finished products.

17. There was no growth when the samples were fresh but as the storage time increased there was the maximum growth in samples after 15 days of storage. Samples after these days of storage can adhered to the ISI specification.

18. Cost of production in different developed formulation varied between 16.25 to 22.50 rupees per kg. The formulations incorporated with carrot had higher price as compared to the other mixes.

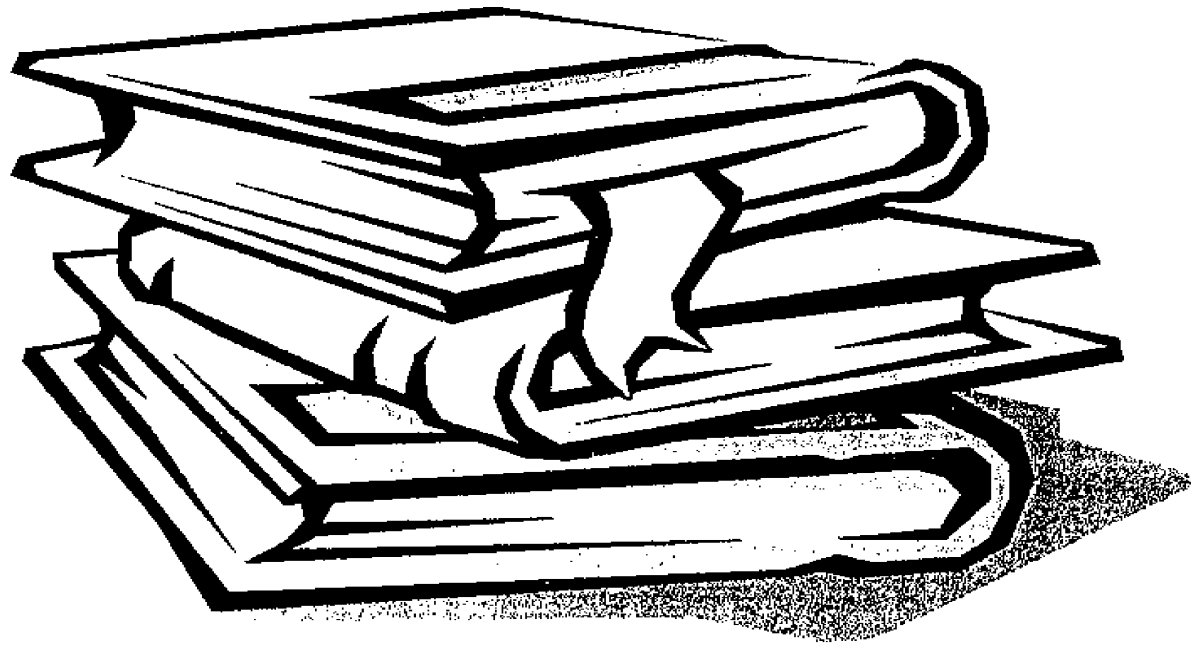
19. The normal breast-fed infant of a well nourished mother receives sufficient quantities of all vitamins except vitamins K and D. Concerning vitamin K, new borne have sterile intestine and can not initially synthesize menaquinones. Because human milk contains only 1-2 mg/liter of phylloquinone, as compared to 10-15 mg/liter in cow's milk-which needs the estimated daily allowances. Trace mineral supplements should be used in therapeutic doses only to treat bonafide deficiencies (e.g., iron, copper and zinc).

On the view of infant's nutritional problems, the humanized bovine milk and its formulations based on commonly consumed, inexpensive, and locally available raw materials are possible. These do not involve any

addition economic burden because they were already available in the house. Being inexpensive, easily available and nutritious, these formulations could be effective in ameliorating the common symptoms associated with the infant feeding problems as well as incidence of protein calorie malnutrition.

Epilogue

Mother and child are two separate physical entities; they constitute a unique biological unit. The composition of breast milk varies with the gestation period at which off-spring is born to support optimal extra uterine growth. According to WHO estimate, 22 million low-birth babies are born every year, mostly in the developing countries. In India, 14 per cent of births are pre-term and about 33 per cent are low-birth weight infants. In advanced countries, pre-term births are regarded natures accident. In developing countries, it is mainly due to physiological stress arising from poor health, biological immaturity and poor nutritional status. Dietary management of such subjects presents special problems. Underdeveloped organ functions coupled with in- adequacy of enzymes associated with intermediate metabolic pathways impose serious constraints, which are critical for the survival of infants.



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APPENDIX

Appendix-I

Composition of milk of some mammals

Mammal	Specific gravity	Water	Total protein	Fat	Sugar	Ash	Ts
Ash	1.032	90.12	1.85	1.37	6.19	0.47	9.88
-do-	-----	----	1.40	0.60	6.10	0.28	-----
Buffalo Indian	1.030	82.22	4.37	7.65	4.82	0.94	17.78
-do-	-----	-----	3.80	7.20	4.80	0.76	----
Bitch	1.035	75.44	11.17	9.57	3.09	0.73	24.56
Cat	------	82.10	9.08	3.33	4.91	0.58	17.90
Camel	1.042	86.57	4.00	3.07	4.59	0.77	13.43
Elephant	1.0313	79.30	2.51	9.10	8.59	0.50	20.70
-do-	----	---	3.10	19.60	8.80	0.65	----
Fox	----	81.06	6.06	5.09	4.09	0.93	-------
Goat	1.0305	86.88	3.76	4.07	4.64	0.85	13.12
-do-	1.0316	----	3.39	4.08	-----	----	12.64
-do-	1.0316	------	3.49	4.50	4.68	0.77	13.50
-do-	----	----	4.00	4.90	5.10	0.76	14.70
Guinea Pig	-----	85.00	5.09	7.31	2.31	0.29	15.00
Liama	-----	86.55	3.90	3.15	5.60	0.80	----
Mare	1.0347	90.58	2.05	1.14	5.87	.36	9.42
Monkey	-----	88.04	2.02	2.07	6.04	0.18	-----
Rabbit	-----	65.00	16.00	14.00	2.00	2.20	35.00
Reindeer	-----	63.30	9.89	17.9	2.82	1.40	36.70
Sheep	1.0355	83.57	5.15	6.18	4.17	0.93	16.43
-do-	-----	-----	5.70	8.60	4.30	0.97	-----
-do-	-----	-----	4.84	6.04	4.99	0.81	16.30
Sow	1.038	83.94	7.23	4.55	3.23	1.05	16.30
Women	-----	-----	1.10	4.50	6.80	0.20	12.60
Whale	-----	64.08	11.10	21.20	1.60	1.70	-----
Zebra	-----	86.20	3.00	4.80	5.30	0.70	-----
Zebu	1.0313	87.27	3.39	3.68	4.94	0.742	12.73

Appendix-II

Analysis of variance by completely randomized design (CRD)

Treatments	Replications				Sum	Mean
	A	B	C	D		
1						
2						
3						
4						
5						
Sum					Grand total	

Analysis of data

Source of Variation	Degree of freedom	Sum of squares	Mean squares	F. calculated
Treatment	(t-1)	C	C/(t-1) = X	X/Y
Error	(t-1) (r-1)	D	D/(t-1) (r-1) = Y	

A. (Grand sum) 2/total number of observations = C

B. (square all observations and add) – C

C. (sum of observations in one treatment squared and add to same for all treatments/No. of replications) – C

D. B – C

Least significance difference (LSD) =

$$\sqrt{\frac{2}{\text{No. of reps.}} \times \text{Mean square error} \times \text{'t' value at error df.}}$$

Appendix – III

Organoleptic scoring quality control form

Sample : _____ Date:_____

Sample	Perfect	Good			Fair			Poor		Off	Remarks
	10	9	8	7	6	5	4	3	2	1	

Note: Make check mark in columns corresponding to your rating of sample, when scoring one factor. However, when scoring 2 or more factors, write in the following letter in the corresponding column of columns (C) colour (E) Flavour (T) Texture (S) Consistency.

Signature

Appendix – VI

Cost of different ingredients

	Price in Rupees
Cow milk	14.00 per kg
Carrot	16.00 per Kg
Sugar	18.00 per kg
Seera	52 per kg
Banana	32 per dozen

Appendix – V

Chemical composition of tryptone glucose beef extract agar

Agar	15 g/L
Beef extract	3 g/L
Tryptone	5 g/L
Glucose	1 g/L
Final pH (at 25 °C)	7.0 ± 0.2