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Determination of the Bio-Chemical Parameters and the Amount of Trace and Toxic Metals in Baby Foods, Jams, Jellies and Juices Available in Bangladesh

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DETERMINATION OF THE BIO-CHEMICAL PARAMETERS AND THE AMOUNT OF TRACE AND TOXIC METALS IN BABY FOODS, JAMS, JELLIES AND JUICES AVAILABLE IN BANGLADESH

A Thesis

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SUBMITTED

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Declaration by the Candidate

I do hereby declare that the whole of the work submitted as a thesis entitled "**Determination of the Bio-chemical Parameters and the Amount of Trace and Toxic Metals in Baby Foods, Jams, Jellies and Juices Available in Bangladesh**" for the degree of **Doctor of Philosophy** in Chemistry is the results of my own and original investigation except where some due acknowledgements have been given. The thesis has not been concurrently submitted for any other Degree, Award, Diploma and Associateship or Fellowship. The work has been carried out under the direct supervision of **Professor Dr. Md. Nazrul Islam**, Department of Chemistry, University of Rajshahi and my Co-supervisor **Professor Dr. M. Saidul Islam**, Department of Chemistry, University of Rajshahi, Rajshahi-6205, Bangladesh.

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Declaration Certificate

This is to certify that the thesis entitled **"Determination of the Bio-chemical Parameters and the Amount of Trace and Toxic Metals in Baby Foods, Jams, Jellies and Juices Available in Bangladesh"** is a bonafide record of research work done by Lokonuzzaman Ahmmed under our joint supervision and guidance. We further certify that no part of this thesis has been submitted to any other University or Institute for any Degree, Diploma, Associateship or Fellowship or similar title for any candidate. The candidate has fulfilled all terms and conditions of the PhD course including presentation of the results of his study in seminars held in the Department of Chemistry, University of Rajshahi, Bangladesh.

We have gone through the final draft of the thesis and recommended its submission for the degree of Doctor of Philosophy in Chemistry since it is in conformity with the regulation of this University and accepted standard in respect of originality and quality.

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ABSTRACT OF THE THESIS

The thesis entitled **'Determination of the Bio-chemical Parameters and the Amount of Trace and Toxic Metals in Baby Foods, Jams, Jellies and Juices Available in Bangladesh'** deals with the estimation of bio-chemical parameters, trace and toxic metals in baby foods and some fruit products, e.g. jams, jellies and juices in the view of nutrition, dietary supplements, necessity of essential metals and the harmful effect of toxic metals for infants as well as adults. The results obtained during the course of PhD program have been compiled into five chapters as described below.

Chapter One presents the importance of bio-chemical parameters, trace and essential metals and their biological activity in human life. Milk proteins represent one of the greatest contributions of milk to human nutrition. Research indicates that children may develop food allergies if solids are introduced too early. Formula and breast milk provide the perfect balance of nutrients that they need.

The knowledge of nutrition in different types of fruit products especially jams, jellies and juices are very significant due to their good food values since these are made from fruit, water and sugar. Jams, jellies and juices provide more essential nutrients in significant amounts as well as some minerals. Our study was to find out some important bio-chemical parameters of baby foods, such as percentages of protein, reducing sugar, non-reducing sugar, total sugar, ash and moisture by using different biochemical methods. The amounts of some trace and toxic metals were also determined by using atomic absorption spectrophotometer (AAS).

Chapter Two covers the experimental part which includes the listing of chemicals, physicochemical methods for the determination of biochemical parameters, and the estimation of trace and toxic metals by AAS. The preparation of calibration curves for metal estimation is also described in this chapter.

Chapter Three describes the estimation of bio-chemical parameters, trace and toxic metals in baby powder milk and baby cereals. Protein, lactose, acidity, trace and toxic metals were measured in baby foods for comparison to the standard values or the values given in the packet/container. Fourteen samples of baby (0−6 months and 6−24

months) powder milk and ten brands of baby (6−24 months) cereal were analyzed and the result have been discussed in this chapter.

Protein found in baby (0−6 months) powder milk of seven brands was in the range of 15.54−18.50%. The amount of lactose and acidity was found to be 51.02 to 62.49% and 0.66 to 1.28% respectively. The content of protein in baby (6−24 months) powder milk was observed between 17.27 and 22.98%. The content of lactose and acidity were in the range of 50.87−58.69 and 1.07−1.20% respectively.

The trace (Co and Zn) and toxic (Pb, Cd, Cr, As and Ni) metals in baby (0−6 months) powder milk of different brands were estimated and the values were as follows: Co, 0.02 to 0.04 mg/kg; Zn, 16.95 to 52.86 mg/kg; Pb, 0.12 to 0.42 mg/kg; Cd, 0.01 to 0.02 mg/kg; Cr, 0.03 to 0.16 mg/kg and As, 0.26 to 0.61 mg/kg. Ni in baby (0−6 months) powder milk was found below the detectable limit. The trace and toxic metals in the baby (6−24 months) powder milk was found to be in the range of 0.38−0.47 mg/kg, 5.40−42.00 mg/kg, 0.01−0.04 mg/kg, 3.58−4.67 mg/kg, 0.12−0.24 mg/kg and 0.65−9.20 mg/kg for Co, Zn, Cd, Cr, As and Ni respectively.

The metal contents in the baby cereals were observed as follows: Co, 1.2−8.2 mg/kg; Zn, 28.2−659.0 mg/kg; Pb, 0.3−20.4 mg/kg; Cd, 0.04−0.59 mg/kg; Cr, 0.15−0.93 mg/kg; As, 0.58−1.68 mg/kg and Ni, 0.2−32.7 mg/ kg.

The bio-chemical parameters of baby powder milks were found to be consistent with their expected values. The concentration of trace metals (Co and Zn) and toxic metals (Pb, Cd, Cr, As and Ni) in baby powder milks were either significantly lower than their tolerance limits, or below their detection limits. These milks can, therefore, be considered as fairly safe for infant health. But the higher concentration of toxic metals in some baby cereal shows alarming signal to their consumer and demands proper investigation of baby cereals after the import. However, the present investigation shows that the baby powder milk which are coming to the markets from abroad contain very negligible amount of harmful metals.

Chapter Four deals with the estimation of bio-chemical parameters, trace, toxic and essential metals in jams, jellies and juices available in the market of Bangladesh. The contents of protein, moisture, total solid, reducing sugar and ash were measured in jams, jellies and juices for comparison to the standard values or values given in the

packet/container. Total twenty seven samples of jams (nine), jellies (eight) and juices (ten) were analyzed and discussed here.

The moisture contents of different jams, jellies and juices ranged from 17.89 to 41.77%, 17.13 to 45.23% and 81.93 to 88.19% respectively. The total solid contents of different jams, jellies and juices ranged from 58.23 to 82.11%, 54.77 to 83.88% and 11.67 to 18.17% respectively. Ash contents in jams, jellies and juices of different brands were found to be in the range of 0.15–1.52%, 0.11–0.42% and 0.05–0.23% respectively.

The protein contents of different jams, jellies and juices were analyzed and found to be 0.00 % to 0.79%, 0.00 % to 0.50% and 0.00 % to 0.35% respectively. The reducing sugar and total sugar contents in jams were found to be in the range of 28.00%−60.30% and 38.86%−62.53% respectively. The values of reducing sugar and total sugar in jellies were in range of 16.32%−49.66% and 28.44%−59.97% respectively. The reducing sugar and total sugar in juices ranged from 2.65 to 11.60% and 10.06 to 14.41% respectively.

The analysis of trace (Co and Zn) and toxic (Pb, Cd, Cr, As and Ni) metals in jams of different brands were as follows: Co, 0.01 to 0.05 mg/kg; Zn, 0.32 to 0.72 mg/kg; Pb, 0.03 to 0.24 mg/kg; Cd, 0.01 to 0.02 mg/kg; Cr, 0.13 to 0.33 mg/kg and As, 0.06 to 0.88 mg/kg. The content of Ni in jams was found below the detectable limit. The trace and toxic metals in jellies of different brands were found in the range of 0.01 to 0.07 mg/kg, 0.16 to 0.46 mg/kg, 0.16 to 0.30 mg/kg, 0.01 to 0.03 mg/kg, 0.14 to 0.17 mg/kg, 0.005 to 0.008 mg/kg and 0.11 to 0.13 mg/kg for Co, Zn, Pb, Cd, Cr, As and Ni respectively. These metals in juice samples of different brands were shown in the ranges of 0.03–0.09 mg/kg for Co, 0.12–0.27 mg/kg for Zn, 0.10–0.23 mg/kg for Pb, 0.01–0.04 mg/kg for Cd, 0.08–0.18 mg/kg for Cr, 0.003–0.007 mg/kg for As and 0.01–0.12 mg/kg for Ni.

The contents of essential metals Na, K, Ca and Mg in jams were found in the range of 1.9−5.2 mg/kg, 9.9−200.0 mg/kg, 8.4−62.0 mg/kg and 0.4−28.9 mg/kg respectively. The levels of Na, K, Ca and Mg in different jellies were found in the range of 2.2−5.5 mg/kg, 12.1−99.1 mg/kg, 9.8−62.2 mg/kg and 0.02−9.7 mg/kg respectively. The

contents of above metals in juices were found in the range of 1.1−4.5 mg/kg, 8.0−38.02 mg/kg, 4.3−85.3 mg/kg and 0.8−2.1 mg/kg respectively.

The nutrients studied in the present work have very significant and specific role in human metabolism and their deficiency can be removed through intake of reasonable amount of fruit products.

Three different criteria proposed for statistical analysis of the experimental results of the studied samples. Descriptive statistics, Analysis of Variance (ANOVA) for the variation study and Duncan's Multiple Range Test (DMRT) have been applied to explain the experimental results precisely obtained from our study.

Chapter Five contains overall conclusion of the experimental results. Fourteen types of infant formula milk, ten types of baby cereals and twenty seven samples of jams, jellies and juices were studied. In the view of the experimental results of the bio-chemical analysis of different milk, it could be concluded that the investigated milk shows good results with a few exceptions.

The results of the study provide information about the concentration of trace and toxic metals in different baby powder milks, baby cereals, jams, jellies and juices. The higher amount of As, Pb, Cd, Cr and Ni were found in some studied samples but other samples are safe considering the recommended value.

The information gained from these measurements will provide a baseline level of toxicity for baby powder milk, baby cereals, jams, jellies and juices. The data obtained from this study will help to make a food list according to the presence of estimated metals. This research will also help consumers, manufacturers and professionals to realize about the possible direct or cumulative effects of the toxic metals to healthcare system.

LIST OF ABBREVIATIONS

mmol.	: Millimole
g	: Gram
mL	: Milliliter
m.p.	: Melting Point
%	: Percent
Max	: Maximum
Min	: Minimum
et al	: And others
e.g.	: As for example
i.e.	: That is
UV	: Ultraviolet
nm	: Nanometer
ng	: Nanogram
WHO	: World Health Organization
K	: Kelvin
L	: Litre
Soln	: Solution
M	: Molarity
ed.	: Edition
P	: Page
Eq.	: Equation
mg	: Milligram
Conc	: Concentration
kg	: Kilogram
hr	: Hour
Sig	: Significant
µg	: Microgram
ppm	: Parts per million
SD	: Standard Deviation
DF	: Degrees of Freedom
SS	: Sum of Square
SE	: Standard Error
MS	: Mean of Square
CI	: Confidence Interval

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CHAPTER ONE

General Introduction

CHAPTER ONE

General Introduction

1.1 General Introduction

Milk is as ancient as mankind itself as it is the substance created to feed the mammalian infant. All species of mammals from man to whales produce milk for this purpose. Many centuries ago perhaps as early as 6000−8000 BC, ancient man learned to domesticate species of animals for the provision of milk to be consumed by them [1]. Human milk is usually the only source of food for infants during the first four to six months of their lives. Many chemicals can be transferred from the body into the breast milk of a lactating mother. Despite the attention focused on environmentally persistent organochlorine compounds in human milk, level of toxic metals in milk is also of growing concern [2].

During early life, infants usually consume a diet predominantly made up of milk, which is the main source of their protein, energy and minerals. Milk is a complex biological fluid including water (87.3%), proteins (3.2%), carbohydrates especially lactose (4.6%), fat (3.9%), and a mineral fraction (0.7%); milk is considered as a rich source of some essential trace metals like zinc, but deficient in copper [3].

Mothers are consistently being reminded about the necessity of feed to their infants. However, some instances, like the presence of hypogalactia, inverted nipple, nipple tenderness and other medical conditions prohibiting breast feeding are inevitable, thus needs the infant formula of milk. Infants, particularly in the 6−12 months age group are vulnerable to infection due their immature immune system. This is also the time when they are weaned from a pure breast milk diet to one with solid food. Infant formula, when in liquid form, may be used either directly or diluted with water before feeding, as appropriate. In powder form it requires water for preparation [4].

Milk may be defined as the normal secretion of the mammary glands of mammals. For centuries, milk has been recognized as an almost indispensable food for mankind. Milk contains all the food constituents required in the human diet and in essentially the proper proportions. Cow, goat, sheep, the camel, and to a small extent, the mare and the llama used as a source of milk for man. The term milk always will be understood as referring to the milk of the cow because the cow supplies such a large

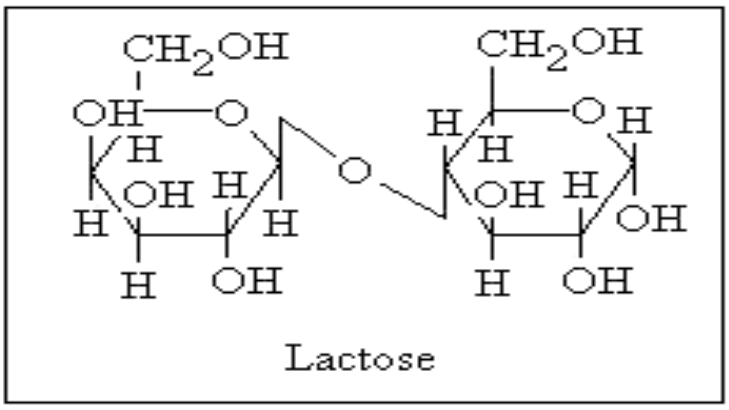
proportion of the product used that little attention need to be directed towards other sources [6]. As a food, milk serves the following broad purposes: (a) growth, (b) reproduction, (c) supply of energy, (d) maintenance and repair, and (e) appetite satisfaction. Nutritionally, milk has been defined as the most nearly perfect food. It provides more essential nutrients in significant amounts than any other single food [5].

Protein is an extremely important class of naturally occurring compound that is essential to all life processes. It performs a variety of functions in living organisms ranging from providing structure to reproduction. Milk proteins represent one of the greatest contributions of milk to human nutrition [5]. Proteins are polymers of amino acids and these proteins are among the most complex of organic substances. They contain carbon, hydrogen, oxygen, nitrogen, sulphur and sometimes phosphorus. They are characterized chiefly by the fact that they contain nitrogen. Protein in indispensable part of the food of animal as it is the chief constituent of the protoplasm which forms the vital part of every living cell. Familiar examples of protein are the white part of an egg and the curd of milk. In milk of average composition, that is, with a fat content of about 3.4 percent, the protein content will be approximately 3 percent. Nearly all samples of normal milk would be between 2.80 and 4.00 percent. The protein of milk is not a single compound but includes two major proteins and small quantities of other. The casein constitutes about 80 percent of the total and lactalbumin of 18 percent. A third protein recognized as present in milk is lactoglobulin. It is present in very small amounts, probably about 0.05 to 0.07 percent. Milk contains a group of nitrogenous substances in addition to the proteins. These substances do not occur in large quantities but they are known to be present. The non-protein nitrogenous substances of milk may be as follows; urea nitrogen, amino nitrogen, creatinine, uric acid, adenine and guanine. Their presence contributes slightly to the protein analysis of milk or milk products of high protein [6].

When milk is freshly drawn from the cow, it shows an amphoteric reaction, that is, it turns red litmus blue, and blue litmus red. Normal fresh milk has a hydrogen-ion concentration of approximately pH 6.5 to 6.6, which indicates that the milk is slightly acidic. When normal fresh milk is titrated with an alkali solution using phenolphthalein as an indicator, it appears acid, showing form 0.10 to 0.26 percent

acid, if it is assumed that the acidity is due to lactic acid. It should be pointed out that perfectly fresh milk contains no lactic acid. The acidity of fresh milk is due to certain constituents of milk some of which give an acid reaction and some of which actually combine with the alkali. The acidity of fresh milk is known to be due to phosphates of milk, the proteins (casein and albumin) and to a slight degree to the presence of carbon dioxide and citrates in milk. The enough free carbon dioxide usually present to account for the acidity equivalent to 0.01 to 0.02 percent lactic acid and acid citrates might account for 0.1 percent acidity in all samples. The acidity due to casein is 0.05 to 0.08 percent. The albumin accounts regularly for a little less than 0.01 percent [7]. It should be clear that the true acidity of milk actually cannot be measured by titrating with a standard alkali solution. The practice of titrating milk with an alkali is followed because it is known that after the acidity of milk reaches a point near 0.18 to 0.20 percent, a large proportion of this acidity is due to lactic acid formed by the action of bacteria on the lactose [6].

Lactose is a disaccharide derived from the condensation of galactose and glucose, which form a β-1→4 glycosidic linkage. Its systematic name is β-D-galactopyranosyl-(1→4)-D-glucose. The glucose can be in either the α-pyranose form or the β-pyranose form, whereas the galactose can only have the β-pyranose form: hence α-lactose and β-lactose refer to the anomeric form of the glucopyranose ring alone**.** Lactose is hydrolysed to glucose and galactose, isomerised in alkaline solution to lactulose, and catalytically hydrogenated to the corresponding polyhydric alcohol, lactitol. The carbohydrate lactose gives milk its sweet taste and contributes about 40% of whole cow's milk's calories [8].



Scheme 1.1: Structure of lactose

In milk, lactose exists in two isomeric forms, called α- and β- lactose respectively. The molecular structures of α- and β -lactose differ in the orientation of a hydrogen- and a hydroxyl group on carbon atom no.1 in the glucose moiety. Both forms change into one another continuously. This phenomenon is called mutarotation. The velocity of mutarotation is determined by factors like temperature, concentration and pH (acidity) of the solution. Lactose solutions strive after a state of equilibrium between the α and β forms. At room temperature the equilibrium results in a ratio of about 40% α-lactose and 60% β-lactose. The fact that two forms of lactose exist which differs in molecular structure has profound effects on various properties of lactose such as crystallization behaviour, crystal morphology, solid state properties and solubility. As the aldehyde group at C-1 position of glucose is free, lactose is known as reducing sugar. Sucrose is made up of α-D-Glucose and β-D- fructose held together by a glycosides bond, between C-1 of α-glucose and C-2 of β-fructose. The reducing groups of glucose and fructose are involved in glycoside bond, so it is a non-reducing sugar [9]. The greater reactivity of lactose, as compared with sucrose, is due to the presence of a potentially free aldehyde group in the glucose portion of the molecule. Milk contains on the average about 4.8 percent of lactose [6]. Processing treatments, with the aim of extending shelf life, have direct influences on the nutritional, biological and functional properties of milk nutrients [10, 11].

Milk is a complex colloidal system in which the dispersion medium, water contains salts and sugar in solution. Therefore milk is heavier than water. The specific gravity of milk is influenced by the relation of its constituents, each of which has a different specific gravity, approximately as follows: fat, 0.93; lactose, 1.666; proteins, 1.346; casein, 1.31; salts, 4.12. Since milk fat is the lightest constituent of milk, the more that is present, the lower will be the specific gravity and in a like manner, the greater the percentage of solids-non-fat, the milk will be heavier. The specific gravity of milk is usually determined with a lactometer. The lactometer is a hydrometer with a scale adapted to the limits of the specific gravity of milk. Any hydrometer works on the principle that a body floating in a liquid sinks to such a level that it displaces a volume of liquid equal in weight to the floating body. In liquid of low specific gravity, the hydrometer sinks further before displacing a great specific gravity.

Cow's milk is ranked first in the world in terms of ideal food. Meanwhile, it is considered one of the main components of the human diet in many parts of the world. It contains all the essential nutrients. Most cows' milk is consumed in the fresh or processed state [12]. To reduce the propensity of milk as a cause of bacterial infection milk is pasteurized. Pasteurization is usually carried out using continuous-flow equipment giving a heat treatment of at least 72℃ for 15 second, which is sufficient to kill all non-sporting pathogens and non-thermoduric organisms; the resistance of spores is one reason why milk needs to be stored at refrigeration temperatures, to prevent the growth of sporting organisms [13]. In pasteurization, milk receives mild heat treatment to reduce the number of bacteria present. UHT stands for ultra-high temperature where the milk has been treated at 130℃ for 1 second and is a method of pasteurization which destroys all bacteria present. The milk is packaged airtight, in sterile boxes and can be stored without refrigeration [14]. Milk has high water content and is an unstable commodity. A number of processes have been developed to reduce the water content and make the product more stable [13]. Powder milk and condensed milk are such type of products. Milk powder is made from cow's whole milk or partly or wholly skimmed milk to which sugar may or may not be added, and which has been evaporated to dryness, either under atmospheric pressure or in vacuum.

The cereal grains are seeds of the grass family. The word cereal is derived from cares, the Roman Goddess of grain. The principal cereal crops are rice, wheat, maize or corn, jowar, ragi and bajra [15].

Cereals are the main sources of energy, contributing 70−80% of the requirement. Hundred grams give more than 340 k.cal of energy. 80% of dry matter of cereals is carbohydrate. The two carbohydrates present in cereal are crude fiber and soluble carbohydrate. Free sugars include simple sugars such as glucose and disaccharides like sucrose and maltose. Of all the cereals, whole wheat, ragi and bark contain high amount of fiber. The protein content of different cereals varies. Rice contains less amount of protein compared to other cereals. The protein content of different varieties of the same cereal also varies. Lipids are present to the extent of 1−2% in wheat and rice, and 3% in maize. More lipids are present in germ and bran than in other parts of the grain. About 95% of minerals are the phosphates and sulphides of potassium, magnesium and calcium. A considerable part of phosphorus in cereals is present in the

form of phytin. Some mineral elements like copper, zinc and manganese are also present in very small quantities in cereals. Cereals are poor sources of calcium and iron, particularly rice is a very poor source of these two elements [15].

A baby's best food is mother's milk, if it is not possible, formula milk is the second choice. Newborns should not take cereal or any other baby foods until they are around six months old. The nutrients are needed for a six months baby until they are best met by mother's milk or formula milk. Human milk contains the right balance of nutrients plus antibodies that help protect the newborn's health. Mother's milk changes as her growing baby's nutritional needs change. Young babies do not get sufficient benefit from solid foods for many reasons. To start, they are not developmentally ready to deal with swallowing solids. Research indicates that children may develop food allergies if solids are introduced too early. Formula and breast milk provide the perfect balance of nutrients that he needs [16].

After around six months, baby is ready for taking solid foods. By that age, he is able to take food in his mouth to swallow and his digestive system becomes accustomed gradually to handle solids. The best first food is usually an iron-fortified rice cereal. It provides iron that is necessary in his diet at this stage [16].

Soon the cereal becomes an important part of his diet. When the baby is well-habituated on rice cereal (mixed with formula or breast milk) then he/she feels easy to take pureed fruits and vegetables. It needs to check after the starting of each new food item. It should be avoided pureed meats until he is at least seven or eight months old, since their protein content may be too high for younger babies. In the early stages of solids, it should be sure that the food is on the runny side. The first foods for babies, other than breast milk or formula, should be cooked fruits and vegetables and mashed bananas. These simple carbohydrates are the easiest foods for baby to digest. The enzymes that break down solid foods develop slowly. It should be start with very simple carbohydrates and gradually introduce more complex carbohydrates and proteins later. The early introduction of grains is associated with later development of allergies and the formation of auto antibodies associated with diabetes. Introducing one new food at a time, it needs to wait two or three days to observe reactions and

then introduce another. Common allergic reactions are a rash around the mouth or anus, runny nose, diarrhoea or fussiness.

The knowledge of nutrition in different types of fruit products especially jams, jellies and juices are very significant due to their good food values as these are made from fruit, water and sugar. Jam, jelly and juice are produced from fruits. These are produced from flowers and flowers are produced from the ripened ovary and ovaries of a plant together with adjacent tissue [17]. Most fruits are made up of an edible portion combined with some refuse. Fruits as a class are valuable, chiefly for their vitamin, mineral content and for their bulk and indigestible fiber. The main energy constituents present in fruits are carbohydrate. Most fruits have only a trace amount of fat, a small amount of protein and water (85%). The major part of the edible portion of fresh fruits consists of water (75−95%) mostly. Fruits are poor sources of protein and oil. The exceptions to these are the olive and also the avocado which may contain as much as 40% oil. Most fruits contain reasonable amount of carbohydrate. The latter may include varying proportions (according to the fruit, maturity, etc.) of dextrose, fructose and sucrose and possibly starch (e.g. banana, apple). The principal acids present in fruits are citric, tartaric and malic acids. The total acidity falls after picking. The pH of fruits varies from 2.5−4.5. Other constituents of fruits include cellulose and woody fibers, mineral salts, pectin, gums, tannins, coloring matters and volatile oils. The main feature of the composition from the nutritional point of view is that certain fruits, particularly blackcurrants, most citrus fruits and strawberries are good sources of vitamin C. Certain specified preservatives are found to be present in bananas, grapes and citrus fruits [18].

Jam processing has been known since the eighteenth century. Barconnot was considered to be the first scientist who noticed the formation of jelly in presence of certain concentration of pectin, sugar, acid and water that had happened in French in 1825 [19]. This industry invented an important method for fruit preservation.

Jam is a food product prepared from cooked fruit or vegetable pulps after removal of stones and seeds with addition of sugar, acid and pectin to make slightly cohesive texture to the extent of total soluble solid not less than 60% and their natural appearance should not be retained.

During the boiling, the proportion of solid matter in the mixture increases (due to the evaporation of water), a proportion of the sucrose is converted to invert sugar and a gel is produced on cooling. It has been shown that three separate components, viz. sugar, pectin and acid, play active function in forming the gel.

There are different types of jam which differ from each other in the raw material used, processing method and additives.

According to the texture jam can be classified into solid, semi solid and liquid.

Jelly is a clear sweet soft fruit-flavoured food that is prepared after the boiling of the fruit with water and then the extract (after filtration) is boiled with the sugar. Artificial coloring matters and preservatives are added to certain varieties of jam and jelly [18].

However every human being requires food for their living as well as for the production of necessary energy like all other animals. Different food contains different proportion of minerals, proteins, carbohydrates and fat. Most of our people are suffering from malnutrition because they are in need of adequate nutritious food. Fruit is occupying a larger proportion of daily food item of modern civilized nation certainly due to their great food values. Jams, jellies and juices also have good food value as these are made from fruit, water and sugar. Jams, jellies and juices provide more essential nutrients in significant amounts as well as some heavy metals.

Minerals are the building blocker of our body. They are required for body structure, fluid balance, and protein structures and to produce hormones. They are keys for the body system and function. They act as co-factors, catalysts or inhibitors of all enzymes in the body. Copper and iron, for example, along with other minerals are required for the electron transport system, and thus needed for all cellular energy production [20].

Minerals are classified into four groups: the macro minerals, or those needed in large quantity, include calcium, magnesium, sodium, potassium, sulphur, iron, copper and zinc. Required trace minerals include magnesium, chromium, selenium, boron, bromine, silicone, iodine, vanadium, lithium, molybdenum, cobalt, germanium, and others up to certain level. Possible required trace minerals include fluorine, rubidium, tin, strontium, gold, silver, and nickel.

Toxic metals include beryllium, aluminium, mercury, lead, cadmium, antimony, bismuth, barium, uranium and others.

Heavy metals are described as those metals which in their standard state, have a specific gravity (density) of more than about 5 g/cm^3 (IOCCC,1969). Heavy metal pollution is a result of increasing industrialization throughout the world, which has penetrated into all sectors of the food industry. The U.S. Environmental Protection Agency for Toxic Substances and Disease Registry (ATSDR) has compiled a priority list in 2001 called the top 20 hazardous substances. The heavy metals arsenic, lead, mercury, and cadmium ranked 1st, 2nd, 3rd, and 4th in the list, respectively [4].

With increasing environmental pollution a heavy metal exposure assessment study is necessary [21]. Heavy metals enter human body through inhalation and ingestion. Intake via ingestion depends upon food habits. There is now growing evidence of the importance of trace elements in human nutrition, and there are reports that suggested the trace elements deficiencies can lead to impaired growth during infancy and childhood [22, 23]. Since the neonatal period is one of the most critical with respect to nutrition, there is needful to know the actual intakes of trace elements by fully breast feeding infants during the 1st month postpartum. It is well established that Pb and Cd are toxic and children are more sensitive to these metals than the adults. In recent years this has led scientists to examine the trace elements content of human milk, the ideal infant food during the first month of life, in order to estimate infant requirements and establish reference values for use in manufacturing infant formulas. While Fe, Cu and Zn are essential, they can be toxic when taken in excess; both toxicity and necessity vary from elements to elements [24, 25, 26].

Heavy metals have no function in the body and can be highly toxic. Once liberated into the environment through the air, drinking water, food or countless human made chemicals and products, heavy metals are taken into the body via inhalation, ingestion and skin absorption [27]. If heavy metals enter and accumulate in body tissues faster than the body's detoxification pathways can dispose of them, a gradual buildup of these toxins will occur. High concentration exposure is not necessary to produce a state of toxicity in the body tissues and over time, it can reach toxic concentration levels [28, 29].

Human exposure to heavy metals has risen dramatically in the last 50 years as a result of an exponential increase in the use of heavy metals in industrial processes and products. Toxic elements can induce impairment and dysfunction include the blood and cardiovascular, eliminative pathways (colon, liver, kidneys, and skin), endocrine (hormonal), energy production pathways, enzymatic, gastrointestinal, immune, nervous (central and peripheral), reproductive, and urinary systems [30]. Heavy metals may alter, remove, or impair the production of specific molecules needed in the body. They may alter the structure of various entities such as the mitochondria or a cell nucleus. Heavy metals may create disturbances in the cell to cell communication occurring between inflammatory mediators, nerve cells or hormones. Toxic heavy metals tend to accumulated at the target sites such as membrane or structural proteins, enzymes, or DNA molecules. Once at the target site, they can displace an important mineral from its binding site and pretend to be this mineral. This is called molecular mimicry; however, they cannot perform the mineral's function and so inhibit any activity at the binding site, affecting cellular function [31, 32, 33]. Since arsenic, lead, and mercury ranked as the top three most hazardous substances in the said priority list, the researchers have emphasized much importance formulated study to determine the presence or absence of these toxic heavy metals in selected samples using atomic absorption spectrophotometer (AAS).

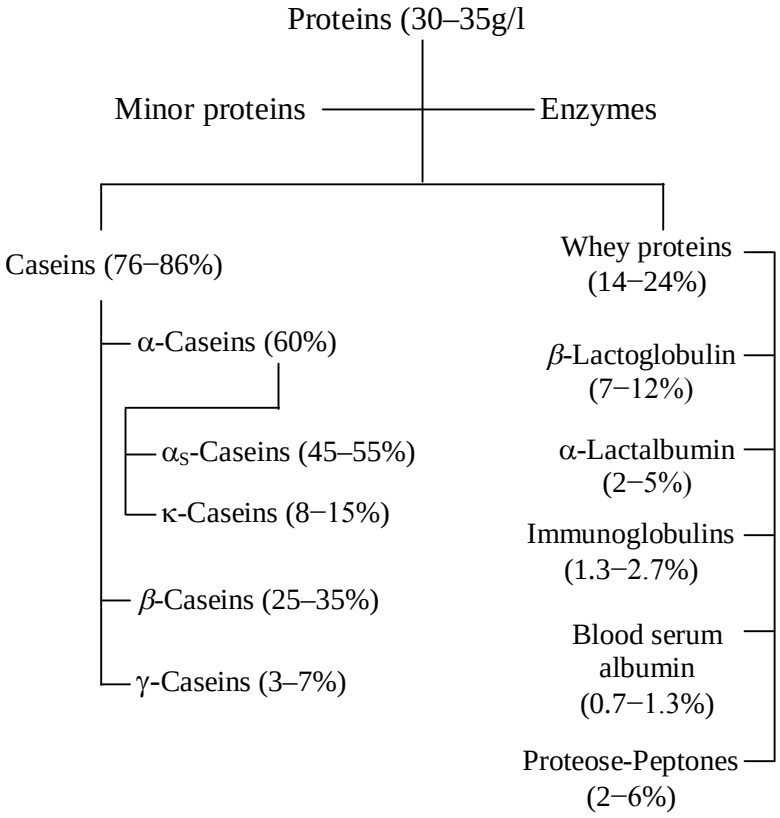
1.2. Milk Proteins

1.2.1 Properties and classification of milk proteins

Proteins are the most valuable components of milk in terms of their importance in human nutrition and their influence on the properties of dairy products containing them. This together with the availability of rapid instrumental methods of measurement has led to increase use of protein as a quality parameter.

Proteins are large molecular weight complex of organic compounds which contain carbon (C), hydrogen (H), oxygen (O), nitrogen (N), sulphur (S), phosphorus (P) and other elements may also be present. Protein molecules are made up of amino acids and they link together via peptide bonds to form long chains [34, 35, 36]. Milk protein and their fractions are shown in Scheme 1.2.

Milk contains 3.3% of total protein. Milk proteins contain all nine essential amino acids required by humans. Total milk protein content and amino acid composition vary with cow breeds and individual animal genetics.



Scheme1.2: Schematic diagram of milk protein with their variety of common fractions

Casein the main fraction is further made up of a number of fractions and is therefore heterogeneous. The whey proteins are also made up of a number of distinct proteins as shown in the Scheme1.2. Milk provides easily digestible proteins of a high nutritional value and is a rich source of essential amino acids. Proteins are the body's building blocks affecting our growth and immunity. Antibodies, enzymes and hormones all contain proteins. Thus the proteins we eat provide the amino acids and enzymes to our body. Body is able to synthesize some amino acids. But our body cannot synthesize nine essential amino acids. Histidine is also considered to be essential for infants.

The essential amino acids have to be supplied through our food. The acid conditions in the stomach untangle proteins laying them open to attack by enzymes called proteases. The broken fragments are then used to provide the body's amino acid requirements. The excess protein of the body is used for energy. Essential amino

acids, their daily requirement, source of milk proteins (in 100 g milk protein) and total amount of liquid milk equivalent for daily requirement of essential amino acids are given in Table 1.1.

Table 1.1: Essential amino acids present in milk proteins and their daily requirements

Amino Acids	Daily Requirement (g)	g/100g milk Protein	Milk for Daily Requirement (g)
Phenylalanine	1.1	5.5	747.63
Methionine	1.1	2.8	1466.66
Leucine	1.1	12.1	414.84
Valine	0.8	7.1	417.09
Lysine	0.8	7.4	570.76
Isoleucine	0.7	6.7	423.82
Tereonine	0.5	4.6	349.57
Tryptophan	0.3	1.4	400.00
Histidine	80.0	2.2	106666.67

1.2.2 Structure of protein

Proteins are biological polymers composed of amino acids. Amino acids linked together by peptide bonds form a polypeptide chain. One or more polypeptide chains twisted into a 3D shape form a protein. Proteins have complex shapes that include various folds, loops, and curves. Folding in proteins happens spontaneously. Chemical bonding between portions of the polypeptide chain aids in holding the protein together and giving its shape. There are two general classes of protein molecules: globular proteins and fibrous proteins. Globular proteins are generally compact, soluble, and spherical in shape. Fibrous proteins are typically elongated and insoluble. Globular and fibrous proteins may exhibit one or more of four types of structure. These structure types are called primary, secondary, tertiary and quaternary [37−41].

1.2.3 Protein structure levels

The four levels of protein structure are distinguished from one another by the degree of complexity in the polypeptide chain. A single protein molecule may contain one or more of the protein structure types.

Primary structure describes the unique order in which amino acids are linked together to form a protein. Proteins are constructed from a set of 20 amino acids. Generally amino acids have the following structural properties:

A carbon (the alpha carbon) bonded to the four groups below:

i) A hydrogen atom (H)

ii) A carboxyl group (−COOH)

iii) An amino group ($-NH_2$)

iv) A variable group or R group

All amino acids have the alpha carbon bonded to a hydrogen atom, carboxyl group, and amino group. The R group varies among amino acids and determines the differences between these protein monomers. The amino acid sequence of a protein is determined by the information found in the cellular genetic code. The order of amino acids in a polypeptide chain is unique and specific to a particular protein. Altering a single amino acid causes a gene mutation which most often results in a non-functioning protein.

Secondary structure refers to the coiling or folding of a polypeptide chain that gives the protein 3D shape. There are two types of secondary structures observed in proteins. One type is the alpha (α) helix structure. This structure resembles a coiled spring and is secured by hydrogen bonding in the polypeptide chain. The second type of secondary structure in proteins is the beta (β) pleated sheet. This structure appears to be folded or pleated and is held together by hydrogen bonding between polypeptide units of the folded chain that lie adjacent to one another.

Tertiary structure refers to the comprehensive 3D structure of the polypeptide chain of a protein. There are several types of bonds and forces that hold a protein in its tertiary structure. Hydrophobic interactions greatly contribute to the folding and shaping of a protein. The R group of the amino acid is either hydrophobic or hydrophilic. The amino acids with hydrophilic R group will seek contact with their aqueous environment while amino acids with hydrophobic R group will seek to avoid water and take position towards the center of the protein. Hydrogen bonding in the polypeptide chain and between amino acid R group helps to stabilize protein structure

by holding the protein in the shape established by the hydrophobic interactions. Due to protein folding, ionic bonding can occur between the positively and negatively charged R group that come in close contact with one another. Folding can also result in covalent bonding between the R group of cysteine amino acids. This type of bonding forms what is called a disulfide bridge. Interactions called van der waals forces also assist in the stabilization of protein structure. These interactions pertain to the attractive and repulsive forces that occur between molecules that become polarized. These forces contribute to occur bonding between molecules.

Quaternary structure refers to the structure of a protein macromolecule formed by interactions between multiple polypeptide chains. Each polypeptide chain is treated as a subunit. Proteins with quaternary structure may consist of more than one of the same type of protein subunit. They may also be composed of different subunits. Hemoglobin is an example of a protein with quaternary structure. Hemoglobin found in the blood is an iron containing protein that binds oxygen molecules. It contains four subunits: two alpha subunits and two beta subunits.

The types and structure of different proteins are shown in Figure 1.1.

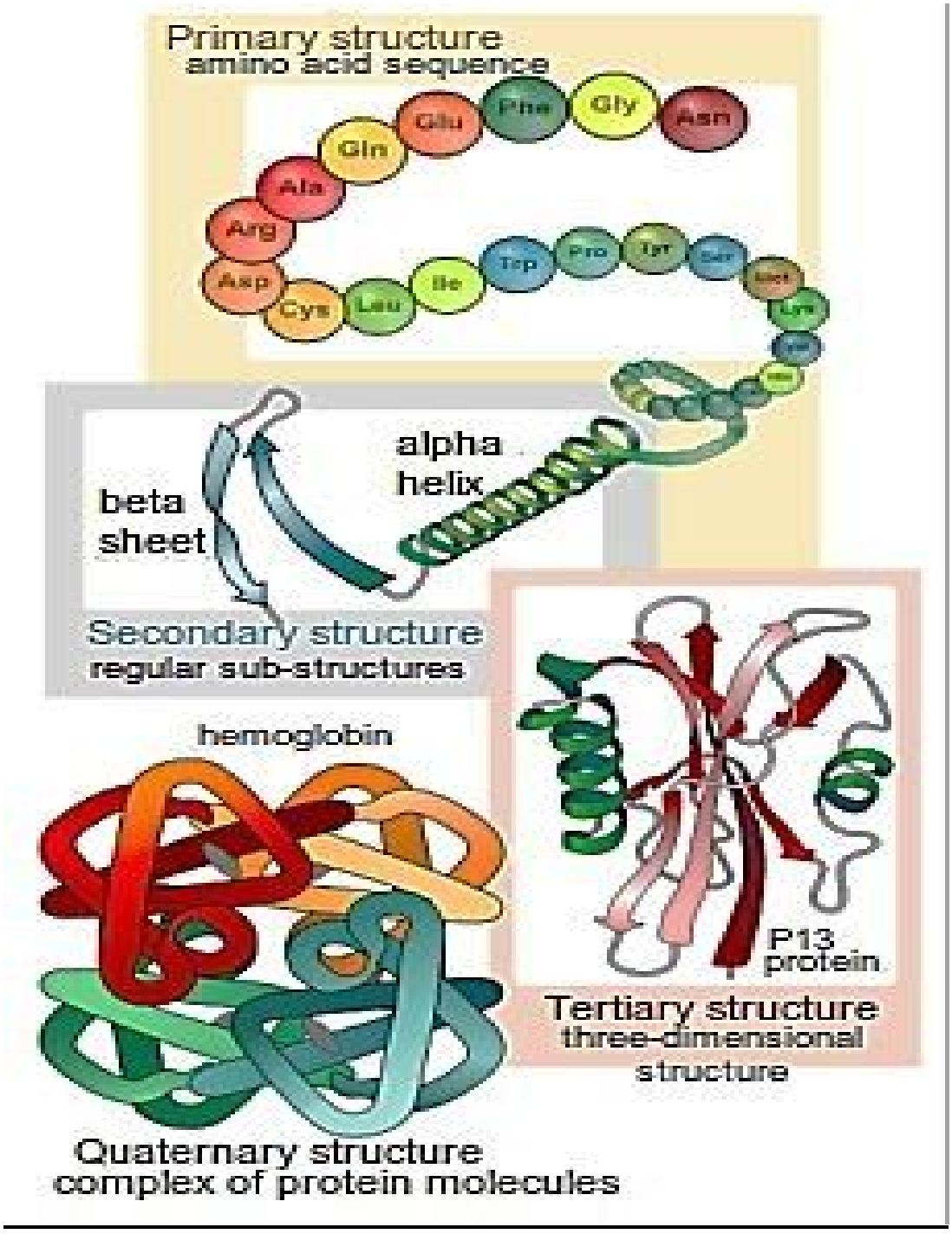


Figure 1.1: Protein structure levels

1.2.4 Role of protein

Protein can be found in animal sources like milk, meat and dairy products or plant sources like beans, nuts and seeds. According to the USDA 10 to 35 percent of our calorie intake should come from protein. Every cell in our body contains protein, so meeting our protein requirement is essential for health [42, 43].

1.2.5 Body tissues and muscles

Protein is necessary in building and repairing body tissues. If we do not receive enough protein in our diet, muscles wasting and other symptoms may result. Exercises like strength training cause micro tears in the muscle and as our body repairs these tears, it causes the muscles to enlarge. Protein is necessary to the immune response that helps to heal the tiny muscle tears. However, consuming extra protein will not help our body to build extra muscle.

1.2.6 Hormones

Hormones are chemicals produced by glands in one part of the body that help coordinate activities and communicate with other areas. Protein hormones bind to receptors on the cell membrane instead of entering the cell directly. Hormonal proteins like insulin and oxytocin play vital roles like controlling blood sugar concentration and stimulating contractions during childbirth. Hormones can also activate muscle growth by increasing protein synthesis or decreasing protein breakdown [44].

1.2.7 Enzymes

Enzymes are proteins that bind to molecules to speed up chemical reactions. They play a role in many activities such as muscle contraction and relaxation and nerve impulse transmissions. Amylase and lipase are enzymes that help to digest carbohydrates and fat. The enzyme ATPase exports cell toxins and is essential for breaking down adenosine triphosphate (ATP) which releases energy [45].

1.2.8 Immune system

Antibodies are specialized protein configurations which provide a specific immune defense against invaders. They are produced by the body once it's exposed to specific antigens such as bacteria, viruses and fungi. Complement proteins support the immune system as a second line of defense. They can create holes in bacterial walls, promote inflammation which attracts macro phases that destroy invading organisms and attach to the foreign substances.

1.2.9 Energy

Protein is broken down into amino acids during digestion and provides four calories per gram. Protein with meals can help to feel more satiated and can keep fuller longer. Although protein can be used as a source of energy. The body's main energy source is carbohydrates. Eating a diet with lean proteins like beans and fish, complex carbohydrates like whole grains and vegetables and healthy fats such as olive oil and avocados is the best way to supply the energy in the body.

1.3 Milk Carbohydrate Chemistry

Milk contains approximately 4.9% carbohydrate that is predominately lactose with trace amounts of monosaccharide and oligosaccharides.

1.3.1 Lactose

Lactose is the major carbohydrate in the milk of most species. It is a disaccharide that composed of monosaccharides D-glucose and D-galactose joined in a β-1, 4-glycosidic linkage.The chemical name for lactose is β-D-galactopyranosyl-(1→4)-D-glucose. It is essentially unique milk sugar-it much less sweet than table sugar and occurs naturally in milk and other dairy products. While one can certainly burn the chemical components of lactose for energy, it doesn't serve unique cellular functions and can burn other carbohydrates instead with no ill effect whatsoever.

1.3.2 Physical properties of lactose

Lactose is dissolved in the serum phase of fluid milk. Dissolved lactose in solution is found in two forms, called the α-anomer and β-anomer that can convert back and forth between each other. The solubility of the two anomers is temperature dependent and therefore the equilibrium concentration of the two forms will be different at different temperatures. At room temperature (e.g., 20°C) the equilibrium ratio of α and β-lactose is approximately 37% and 63% respectively. At temperatures above 93.5°C the β-anomer is less soluble. So there is a higher ratio of α- to β-lactose. The type of anomer present does not affect the nutritional properties of lactose. Lactose crystallization occurs when the concentration of lactose exceeds its solubility. The physical properties of lactose crystals are dependent on the crystal type and can greatly influence their use in foods. Temperature affects the equilibrium ratio of the

α- and β-lactose anomers as described above. Lactose crystals formed at temperatures below 20 °C are mainly α-lactose crystals. The α-monohydrate lactose crystals are in very hard form, for example, when ice-cream goes through numerous warming and freezing cycles. This results in an undesirable gritty, sandy texture in the ice-cream. Gums are often used in ice-cream to inhibit lactose crystallization. The crystal form of ß-lactose is sweeter and more soluble than the α-monohydrate lactose and may be preferred in some bakery applications. When a lactose solution is rapidly dried it does not have time to crystallize and forms a type of glass.

1.3.3 Structure of lactose

Milk sugar commonly designated by the chemist as "lactose" is found only in milk. It is a reducing disaccharide which upon hydrolysis yields one molecule of glucose and one molecule of galactose. The carbohydrate lactose gives milk its sweet taste and contributes about 40% of whole cow's milk's calories [46, 8]. It has the same molecular formula ($C_{12}H_{22}O_{11}$) as ordinary cane sugar or sucrose. It differs from sucrose in molecular configuration, relative sweetness, solubility and chemical reactivity [Structure of lactose: Scheme 1.1].

Lactose is made up of β-D-galactose and β-D-glucose held together by β (1 → 4) glycosidic bond. As the aldehyde group at C-l position of glucose is free, lactose is known as reducing sugar. Sucrose is made up of α-D-glucose and β-D-fructose held together by a glycosidic bond between C_1 of α-glucose and C_2 of β-fructose. The reducing groups of glucose and fructose are involved in glycosidic bond. So it is a non-reducing sugar [47−50].

Sucrose is about six times as sweet as lactose. Lactose is in true solution in the milk serum. Prolonged heating of aqueous solutions of lactose at temperatures from 100 °C to l30 °C results in a decomposition which is indicated by a light-brown or caramel color. In heat-treated milk in the presence of proteins and certain mineral salts, brown color developed that quite readily gives rise to the browning of the sterilized milk and of certain condensed and dried milk products during storage. The greater reactivity of lactose as compared with sucrose is due to the presence of a potentially free aldehyde group in the glucose portion of the molecule. Milk contains on the average about 4.8 percent of lactose [6].

1.3.4 Role of lactose

In addition to burning the components of lactose into glucose and galactose for immediate energy, we can use them to make one of two energy-storage molecules. Our cells can make glycogen from both glucose and galactose where glycogen is a form of carbohydrate storage used by the liver and muscles. This provides a supply of energy for periods of fasting. We can also convert both glucose and galactose into fat for energy storage purposes. When lactose reaches the digestive system the lactase enzyme breaks down lactose into glucose and galactose. The liver then changes the galactose into glucose. If this process occurs normally the glucose enters the bloodstream and raises the glucose level of blood.

1.4 Sources of Trace and Toxic Metals

1.4.1 Sources of zinc

The best and most abundant natural food source of zinc is oysters. Zinc is found in most animal proteins such as beef, pork and poultry. Other food sources of zinc include beans, nuts, whole grains, meat, eggs, seafood, black-eyed peas, tofu, and wheat germ, pumpkin seeds and sunflower seeds.

1.4.2 Sources of cobalt

Small amounts of cobalt are naturally found in most rocks, soil, water, plants, and animals, typically in small amounts. Cobalt is also found in meteorites. Elemental cobalt is a hard, silvery grey metal. However, cobalt is usually found in the environment combined with other elements such as oxygen, sulfur, and arsenic. Small amounts of these chemical compounds can be found in rocks, soil, plants, and animals, cobalt is even found in water in dissolved or ionic form, typically in small amounts.

1.4.3 Sources of lead

Lead has several oxidation states, but only the compounds of Pb(II) are responsible for toxic effects. The probable sources of lead exposure into the environment include the disposed storage batteries, bone meal, canned fruits, ceramic glazes, cigarette smoke and ash, utensils, auto exhaust, lead gasoline, hair dyes, insecticides, lead

crystal dishes and glassware, refineries, smelters, water pipes, lipstick, mascara, lead-based paints, PVC containers, rainwater, snow, solder, tobacco, toothpaste, toys, wine, water and more [51].

1.4.4 Sources of cadmium

The man-male sources of cadmium pollution are dental alloys, batteries, cadmium vapor lamps, candy, ceramics, cereals and grains (refined), cigarette smoke, coffee and tea, colas, electroplating, fertilizers, fungicides, incineration of tires, rubber, plastics, marijuana, ocean fish, oysters, paint pigments, pesticides, pipes (galvanized), polyvinyl plastic, processed foods, rubber carpet backing, rust-proofing, silver polish, solders (canned foods), tools, vending machine soft drinks, welding material, water (municipal, softened & well), and more.

1.4.5 Sources of chromium

Chromium is a naturally occurring element found in rocks, animals, soil and in volcanic dust and gasses. Among several different forms, the most common are trivalent chromium Cr(III) and hexavalent chromium Cr(VI). Chromium is steelgrey, lustrous and hard and is used on a large scale in the metallurgical and chemical industries. The metallurgical industry commonly uses chromium for the production of stainless steels, alloy cast iron and nonferrous alloys as well as for plating steel. In the chemical industry, chromium is used primarily in pigments {Cr(VI) and Cr(III)}, metal finishing and electroplating as well as in wood preservatives {Cr(VI) only} and leather tanning {Cr(III) only}. In the past, chromium was also used in cooling towers as a rust and corrosion inhibitor and as a fungicide. Chromium comes to the environment through these products [52, 53, 54].

1.4.6 Sources of arsenic

Arsenic has +III and +V oxidation states. While As(V) is, in some cases, essential for health As(III) compounds, such as arsenic trioxide, arsenates, arsine etc. are poisonous for human bodies. These compounds come from coal burning in industries and brick fields, groundwater, leaching and research laboratories, wood preservatives and medicines and pollute environment. Arsenic enters into the human body mainly with drinking water and smoking, and accumulates in liver, muscle, hair, nail and skin [51].

1.4.7 Sources of nickel

Metallic nickel Ni(0) itself as well as its compound such as $Ni(CO)_4$ are found to be enemy for health. The main sources of metallic nickel are electronic goods, coins, batteries, jewelries, butter, fertilizers, food processing, fuel oil combustion, hydrogenated fats and oils, imitation whipped cream, industrial waste, kelp, margarine, nuclear device testing, oysters, stainless steel cookware, tea, tobacco smoke, unrefined grains and cereals, vegetable shortening [51].

1.5 Role of Zinc and Cobalt (Trace Metal) in Human Body

Zinc is present in all body tissues and fluids. The total zinc content in the body has been estimated to be 30 mmol (2 g). Skeletal muscle accounts for approximately 60 percent of the total body content and bone mass, with a zinc concentration of 1.5−3 mmol/g (100−200 mg/g), for approximately 30 percent. Zinc concentration of lean body mass is approximately 0.46 mmol/g. Plasma zinc has a rapid turnover rate and it represents only about 0.1 percent of total body zinc content. This level appears to be under close homeostatic control. High concentrations of zinc are found in the choroid of the eye 4.2 mmol/g and in prostatic fluids 300−500 mg/L [55].

Zinc is an essential component of a large number (>300) of enzymes participating in the synthesis and degradation of carbohydrates, lipids, proteins, and nucleic acids as well as in the metabolism of other micronutrients. Zinc stabilizes the molecular structure of cellular components and membranes and contributes in this way to the maintenance of cell and organ integrity. Furthermore, zinc has an essential role in polynucleotide transcription and thus in the process of genetic expression. Its involvement in such fundamental activities probably accounts for the essentiality of zinc for all life forms. It plays a central role in the immune system, affecting a number of aspects of cellular and humeral immunity [56]. The role of zinc in immunity was reviewed extensively by Shankar *et al* [56].

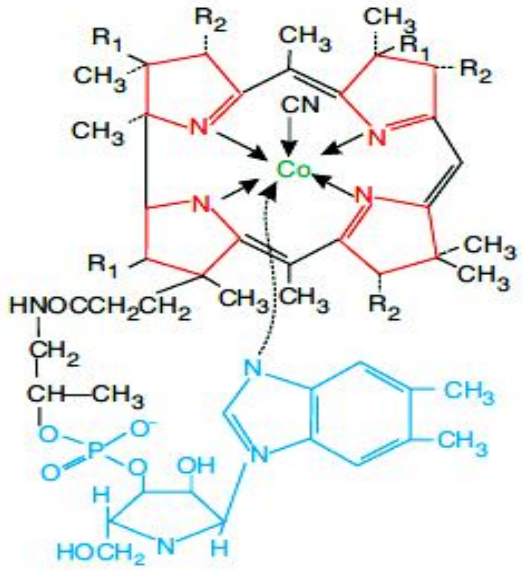
The clinical features of severe zinc deficiency in humans are growth retardation, delayed sexual and bone maturation, skin lesions, diarrhoea, alopecia, impaired appetite, increased susceptibility to infections mediated via defects in the immune system, and the appearance of behavioral changes [48]. The effects of marginal or

mild zinc deficiency are less clear. A reduced growth rate and impairments of immune defense are so far the only clearly demonstrated signs of mild zinc deficiency in humans. Other effects, such as impaired taste and wound healing, which have been claimed to result from a low zinc intake, are less consistently observed.

Cobalt has both beneficial and harmful effects on human health. Cobalt is beneficial for humans as it is part of vitamin B_{12}, which is essential to maintain human health. Cobalt (0.16−1.0 mg cobalt/kg of body weight) has also been used as a treatment for the anemia for the pregnant women, because it causes red blood cells to be produced. Cobalt also increases red blood cell production in healthy people, but only at very high exposure levels. It is also essential for the health of various animals, such as cattle and sheep. Exposure of humans and animals to levels of cobalt normally found in the environment is not harmful. When too much cobalt is taken into our body, however, harmful health effects can occur. Workers who breathed air containing 0.038 mg cobalt/m^3 (about1,00,000 times the concentration normally found in ambient air) for 6 hrs had trouble breathing. Serious effects on the lungs, including abstinent, pneumonia, and wheezing, have been found in people exposed to 0.005 mg cobalt/m^3 while working with hard metal, a cobalt-tungsten carbide alloy. People exposed to 0.007 mg cobalt/m^3 at work have also developed allergies to cobalt that resulted in asthma and skin rashes. The general public, however, is not likely to be exposed to the same type or amount of cobalt dust that caused these effects in workers. In the 1960s, some breweries added cobalt salts to beer to stabilize the foam (resulting in exposures of 0.04−0.14 mg cobalt/kg). Some people who drank excessive amounts of beer (8−25 pints/day) experienced serious effects on the heart. In some cases, these effects resulted in death. Nausea and vomiting were usually reported before the effects on the heart were noticed. Cobalt is no longer added to beer so you will not be exposed from this source. The effects on the heart, however, may have also been due to the beer-drinkers had protein-poor diets and may have already had heart damage from alcohol abuse. Effects on the heart were not seen, however, in people with anemia treated with up to 1 mg cobalt/kg, or in pregnant women with anemia treated with 0.6 mg cobalt/kg. Effects on the thyroid were found in people exposed to 0.5 mg cobalt/kg for a few weeks.

1.6 Structure of Vitamin B_{12}

Vitamin B_{12} is the only known biomolecule with a stable carbon-metal bond. It is an organometallic compound. The core of the molecule is a corrin ring with various attached side groups. The ring consists of 4 pyrrole subunits, joined on opposite sides by a C−methylene link, on one side by a C−H methylene link, and with the two of the pyrroles joined directly. It is thus like a porphyrin, but with one of the bridging methylene groups removed. The nitrogen of each pyrrole is coordinated to the central cobalt atom. The sixth ligand below the ring is a nitrogen of a 5, 6-dimethylbenzimidazole. The other nitrogen is linked to a five-carbon sugar, which in turn connects to a phosphate group, and thence back into the corrin ring. The base ligand thus forms a 'strap' back into the corrin ring.



Scheme 1.3: Structure of vitamin B_{12}.

1.7 Effect of Toxic Metals in Human Body

Lead and lead compounds can be highly toxic when eaten or inhaled. The four major target organs and systems are the central nervous system, the peripheral nerves, the kidney and the hematopoietic system. In all four cases the effects have been observed in man and have been studied extensively.

There are numerous reports often in fatal condition commonly referred to as lead encephalopathy, occurring as a result of chronic or subchronic exposure to high doses of inorganic lead. The major features are dullness, restlessness, irritability headache,

muscular tremor, ataxia and loss of memory. A high incidence of residual damage is seen, including epilepsy, hydrocephalus and idiocy. A major concern today is subtle behavioral effect particularly in children at levels of exposure below those causing encephalopathy. Epidemiologic studies suggest that only moderately elevated lead exposure in infants and young children may cause deficits as reflected in psychometric performance tests and in certain neurologic tests [57].

The major manifestation of lead palsy is weakness of the extensor muscles. Sensory disturbances also occur, e.g. hyperesthesia and analgesia. Functionally, nerve conduction velocity is slowed, even in the absence of palsy, an effect seen in both children and adults even with no discernible impairment of myoneural function [58, 59].

Two distinct types of renal effect have been observed in man. In the first type, the effects are manifestation of damage to the proximal tubules. Tubular reabsorption of glucose, amino acids and phosphate is depressed. These effects are readily reversible with chelation therapy. The other type of renal effect occurs with prolonged high lead exposure. It is a progressive disease characterized by interstitial fibrosis, sclerosis of vessels and glomerular atrophy. Death may ensue due to renal failure.

It has long been known that anemia is one of the early manifestations of lead poisoning. It results from reduction of the lifespan of circulating erythrocytes as well as from inhibition of synthesis of hemoglobin. The shortened lifespan of erythrocytes is inconstant, occurring only in some cases of lead induced anemia. Erythrocytes exposed to lead in vitro show increased osmotic resistance but also show increased mechanical fragility. In addition it has been shown in vitro that, even in moderate lead exposure, erythrocyte Na-K-ATPase is somewhat inhibited, suggesting a loss of cell membrane integrity. This may account for the shortened lifespan of erythrocytes that sometimes occurs [60].

Cadmium metal and some of its compounds as carcinogens have been cited by the International Agency for Research on Cancer lists [61]. Recent studies have suggested that overall nutritional status is a more important determinant of cadmium uptake into the body than is the actual amount of cadmium ingested. For example, women subsisting upon a vegetarian diet and with reduced iron stores have increased uptake

of ingested cadmium. For these women, iron deficiency is a more important determinant of cadmium uptake than is the actual amount of cadmium ingested [62]. Acute poisoning by inhalation may lead to respiratory manifestations such as severe bronchial and pulmonary irritation, sub-acute pneumonitis, lung emphysema and in the most severe situations, death from pulmonary edema may occur [63]. Chronic obstructive airway disease has been associated with long-term high-level occupational exposure by inhalation. For chronic cadmium exposure, effects occur mainly on the kidneys, lungs and bones. A relationship has been established between cadmium air exposure and proteinuria (an increase in the presence of low molecular weight proteins in the urine and an indication of kidney dysfunction) [64, 65]. Recent work [66] has demonstrated that these effects are reversible at low exposure levels once the cadmium exposure has been removed or reduced.

Nutritionally, Cr(III) is an essential component of a balanced human and animal diet for preventing adverse effects in the metabolism of glucose and lipids (e.g. impaired glucose tolerance, elevated fasting insulin, elevated cholesterol and triglycerides, and hypoglycemic symptoms) [67,68]. This was identified as the active component of a molecule called the glucose tolerance factor (GTF) [69]. GTF acts as a cofactor to bind insulin to receptor sites on membranes and therefore improves the efficacy of insulin [70]. Although Cr(III) in small amounts is an important nutrient needed by the body, swallowing large amounts of Cr(III) may also cause health problems, e.g**.,** lung cancer [53, 71]. Hexavalent Cr compounds have been considered to be 10−100 times more toxic than Cr(III) compounds [70]. The LD_{50} for oral toxicity in rats is reported to be 1900 to 3300 mg/kg for Cr(III) and from 50 to 100 mg/kg for Cr(VI) [72]. This may be due to the fact that Cr(VI) penetrates mammalian cells more readily than Cr(III) [73]. Skin contact of Cr(VI) compounds can induce skin allergies, dermatitis, dermal necrosis and dermal corrosion [74]. The mechanism of cancer formation caused by Cr(VI) is not known for certain; however, it has been postulated that Cr(VI) binds to double stranded deoxyribonucleic acid (DNA), therefore altering gene replication, repair and duplication [75].

Toxic effects of arsenic largely depend on its chemical and physical form and how it is exposed. Compounds of arsenic may be absorbed after ingestion or by inhalation. It has been shown in some instances, however, that the arsenate is reabsorbed by the

proximal renal tubule and excreted as the arsenate [76]. Arsenates bind to tissue proteins and are concentrated in the leukocytes. They accumulate in the body primarily in the liver, muscles, hair, nails and skin, perhaps because of combination with sulfhydryl groups excreation via the bile duct. In man, the symptom of acute inorganic arsenic poisoning occurring as a consequence of accidental or homicidal ingestion consist of burning and dryness of the oral and nasal cavities, gastrointestinal disturbance and muscle spasms. Vertigo, delirium and coma may occur. Increased arsenic content of hair, nails and urine is frequently present for long periods after exposure has been discontinued [77]. Arsenic neuropathy is a recognized complication of As toxicity. Peripheral neuropathy (an abnormal and usually degenerative state of the peripheral nerves) due to chronic As exposure is one of the most common complications of the nervous system. The neuropathy is a usually sensor (affects sensation) and the cares of development is chronic. Patients can suffer from constant pain, hypersensitivity to stimuli, muscle weakness or atrophy [78, 79]. Sensory and sensorimotor (sensation and muscles are affected) neuropathy have also been observed [80]. The International Agency for Research on Cancer (IARC) has listed arsenic as human carcinogen since 1980 [81]. Arsenic is a unique carcinogen. It is the only known human carcinogen for which there is adequate evidence of carcinogenic risk by both inhalation and ingestion [82]. A significant relationship between As exposure and skin cancer has been observed. Arsenate can play a role in the enhancement of UV-induced skin cancers [83]. The mechanism of action may involve effects on DNA methylation and DNA repair. Epidemiological evidence indicates that As is associated with cancers of skin and internal organs, as with vascular disease [84]. Mortality from lung cancer was significantly increased with increasing As ingestion [85].

The health effects are highly dependent on the manner and degree of exposure and on the exact chemical `species' in which nickel atoms are present. Nickel is essential in small quantities, but when the uptake is too high it can be a danger to human health. Appropriate amount of nickel on human body play an important role in regulating prolactin and stabilization of RNA and DNA structures [86]. An uptake of too large quantities of nickel has the following consequences [87].

- Higher chances of development of lung cancer, nose cancer, larynx cancer and prostate cancer.
- Sickness and dizziness after exposure to nickel gas.
- Lung embolism.
- Respiratory failure
- Birth defect
- Asthma and chronic bronchitis
- Allergic reactions such as rashes, mainly from jeweler
- Heart disorders

The dermatitis is a sensitization reaction and contact may, in some cases, produce paroxysmal asthmatic attacks and pulmonary eosinophilia [88].

Nickel carbonyl is the most toxic compound following acute exposure. The symptoms of acute exposure to nickel carbonyl occur in two stages, immediate and delayed [89, 90]. The immediate toxic effects of nickel carbonyl exposure are respiratory tract irritating and neurological symptoms include dizziness, frontal headache, nausea, vomiting, irritability and upper airway irritation [91, 92]. Following the immediate symptoms there is an asymptomatic period before the onset of the delayed pulmonary symptoms, similar to those of a viral pneumonia [81, 82]. Symptoms include chest pain, cough, dyspnoea, tachycardia, weakness and fever with leukocytosis. Pulmonary haemorrhage, cerebral oedema, toxic myocarditis, pulmonary oedema and pneumonitis may occur in severe cases [89, 91]. Neurasthenic syndrome and weakness may develop following a severe exposure to nickel carbonyl and may persist for upto 6 months [91].

The respiratory tract is the primary site of toxicity following inhalation of nickel and its compounds. Rhinitis, sinusitis, asthma, chronic bronchitis, emphysema and nasal septal perforations have frequently been reported in individuals occupationally exposed to nickel or nickel compounds. Hyposmia or anosmia was also noted in many of the workers with sinusitis. Pulmonary changes with fibrosis were also observed in workers exposed to nickel dust or fumes [89, 92].

The IARC concluded that there is sufficient evidence in humans for the carcinogenicity of nickel sulphate and of the combinations of nickel sulphides and

oxides encountered in the nickel refining industry. Nickel compounds are classified as carcinogenic to humans [93]. There was limited evidence for carcinogenicity of nickel alloys in animals. IARC did not give any overall classification for nickel alloys [93].

1.8 Function of Sodium, Potassium, Calcium and Magnesium in Biological System

Sodium mainly works as a principal caution in extracellular fluid. It regulates plasma volume, acid-base balance, nerve and muscle function, Na^+/K^+ ATPase and organizes membrane transport systems in animal tissues or plant tissues.

Sodium accounts for 90% of the basic ions in the extracellular fluids and helps to maintain body neutrality by contacting the effect of the acid-forming elements. When as excess of acid- forming elements appears in the body fluid, sodium can be released from the sodium reserves in the bone to offset the acid. One of the major causes of alkalosis, or an excess of base-forming elements, is the ingestion of sodium-containing antacid preparations.

In the transmission of nerve impulses a change in the permeability of the nerve cell membrane allows sodium to enter and for a temporary period this changes the electrical charge on the membrane. This charge travels down the nerve fiber as a nerve impulse, or message. If the valance between sodium outside and inside the cell were upset, this transmission of nerve impulses could not occur. Similarly, the contraction of muscles involves a temporary exchange of sodium and potassium in the contracting muscle cell. Sodium is also essential for the absorption of glucose and in the transport of other nutrients across membrane [94−100].

Within the cell potassium acts as a catalyst in many biological reactions, especially those involved in the released energy and in glycogen and protein synthesis. If the sodium level increases in the intracellular material, it may counteract the catalytic effect of potassium and may interfere with cellular metabolism, especially protein synthesis. Potassium is a major factor in maintaining the osmotic pressure of the cell essential to the regulation of fluid balance. Its presence within the cell is important in the maintenance of acid-base balance, although it is not as readily mobilized as sodium to be a reserve base in offsetting an excess of acid-forming elements. It also

plays an important role in transmission of nerve impulses and in the release of insulin from pancreas.

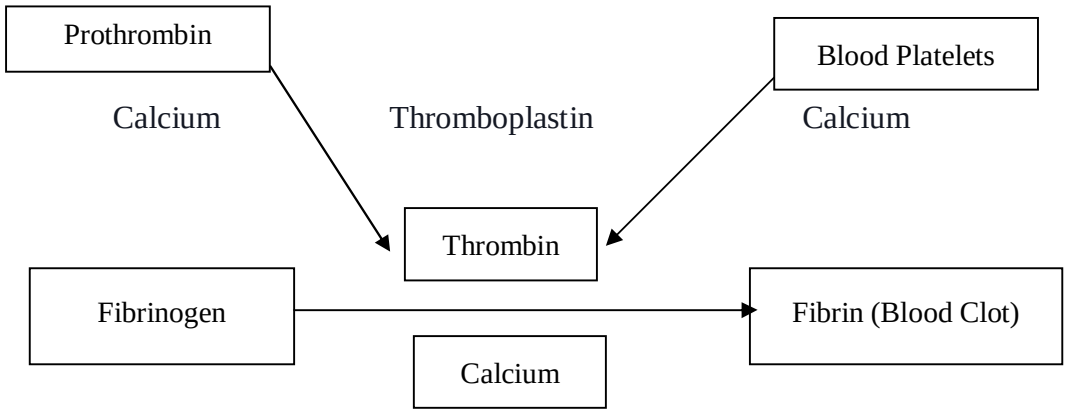
Potassium is not known to be a constituent of important metabolites such as protein, chlorophyll, fats and carbohydrates etc. That is why it is difficult to assign its particular role. It is frequently found in all the parts of plant but fairly large proportion in growing points. It is considered that potassium is present in soluble form and most of it seems to be contained in the cell sap and cytoplasm. It is readily mobile within the plant tissues. It's utilization in plant is concerned with the formation of carbohydrate and proteins, photosynthesis, transpiration regulation, enzyme action, syntheses of nucleic acid and chlorophyll, oxidative and photophosphorylation, translocation of solute etc. The element potassium is believed to be related in some manner, probably catalytically, to the formation of sugar and starch, although the evidence is not very clear [101−106].

Calcium is one of the few essential elements entering into the frame work of the plant. Combining with peptic acid it forms calcium pectate, a constituent of the middle lamella. No new cell walls are laid down when calcium becomes limiting, although other manifestations of cell decision, including nuclear division, may take place. The essentiality of calcium for microorganisms has not been definitely established. It has been suggested that calcium has some role in sugar transport or is directly concerned in starch sugar changes. However, growth is greatly retarded in the absence of calcium and the reduction of growth from many causes results in starch accumulation. It is probable, therefore, that the supposed influence of calcium on translocation is an indirect one, calcium has important roles in connection with permeability and antagonistic action.

Calcium is a relatively inert inorganic mineral element which usually associates with bone and tooth formation. The use of term calcification to describe the process by which these structures assume strength and rigidity has tended to reinforce the importance of calcium in bone formation. Calcium does play an important role in this process, but it is only one of many nutrients necessary for effective bone and tooth formation. Teeth don't have the ability to repair themselves once they have erupted, there is no further need for a dietary source of calcium to maintain or repair as a

weakness in structure with increase susceptibility to teeth decay even through the teeth appear normal histological. As in the case of one the integrity of tooth structure involves nutrients in addition to calcium [108].

The role of calcium in the blood clotting mechanism is one of the more clearly, representing over half the total blood, calcium stimulates the release of a phospholipids, thromboplastin, from the blood platelets. Thromboplastin in turn catalyzes the conversion of prothrombin, a normal blood constituent, to thrombin.



Scheme 1.4: Schematic representations of blood clotting mechanism

Thrombin then aids in the polymerization fibrinogen to fibrin the clot. A schematic representation of the blood-clotting mechanism (Scheme 1.4) shows that calcium must be present at each step in the series of changes needed for the formation of the clot. Under normal conditions blood calcium levels are maintained at a level sufficiently high to facilitate the blood-clotting process, therefore an increase in dietary calcium will have little direct effect on blood clotting time [112].

Calcium occurs in the cell membrane closely bound to the phospholipids lecithin. Here it governs the permeability of the cell membrane to various nutrients and thus controls the uptake of nutrients the cell [107−112].

Magnesium is the second most common intracellular caution. It has dozens of biological functions. It has a vital role in practically all major metabolic pathways of the new tissues and other essential biomolecules required by the body [113]. Magnesium activates many enzymes in reactions requiring ATP, and especially those, which bring about the linking of phosphate groups to glucose in the formation and

breakdown of glycogen and release of energy. Magnesium is also necessary of regulation of temperature of the body [113].

Evidence accumulates that magnesium has an anticancer effect. Magnesium deficiency leads to the impairment of calcium and potassium homeostasis. Hypomagnesaemia in diabetes may be one of the risk factors in the development of diabetic retinopathy. Magnesium may be a physiologic calcium channel antagonist.

1.9 Literature Review

Khier, *et al.* (2009) carried out some analysis to assess the quality of powder milk packed in Sudan. Physicochemical, microbiological and sensory characteristics of milk powders packed in Sudan were investigated and compared with international quality standards. The compositions (moisture, fat, protein, ash and lactose) of the locally packed milk powders were almost insignificantly different. Despite the significant variations in acidity and pH in milk powder samples their levels remain within the acceptable standard levels [12].

Ibtisam, *et al*. (2009) analyzed the effect of pasteurization of milk on the keeping quality of fermented camel milk in Sudan. The processed Garris samples from non-pasteurized and pasteurized camel milk showed mean values for fat content of 3.0± 0.445% and 3.0± 0.076% respectively. The protein contents were found as 3.1± .14% and 3.2± 0.311%, the ash values were 0.64± 0.108 and 0.71± 0.067% and the total solids were 9.6± 0.445% and 10.0±0.801%, respectively [114].

Imran, *et al*. (2008) worked on the physicochemical characteristics of various milk samples available in Pakistan. Milk samples were analyzed for their physical features including moisture, total solids, specific gravity, conductivity, viscosity and titratable acidity (lactic acid equivalent) and chemical components and macrominerals including total protein, casein, lactose, ash and minerals (Na, K and Mg). These items were compared with the physicochemical characteristics of the fresh natural milk samples from buffalo, cow and goat. All the physical features and chemical components of commercially available milk in Pakistan markets meet WHO's requirements except for Na, K, Ca and Mg which are below the standards [115].

Fabro, *et al.* (2006) determined acidity in whole raw milk by two different analytical methods and compared their results. In Argentina, one analytical method was usually

carried out to determine the acidity in whole raw milk in the Institute of National de Racionalization de Materiales standard (no. 14005) based on the Dornic method of French origin. In a national and international regulation the Association of Official Analytical Chemists International method (no. 947.05) was proposed as the standard method of analysis. Although the foundation of these methods was same and there was no evidence of equivalent results obtained using the 2 methods. The statistical study was performed to verify the equivalence of the obtained results. The existence of significant differences between the results obtained by both the methods was determined [116].

Kittivachra, *et al.* (2006) determined the amount of essential nutrients in raw milk. This study was to assess the composition of raw milk produced in Thailand which included fat, protein, lactose, solid-not-fat (SNF) and total solid (TS). The raw milk was analyzed by the Fourier Transform infrared analysis (FTIR). The results showed the average fat content of 3.50 ± 0.47%, protein of 3.13 ± 0.16%, lactose of 4.59 ± 0.12%, SNF of 8.42 ± 0.20%, and TS of 11.92 ± 0.54%. The samples were superior in all of the nutrients as compared to the standard levels set by the department of livestock development except for TS [117].

Kamizake, *et al.* (2003) determined the total amount of proteins in cow milk powder samples and had a comparative study between the Kjeldahl method and Spectrophotometric methods. Bradford method could be used for the determination of total proteins in skim milk powder and whole milk powder samples (without extraction of lipids) instead of the Kjeldahl method. The Bradford method showed the highest sensitivity of the spectrophotometric methods. Using casein and BSA as standard proteins, the Lowry method showed the lowest variation of specific absorbance indicating either casein or BSA could be used as a standard. The UV-220 nm method with previous extraction of lipids showed the best results for the determination of total proteins in all the samples; and the results were not statistically different ($P > 0.05$) from those obtained by the total protein nitrogen (TPN) without extraction of the lipids. However, when these results were compared to TPN they were statistically different from each other ($P<0.05$) for the buttermilk powder and whey protein powder samples. The determination of total proteins using the Bradford method for the whole milk powder and whey protein powder samples with and without the

extraction of lipids was not statistically different ($P<0.05$) from each other [118].

Macedo, *et al.* (1997) have performed a comparative study on some analytical methods and thermal decomposition of powder milk. Protein content was determined conventionally (Kjeldahl) and colorimetric methods with biuret reagent at 540 nm and integral quality by thermogravimetric and biological methods. A method was developed for the protein separation of powder milk. Powder milk was submitted to degradation processes at 45, 60 and 80 °C for 20 days. The results indicated that protein content values were inconsistent if determined by Kjeldahl and colorimetric methods. Their biological tests were also compared [119].

Patel, *et al.* (1997) measured the physicochemical and structural properties of ultrafiltered buffalo milk and milk powder. Buffalo skim milk containing 10.20% total solids (TS), 3.96% protein and 5.22% lactose was ultrafiltered at 50°C to 23.50% TS and 16.44% protein. Contents of TS, fat, ash and protein increased during UF and lactose content decreased. The composition was influenced by the concentration factor during UF of milk. With the increasing of the concentration factor TS, protein, ash and lactose content is also increased. The rejection coefficients after 77% weight reduction were 52.52% for TS, 94.95% for protein, 100% for fat and 52.04% for ash [120].

Fleming, *et al.* (1921) determined the value of a titration test for acidity at the receiving platform. Brew had pointed out that this apparent acidity might be as high as 0.25 percent and yet the milk be processed successfully though his evidence on that score was negative. Sommers and Hart found variations on titrable acidity of 0.102 to 0.257 percent, 52 percent being above 0.18 percent and showed that this bears no relation to the heatcoagulation of milk [121].

Lago, *et al*. (2006) analyzed the processing, physiochemical properties and sensory evaluation of jambolan (Syzgium cumini Lamarck) jelly. The fruit showed the following chemical composition: ash, 0.34%; lipids, 0.30%; proteins, 0.67%; carbohydrates, 10.07%; fibers, 0.28%; moisture, 87.75%; fructose, 0.4%; glucose, 0.6%; total anthocyanins, 0.276%; pectic substances, 0.245%; acidity (direct titration), 5.91%; soluble solids, 9.00% and pH 3.9. The jelly showed the following composition: reducing sugars, 20.99%; non-reducing, 18.01%; total sugars, 39.00%; pH, 3.42; soluble solids, 67 °Brix; acidity (direct titration), 5.47% and moisture,

29.63%. The jambolan jelly had a satisfactory acceptability based on the sensory analysis [122].

Ahmed, *et al*. (2009) analyzed the nutrients present in Bangladeshi Processed Foods. Moisture content ranged between 3 to 11%. Ash content was 0.5 to about 2.5%, which was within the permissible limit. The fiber content of the noodles was negligible. The protein content of the samples varied between 5.51 to 14.01%. Fat content varied widely and depended on the composition of the samples. Similarly the calorific content also varied. Denaturation of the protein ranged between 40 to 92%. Out of the eleven samples, seven samples had more than 75% denatured protein. Six samples contained fat less than 1%. The acid value of the fats was about 2 to 3% [123].

Basar, *et al*. (2007) studied on the microbiological quality of processed fruit juice. The study involves the evaluation of microbiological quality of industrially processed packed juices such as mango, orange and lemon of five different local companies. Before and after neutralization of pH, aerobic plate count (APC) in juice samples was nil. After enrichment of couple of samples, growth of bacteria was observed on nutrient agar and McCaskey agar media. Sample A showed 120 cfu/mL after 3 hrs on nutrient agar and 20 cfu/mL. After 5 hrs on McCaskey agar medium. These findings indicate that manufactures might use high amount of preservatives that had bacteriostatic effect on microbes. It can be suggested that processed juices should be prepared under hygienic condition without use of high amount of preservatives that might cause health hazard [124].

Joya-Saikia *et al*. (2002) measured the utilization of crambo fruit for the preparation of value added product. To prepare a value based product-jelly from indigenous crambo fruit (Overheat crambo) to evaluate its quality changes during storage, a study was conducted. At room temperature, the jelly was stored for 6 months. The quality parameters were evaluated at monthly interval. An increasing trend was observed in percent reducing sugars and percent total sugars. The ascorbic acid, percent non-reducing sugar and percent acidity showed decreasing trend during extended storage. The jelly was ranked very well in organoleptic evaluation and total cost of processing was calculated [125].

Folegatti, *et al*. (2003) studied on the industrial use of umbu jelly and fruit-in-syrup processing. The study involved the adaptation of conventional processing

technologies for umbu (*Spondais tuberosa*) jelly and fruit-in-syrup. For the umbu jelly, pulp: sugar proportions of 50:50 and 40:60, and fruit pulp with 0, 50 and 100% insoluble solids were used. Umbu fruit-in-syrup with different concentrations of soluble solids (25, 30 and 35 degrees Brix) with added 1% calcium chloride were prepared. The pulps, jellies and fruit-in-syrup were submitted to physical, physicochemical, chemical, and sensorial analyses. The final umbu jelly products presented total titratable acidity values between 0.60 and 0.90% and soluble solid content between 66.3 and 68.6 degrees Brix. Thee jellies prepared with 50:50 ratio of pulp: sugar were significantly (P<=0.05) the most acceptable products in relation to appearance, color and texture. The global acceptability of the umber fruit-in-syrup was highest for the treatments with 30 and 35 degrees Brix [126].

Eremin, *et al*. (2007) analyzed the fruits of Russian plum-precious raw material for preservation. Information is presented on plum cultivars recently developed and widely used in Russia, especially in the north Caucasus region. All the cultivars studied showed large-size fruit, particularly Globus (61 g), Obil'naya (58 g), Podarok sad-Gigantu (44.2 g) and Dynnaya (40.4 g). Data are tabulated on chemical composition of Globus, Dynnaya, Evgeniya, Iyul'skaya roza, Kolonnovidnaya, Kometa Pozdnyaya, Kubanskaya Kometa (standard), Obil'naya (standard) and Podarok Sad-Gigantu during 2000−02. Technological characteristics of plums are also outlined, i. e. maturation time, fruit weight, and taste of fresh fruits, juice, compote and jam. Information is also included on percentage of dry matter content, acidity and marketing grade (1st and highest) of juice, compote and jam. Data are tabulated on visual aspect, color, consistency, taste and total mark for Globus, Dynnaya, Evgeniya, kometa pozdnyaya, Kubanskaya kometa, Obil'naya and podarok sad-Gigantu. Chemical composition (acidity, sugar and pectin substances) and taste of fruit in jelly of Globus and Kolonnovidnaya are also outlined [127].

Khalid (2009) carried out some analysis to assess the suitability of some mango (*Mangofera indica* L.) varieties for jam production. The physical and chemical properties of three mango varieties Abusamaka, Gulb-Eltour and Molgoba were studied to show the varietal differences. The results revealed that the highest level of pulp (75.48%) and soluble solids (17 °Brix) were extracted from Molgoba variety, while Abusamaka showed 13^0 Brick. The highest level of total sugar (11.95%) was

recorded by Algoma and the lowest (10.18%) by Abusamaka, while the highest level of reducing sugars (3.45%) was found in Abusamaka and the lowest (2.96%) in Gulb-Eltour. The organoletic quality of mango jams tested at zero time (fresh) showed that Gulb-Eltour superior in flavor. While the organoleptic quality of mango jams compared to three commercial jams showed that mango jams are comparable to the highest quality jam in Sudan and better than the other two imported commercial jams [128].

Puminat, (2008) determined the conditions and properties of gel in jam. Gel of jam was produced from dehydrated fruits (Tamarind, Guava and Kumquats), pectin, buffer of fruit aced and six groups of various sugar formulae. In the experiments, the data of statistical analysis of gel in jam was as follows: pH range 3.03−3.18, TSS 63.3− 64.7%, TS 65.3−66.2%, moisture 28.6−30.1%, reducing sugar 15.9−23.5% and gel strength 211.67−287.65 g.f. by estimated method (minimum-maximum) at confidential level 95%. Gel strength of jam which were prepared by various sugar formulae were no significant difference at $\alpha = 0.05$. Gel strength of texture is related to percentage quantities of total solid on the high level [129].

Itodo *et al.* (2010) selected branded canned milk (B_1, B_2, B_3 and B_4) to analyze in triplicate, using market basket approach. The samples were pre-treated and analyzed for heavy metals. Their physicochemical variables were estimated. The metal concentration (in µg/g, using AAS) of some toxic metals compared to those of uncanned dairy products include : 0.02±0.008 (006±0.003); 1.61±0.21 (0.01±0.01); 1.47±0.73 (0.01±0.01); 1.64±0.66 (0.05±0.03) and 1.75±0.29 (1.54±1.2) for Cd, Co, Cr, Ni, and Pb found in canned and (uncanned) milk products respectively. Further analysis revealed that nickel content in milk is less, compared to those of canned fish products. Unlike Cd contents, Cr and Pb concentration were above the threshold limit values (TLV) of 2.0 µg/g [130].

Gian *et al.* (2009) determined the presence or absence of toxic heavy metals (lead, arsenic and mercury) in selected infant formula milk and their levels within or beyond standards set by the WHO. Of the three infant formulas tested, all were negative for arsenic, lead, while two out of the three infant formulas tested positive for mercury with levels of 0.6333 ppm and 0.8333 ppm. The levels of mercury obtained, expressed in parts per million (ppm), form the two infant formulas tested were above

the Provisional Tolerable Weekly Intake of total mercury, which is 0.005 ppm, as set by the Food and Agriculture Organization or World Health Organization Joint Expert Committee of Food Additives JECFA, 2003 [3].

Okoye, *et al* (2009) found out the levels of essential and toxic metals in milk and baked products. Seven essential metals (Ca, Cr, Cu, Fe, Ni, Se, and Zn) and two toxic ones Cd and Pb were determined in milk and some baked products (bread, meat pie and sausages) by atomic absorption spectrophotometer using air-acetylene flame. The mean concentrations (mg/kg, wet matter) were in the following ranges: Ca (29.12−7894.45; Zn (2.75−18.00); Fe (1.05−33.12); Ni (0.75−16.82); Cu (0.03−0.08); and Pb (0.001−0.003). Cadmium, chromium and selenium were below detectable levels. The levels of Cu were too low that none of the food could be considered a good dietary source of the metal. The lead levels were too low in comparison with the standards set by the Food Standards of Australia and New Zealand (FSANZ), indicating that analyzed samples were not polluted with lead and were safe for human consumption [131].

Birghila, *et al* (2008) milk products are a very important human nutrient since their consumption has increased in recent years. Good quality measurements are essential to control and maintain milk products and processes quality, both in manufacturing, trade and in research. The presence of toxic elements in powder and liquid milk may create significant health problems for people. The aim of this paper was to determine the content of major and minor elements in different milk samples, sold in major supermarket chains in Romania. Inductively coupled plasma atomic emission spectrometry (ICP-AES) was used for the quantitative determination of elements in this matrix. Analyses were performed after the chemical mineralization of the samples with nitrogen acid. Detection limits ranged from 0.4 to 7.03 ng/g [132].

Li, *et al*. (2005) determined heavy metals in Wisconsin dairy feeds. Heavy metals such as zinc (Zn), copper (Cu), chromium (Cr), arsenic (As), cadmium (Cd) and lead (Pb) were potential bio-accumulative toxins of the dairy production system. This survey determined the heavy metal content of 203 typical dairy ration components sampled from 54 dairy farms in Wisconsin. Lowest heavy metal concentrations were found in homegrown alfalfa (*Medicate sativa L.*) hay , corn (*Zea mays L.*) grain and silage. Highest metal concentrations were found in purchased feeds, particularly

mineral supplements and to a lesser extent cornor soybean-based concentrates. Zinc and Cu were found at the highest concentration in complete dairy (total mixed and aggregated component) rations and reflected the deliberate addition of these metals to meet animal nutrient requirements although more than half the farms fed Cu and Zn above US recommended levels. Concentrations of Cr, As, Cd and Pb were present in much lower concentrations and decreased in the order Cr >As >Pb > Cd. No complete Wisconsin dairy ration contained heavy metal concentrations above US maximum acceptable concentration and would be unlikely to induce any toxic effects in dairy cattle [133].

Sroor, *et al.* (2003) analyzed the amount of major and trace elemental in milk powder by Inductively Coupled Plasma-Optical Emission Spectrometry (ICP-OES) and Instrumental Neutron Activation Analysis (INAA). The concentration of 24 elements was determined by ICP-OES method, from these elements 9 elements determined by INAA. The determination of trace element contents of foodstuffs, especially milk as daily drink for all peoples age which being a complex food had great importance. The major elements were Ca, K, Mg, Na, P and S. While trace element were B, Ba, Co, Cr, Cu, Fe, Li, Mn, Mo, Ni, Sb, Se, Sn, Sr, V, W and Zn. ICP-OES technique was shown to be a powerful tool for trace determination in powder samples [134].

Zamir, *et al*. (2001) measured the amount of lead and cadmium in powder milk in Quetta (Pakistan) by atomic absorption spectrometer. The samples of different brands of powder milk and infant formula milk were collected from Quetta local market and atomic absorption spectrometer was used for the determination of toxic metals e.g. lead and cadmium. The concentration of lead and cadmium were found to be within the same limits as recommended by the WHO. The intake of lead was quite higher than cadmium from whole cream milk and infant baby formula [135].

Karadjova*, et al.* (2000) determined the amount of Cd, Co, Cr, Cu, Fe, Ni and Pb in milk, cheese and chocolate. Combined analytical procedures consisting of wet digestion step followed by instrumental determination-Differential Pulse Cathodic Stripping Voltammetry (DPCSV) or Electrothermal Atomic Absorption Spectrometry (ETAAS) as well as a direct analysis method slurry sampling ETAAS-for the determination of Cd, Co, Cr, Cu, Fe, Ni and Pb in milk, cheese and chocolate were compared, wet digestion using a mixture of HNO_3-$HCIO_4$-H_2O_2 was proposed for

complete matrix decomposition prior to trace analyte determination by DPCSV of ETAAS. A mixture of HNO_3-H_2O_2 was used for slurry preparation. Results obtained were in good agreement with the certified values and the relative standard deviations (for this result) were in the range 5−10% for wet digestion DPCSV or ETAAS and 3−9% for slurry sampling ETAAS in the range of 2 µg/g (Cd) to 12 µg/g (Fe) [136].

Tripathi, *et al.* (1999) determined the concentrations of the essential elements Zn and Cu and potentially toxic elements Pd and Cd in n different milk samples and baby food materials were measured, primarily to assess whether the intakes comply with recommended desired levels for essential and permissible levels for toxic elements. The geometric mean concentrations of Pd, Cd, Cu and Zn in different types of milk were found to vary from 1.70 to 3.35, 0.07 to 0.10, 43.2 to 195 and 1772 to 4230 µg/L, while the same in different baby foods had values from 39.5 to 77.7, 0.45 to 17.7, 1106.3 to 3157.3 and 9367 to 34 492 µg/kg, respectively. The concentration of Cd was found to be very low (0.1 µg/L) and fairly constant in all types of milk. The lead content in cow milk was observed to be the lowest even in comparison with breast milk. Concentrations of all these metals are approximately one order of magnitude higher in baby food products than those observed in different types of milk owing to higher fat content. The infant baby food amul spray contains low concentrations of toxic (Pb and Cd) and high concentrations of essential (Cu and Zn) elements. The daily intakes of Pb, Cd, Cu and Zn by infants through milk and baby foods marketed in Mumbai city have also been estimated. The daily intakes of Pb (1.1 µg/kg) and Cd (0.01 µg/kg) for infants through baby foods are well below the recommended tolerable levels of 3−5 mg and 0.5−1.0 mg for Zn and Cu respectively. Milk from an Indian mother also does not provide adequate levels of essential elements to the infants [24].

Cabrera, *et al.* (1996) determined the level of some metal in milk and dairy products by atomic absorption spectrometer. The level of chromium, copper, iron, manganese, selenium and zinc in 60 samples of 10 widely consumed dairy products was determined. In analyzed samples, mean values ranged from not detectable to 0.950 µg/g for Cr, from 0.0020 to 2.800 µg/g for Cu, from 0.750 to 20.0 µg/g for Fe, from 0.010 to 0.900 µg/g for Mn, from not detectable to 0.140 µg/g for Se, and from 0.250 to 4.5 µg/g for Zn. The highest levels for Cr, Cu, Fe, Mn and Zn were detected in

children's milk. Increased concentration of Cr, Cu, Fe and Mn were detected in products packaged in glazed ceramic containers [137].

Dabeka, *et al.* (1988) Lead and cadmium levels were determined in 131 infant foods. Mean lead and cadmium levels were 19.3 and 3.3 ng/g for meats, 8.4 and 4.1 ng/g for vegetables, 14,9 and 0.58 ng/g for fruits and desserts, 9.6 and 0.53 ng/g for juices and drinks, and 32.8 and 33.6 ng/g for dry infant cereals. These data, combined with those from other recent surveys, yielded average dietary (food and water) intakes of lead and cadmium by infants of 0−1 year old of 2.4 and 0.37 microgram/kg/day, respectively. Lead intakes were most strongly influenced by storage of infant formulas in lead-soldered cans. For infants 0−1 month old, the ranged from 0.5 microgram/kg/day when human or cow milk was fed to infants to 5.3 micrograms/kg/day (exceeding the FAO/WHO provisional tolerable daily intake, PTDI, of lead by children of 3.5 micrograms/kg) when ready to use formula stored in lead-soldered cans was fed. Cadmium intakes were most strongly affected by soya based formulas, and ranged, for 0−1 months old, from 0.16 microgram/kg/day for infants fed human or cow milk to 0.50 microgram/kg/day for infants fed soya-based concentrated liquid formula. Cadmium intakes were all below the FAO/WHO PTDI of cadmium by adults of 0.96−1.2 micrograms/kg [138].

Winiarska-Mieczan, *et al.* (2008) determined the content of some minerals in fruit and vegetable baby juices. The research material consisted of juices, all before their use-by fate, purchased in grocer shops in Lublin in January 2006. Eight of the examined juices were labeled as special purpose food, two were recommended by the National Food and Nutrition Institute and ten juices were labeled as 'food for young children'. Juices make an important source of minerals in the diet of infants and young children. The content of dry mass was 7.35−14.40%. Juice contained very little ash and few minerals in comparison with the other juices. The level of calcium in juices ranged from 0.025 to 0.084 mg/g. The content of magnesium in juices ranged from 0.035 to 0.145 mg/g. The content of sodium in juices ranged from 0.013 to 0.168 mg/g. The highest amount of potassium was detected in juice B-3 (1.380 mg/g) and its lowest level was observer in juice H (0.398 mg/g). The amount of iron in juices ranged from 4.57 mg/g to 0.36 mg/g zinc was found in juice 2−4 (1.10 mg/g). The largest content of copper was observed in juice I (0.915 mg/g) and the lowest in juice

F-2 (0.090 mg/g). Most manganese was present in juice B-2 (2.59 mg/g) and this value was significantly different from the other results. This was most probably caused by the presence of wild rose fruit as an ingredient of the juice. The lowest amounts of manganese were detected in juices K (0.339 mg/g) and A-4 (0.370 mg/g). Differences in the content of particular mineral elements in juices result primarily from their composition. Although juices for infants and treated with caution as it cannot be excluded that some may contain prohibited compounds, e. g. calcium ascorbate or calcium chloride [139].

Hassan, *et al*. (2003) evaluated some metals in commonly consumed spices in Bahrain. 17 commonly consumed spices in Bahrain were analyzed using atomic absorption spectroscopy and the levels of some heavy and essential metals were determined. Sample was collected from different retail outlets in the local spice market. The data showed wide variation in metal contents among the various spice samples. The maximum mean level of elements among all spices based on plant parts fall in the magnitude of the order: iron > zinc > copper > nickel > lead>cadmium, in leaves, rhizomes, seeds, buds, fruits and barks, respectively. The highest level of lead (2.2 µg/g) was found in caraway. The highest cadmium level (0.9 µg/g) was found in green cardamom. With essential metals, concentration of iron, zinc, copper and nickel were highest in cumin (13.6 µg/g), black cumin (52.2 µg/g), black pepper (17.3 µg/g) and black cumin (4.9 µg/g). By analyzing data from literature, the reliability of the findings and approach was confirmed [140].

Williams, *et al*. (2009) measured the trace metal levels in fruit juices and carbonated beverages in Lagos, Nigeria using atomic absorption spectrophotometer (Unicam model 1969). Trace metals Cr, Cu, Pb, Mn, Ni, Zn, Fe, Cd and Co in grape, pineapple, apple, orange and lemon juices were analyzed. Trace metal contents of fruit juices were found to be more than the metallic contents of carbonated beverages. Pb level in the fruit juices ranged from 0.08 to 0.57 mg/L but were not detected in the carbonated drinks. Concentrations of Pb in lemon juice and Mn pineapple juice were relatively high. Cd and Co were not detected in the selected juices and beverages. Additionally Pb, Cu, Cr and Fe were not detected in canned beverages but were present in bottled beverages. Except for Mn in pineapple juice and Pb in lemon juice, the metal levels of selected fruit juices and carbonated beverages were within permissible levels [141].

Mahdavian, *et al.* (2008) measured the levels of heavy metal contamination of fruits in Bangalore markets and assessed how the heavy metal contamination might have impacted food safety standards viz. a heavy metals on urban consumers. The results show that urban consumers are at greater risk of purchasing fresh fruits with high levels of heavy metals beyond the legally permissible limits as defined by the Indian Prevention of Food Adulteration Act, 1954. It must be noted here that these norms are less strict than international food safety norms like Codex Alimentarius or European Union standards. It is therefore suggested here that care should be taken in the following: reduce pollution at water source points; improve post harvest handling; enhance better coordination in fresh crops trading system to improve food safety standards; improve sanitary conditions for the city food markets; and increase awareness in consumers and policy makers on the dangers of heavy metal contamination in the food intake [142].

Krejpcio, *et al*. (2005) determined the content of Pb, Cd, Cu and Zn in fresh fruit and juices of Poland using atomic absorption spectrometer (AAS). This determination estimates the level of safety of some food available in the Polish market. It was found that most fruit samples (90.4%) contained low levels of heavy metals. However, the remaining 9.6% had increased heavy metal contents (Pb 2.2%, Cd 4.4%, Cu 1.5%, Zn 1.5%). Most fruit juice sample (88%) met the national standard criteria, but 12% exceeded the permissible limits for Pb and Cd (3% and 9%) respectively [143].

1.10 Statistical Analysis of the Experimental Results of Sample

Three different criteria proposed for statistical analysis of the experimental results of the studied samples are stated below:

1.10.1 Descriptive statistics of the experimental results of sample

We were aimed to test about H_0 (Null hypothesis) and H_1 (Alternative hypothesis). Here, H_0 = the mean value of the experimental results of each studied sample is same and H_1=the mean value of the experimental results of each studied sample is different.

1.10.2 Analysis of variance (ANOVA) for the variation study of the experimental results of sample

It provides statistical information of the experimental value of the studied sample and indicates a significant difference of the experimental value of the selected studied

sample. It also indicates that the level of significant or insignificant of the variance of the experimental value of each studied sample.

1.10.3 Duncan's Multiple Range Test (DMRT) of the experimental results for mean comparison of sample

DMRT was performed that within the group and between the groups the mean value of the experimental results is significantly different or insignificantly different as well as it is mentioned the interaction effect between and within the groups by DMRT.

1.11 Aim of the Present Investigation

The food represents one of the most important needs of the human being. In order to obtain a good assimilation of food for an optional functioning of the organism, the supplying of all nutritive elements in a balanced proportion is required. The nutritional imbalance either over nutrition or under nutrition leads to nutritional diseases.

Milk is the fundamental food for infants. The intake of milk must always be fulfilling to produce the necessary energy by metabolic reaction for living. The proportions of essential component are not same in all the powdered milk available in the market. Indeed infant body requires definite amount of minerals for functioning of some important biomolecules such as hemoglobin, myoglobin, cytochrome, ferredoxin and many other macromolecules. Toxic metals such as Pb, Cd, Cr, Ni and As etc. are found in milk along with the required minerals. As a result, when this powder milk is used as baby feed, these toxic metals are also entered into the infant's body. These metals have no specific useful function in infants' body. Moreover they act as toxic if their presence is beyond the tolerance level in the body and produce various severe problems. So, we should be alert about them. But we do not have enough information about the minerals and toxic metal contents in different brands of baby powder milk available in the market. In order to know the level of toxicity of the different brands of baby powder milk, it has been endeavored to estimate the amount of some metals (trace and toxic), specially the toxic metals e.g. Pb, Cd, Ni, Cr, and As in different brands of baby powder milk, which may help to aware the people about the baby powder milk. Also the objective of this study was to find out some important biochemical parameters such as percentages of protein, reducing sugar, non-reducing

sugar, total sugar, ash and moisture by using different biochemical methods as well as the amounts of some trace and toxic metals in jam, jelly and juice available in Bangladesh by using atomic absorption spectrophotometer. The nutrients studied in the present work have very significant and specific role in human metabolism and their deficiency can be removed through intake of reasonable amount of jam, jelly and juice. So the main points of this research work are given below in brief:

1. Determination of the percentages of protein, lactose and acidity of the selected baby powder milk.
2. Determination of the percentages of protein, total sugar and reducing sugar of the selected brand of jams, jellies and juices.
3. Determination of the amount of trace (Co and Zn) and toxic metals (Pb, Cd, Cr, As and Ni) in baby (0−6 months and 6−24 months) powder milk available in the market.
4. Estimation of the amount of Co, Zn, Pb, Cd, Cr, As and Ni in baby (6−24 months) cereals.
5. Determination of the amount of trace and toxic metals (Co, Zn, Pb, Cd, Cr, As and Ni) in jams, jellies and juices available in Bangladesh.
6. To prepare baby powder milk list according to the concentration to toxic metals which helps the parents to select better quality food items for their babies.
7. Determination of the amount of essential metals (Na, K, Ca and Mg) of jams, jellies and juices available in Bangladesh
8. To formulate a chart for comparative study about toxicity of baby powder milk of different brands available in the market.
9. Making a food list according to the concentration of trace and toxic metals which helps the people to select better quality food items.
10. This will also increase public awareness about these food items.

1. 1.12 References

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CHAPTER TWO

Experimental

CHAPTER TWO

Experimental

2.1 Materials

2.1.1 Baby (0−6 months and 6−24 months) powder milk and baby cereals (6−24 months) under study

Total twenty four samples of baby (0−6 months and 6−24 months) powder milk and baby cereals are studied that are available in the market. Fourteen samples of baby (0−6 months and 6−24 months) powder milk and ten brands of baby (6−24 months) cereal are analyzed. The brand name and country of origin of powder milk and cereal are given in Table 2.1 and 2.2 respectively.

Table 2.1: Baby (0−6 months and 6−24 months) powder milk of different brands

Age of the baby	Serial No	Name of the brands	Country of origin
0−6 months	1	Biomil-1	Belgium
	2	Lactogen-1	Switzerland
	3	Mother's smile-1	Australia
	4	Eldorin-1	Netherland
	5	Mamilag-1	Poland
	6	Baby care-1	Korea
	7	Biomil soy	Belgium
6−24 months	8	Biomil-2	Belgium
	9	Lactogen-2	Switzerland
	10	Mother's smile-2	Australia
	11	Eldorin-2	Netherland
	12	Mamilag-2	Poland
	13	Baby care-2	Korea
	14	Lailac-2	France

Figure 2.1: Baby powder milk (Lactogen-1)

Table 2.2: Investigated baby (6–24 months) cereals.

Serial No	Brand name	Country of origin
1	Biomil-1 (Wheat+3 Fruits+Milk	Belgium
2	Nestle -1 (3 Fruits + Wheat +Milk)	India
3	Nestle - II (Rice + Milk)	India
4	Nestle -III (Rice+ Mixed vegetable)	India
5	Nestle - IV (Wheat + Dal + Palank)	India
6	Nestle - V (Wheat + Milk)	India
7	Nestle-VI (Wheat + Apple + Cornflakes)	India
8	Mother's smile - I (Rice)	Australia
9	Mother's small-II (Relax + Fruits +Wheat)	Australia
10	Mother's smile-III (Honey + Wheat)	Australia

Figure 2.2: Biomil Fruits-First Solid Food

2.1.2 Jams, jellies and juices under present study

Total twenty seven samples of jams, jellies and juices are studied that are available in Bangladesh. Nine jams, eight jellies and ten juices are analyzed. Brand name, country of origin and the companies that involved in producing, packaging and marketing of jam, jelly and juice are given in Table 2.3 and 2.4.

Table 2.3: Jams and jellies of the present study

SI. No	Sample	Country	Name of Company
1	Shezan Mango Jam	Bangladesh	Hashem Foods Ltd.
2	Pran Mango Jam	Bangladesh	Pran Agro Ltd.
3	Freswel Mango Jam	Pakistan	Ahmed Foods Uan.
4	Nur Apple Jam	Bangladesh	Nur Food Products
5	Rajshahi Mango Jam	Bangladesh	Raj. Mango Products (Pvt.) Ltd.
6	BF Orange Marmalade Jam	Import	Pretty Engineering Limited
7	Agrokomerc Pineapple Jam	Bangladesh	Agrokomerc Foods
8	Shezan Mixed Jam	Bangladesh	Hashem Foods Ltd.
9	Nur Mixed Jam	Bangladesh	Nur Food Products
10	Agrokomerc Mango Jelly	Bangladesh	Agrokomerc Foods
11	BD Orange Jelly	Bangladesh	BD Foods Limited
12	Shezan Orange Jelly	Bangladesh	Hashem Foods Ltd.
13	Pran Orange Jelly	Bangladesh	Pran Agro Ltd.
14	Ahmed Guava Jelly	Bangladesh	Ad Mango Products(Pvt.) Ltd.
15	Pran Apple Jelly	Bangladesh	Pran Agro Ltd.
16	Friends Mango Jelly	Bangladesh	Friends Foods corporation Ltd.
17	Friends Orange Jelly	Bangladesh	Friends Foods corporation Ltd.

BF- Best Food, Raj- Rajshahi, Ad-Ahmed

Table 2.4: Fruit juices of the present study

SI.No	Sample	Country	Name of Company
1	Danish Mango Juice	Bangladesh	Danish condensed milk BD Ltd.
2	Shezan Juicepac (Mango)	Bangladesh	Hashem Foods Ltd.
3	Starship (Mango)	Bangladesh	AK Cond. Milk and Beverage Ltd.
4	Acme Premium Mango Juice	Bangladesh	Acme Agrovat and Beverage Ltd.
5	Pran Premium Mango Juice	Bangladesh	Agricultural Marketing Co Ltd.
6	Aarong Orange Flavor	Bangladesh	Brac Dairy and Food Project
7	Aarong Tamarind Juice	Bangladesh	Brac Dairy and Food Project
8	Frutika Red Grape Juice	Bangladesh	Akij Food and Beverage Ltd.
9	Pran Junior Juice (mango)	Bangladesh	Agricultural Marketing Co Ltd.
10	Pran Frooto Mango Juice	Bangladesh	Pran Beverag

AK- Abul Khair, Cond - Condensed

Bottle/ Packet of jams, jellies and juices of different brands of present study are given below:

Figure 2.3: Pran Mango Jam

Figure 2.4: Friends Orange Jelly

Figure 2.5: Freswel Mango Jam

Figure 2.6: Shezan Juicepack Mango Classic

2.4 Reagents and Chemicals

Reagents and chemicals used for all analysis were usually for the analytical grade. Solutions for metal were prepared according to standard procedure. Redistilled water was used through the present investigation. The standard solutions of Co, Zn, Pb, Cd, Cr, As and Ni were prepared according to standard procedure before Spectrophotometric determination. Reagents and chemicals used in different experiments have been listed in the respective chapters.

2.4.1 Collection and preservation of samples

A market survey was conducted to list the name of baby powder milk and baby cereals of various brands available in market. For the present study commercially available baby (0−6 months and 6−24 months) powder milk samples of 14 different brands were collected from the renowned medical colleges and hospitals of Bangladesh. These hospitals were selected randomly. Doctor and nurse helped to collect the powder milk from hospitals. The ten different brands baby cereals available in the markets were purchased. Various types of jams, jellies and juices were purchased from the different market of Bangladesh. These were kept in polyethylene bags separately and stored in refrigerator for preparation of solutions.

2.5 Methods

2.5.1 Research plan

This research work was conducted through the inception and implementation of the following research plan which have been represented in the form of flow chart according to the sequence of the works.

2.5.2 Analysis of baby powder milk

a) Powder milk contains four components for analysis and these are:

1. Protein
2. Acidity
3. Lactose and
4. Metals.

b) Metals are classified into two groups:

i) Trace Metals: Co and Zn and

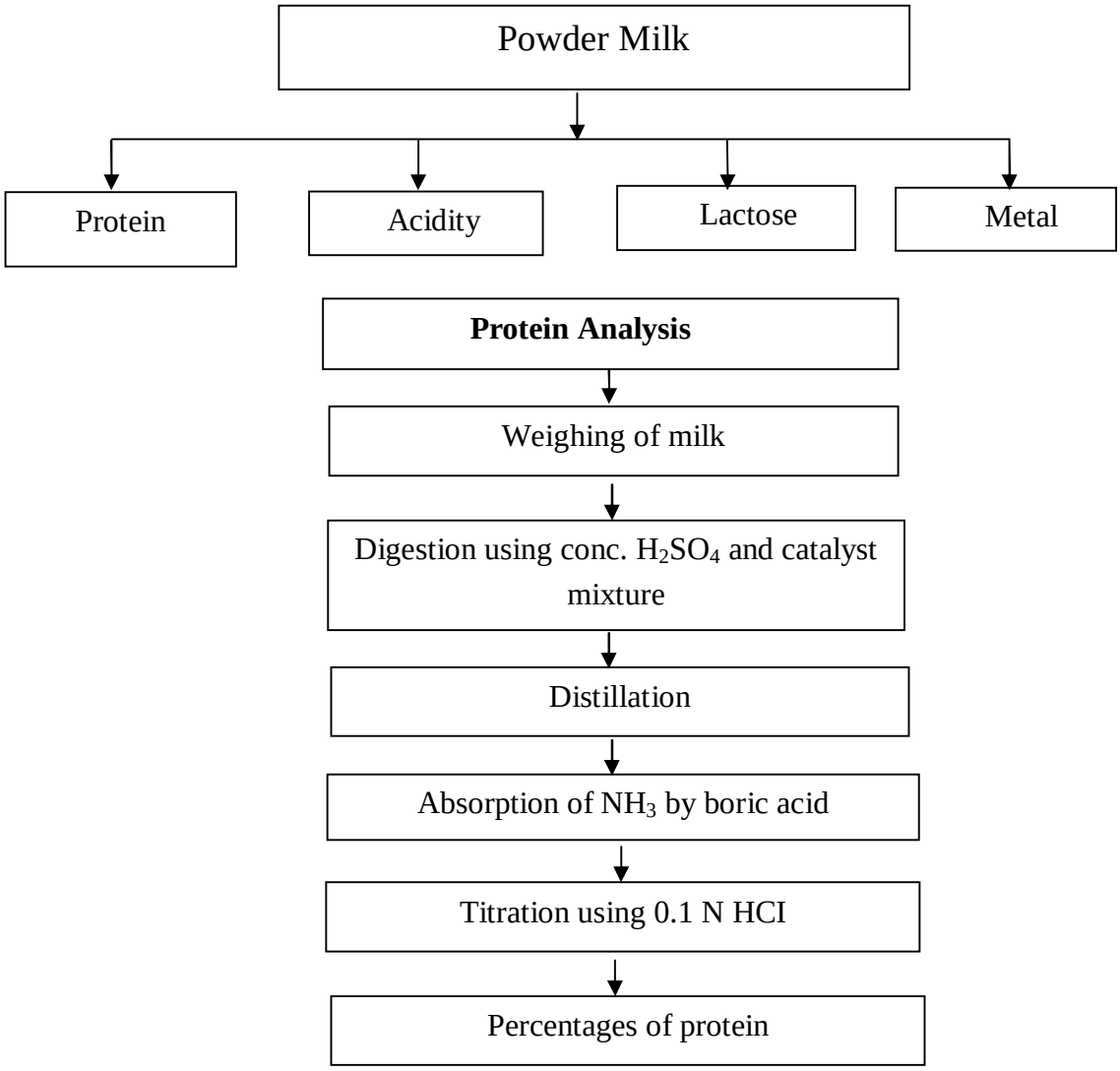
ii) Toxic Metals: Pb, Cd, Cr, As and Ni.

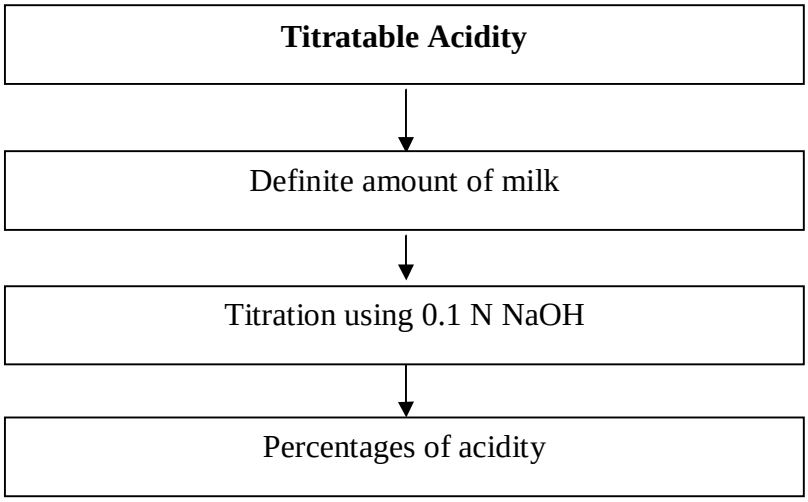
2.5.3 Analysis of jam, jelly and juice

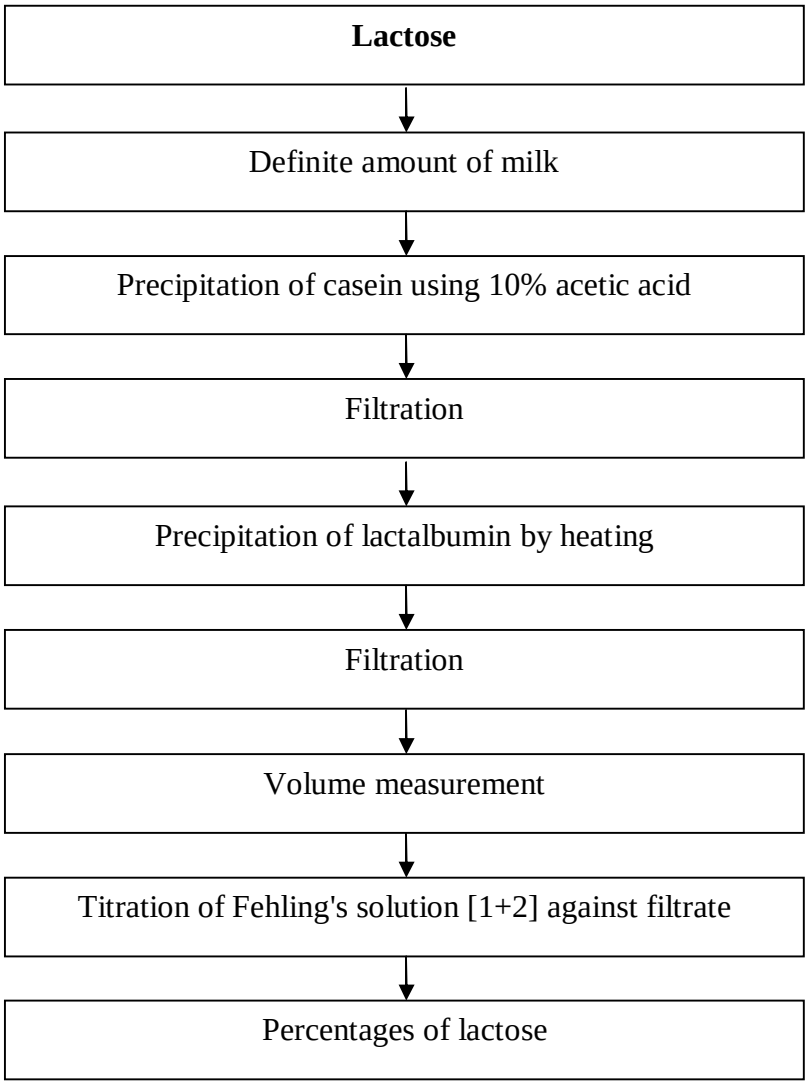
Jam, jelly and juice contain five components for analysis and these are:

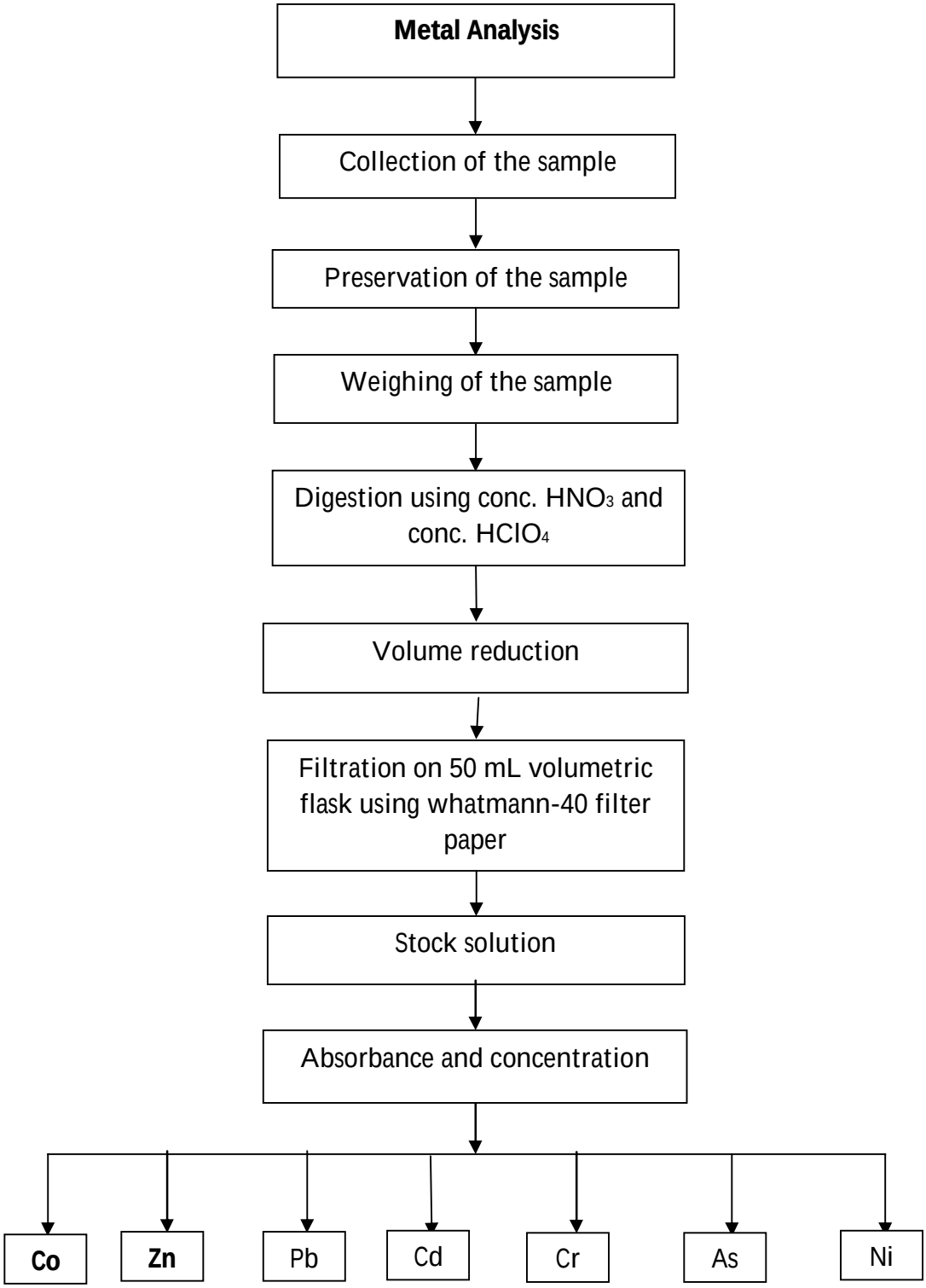
1. Protein
2. Sugar
3. Ash
4. Moisture and
5. Metals (essential metals, trace metals and toxic metals)

2.5.4 Research plan for baby powder milk

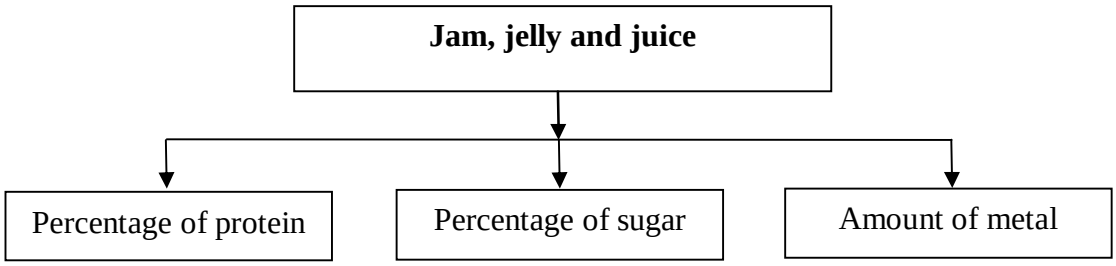


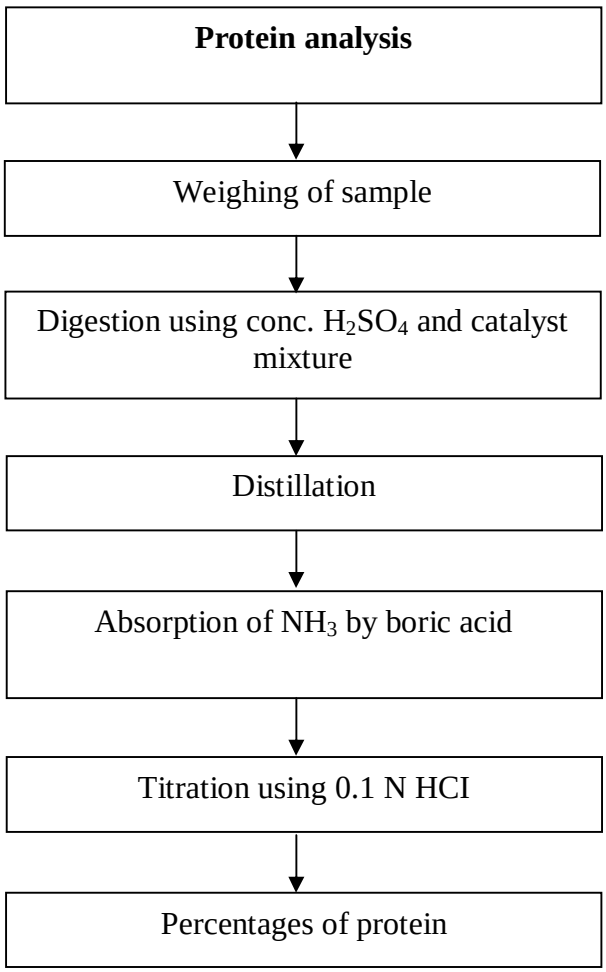

Titratable Acidity
Definite amount of milk
Titration using 0.1 N NaOH
Percentages of acidity

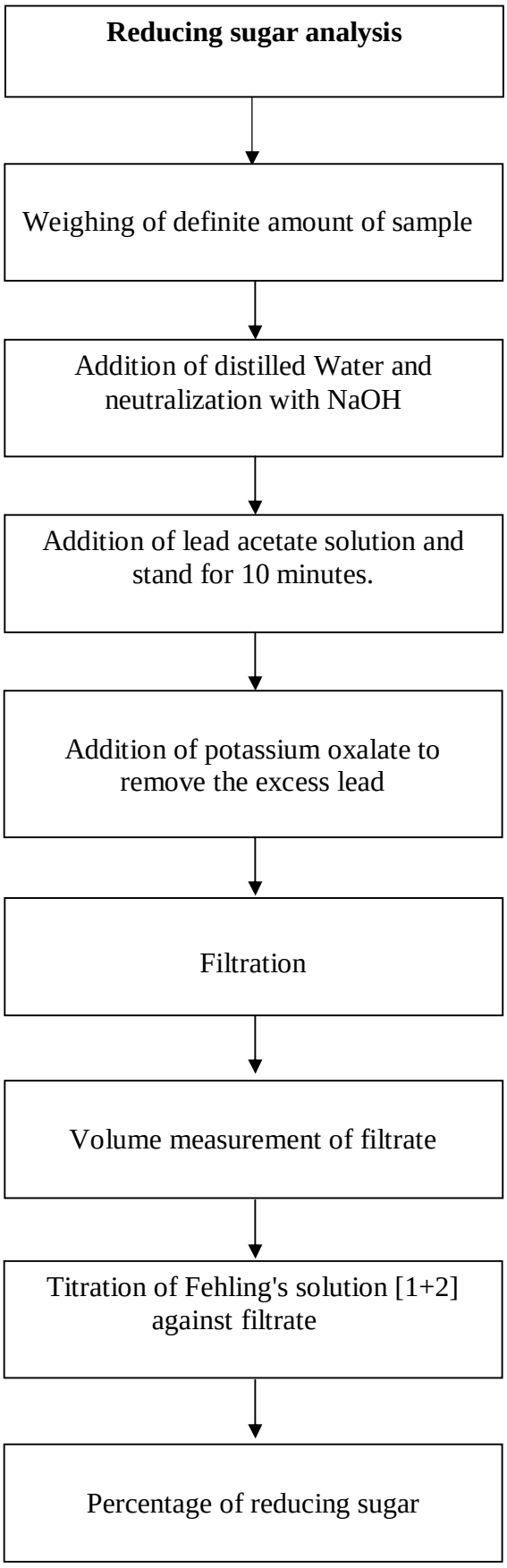

Lactose
Definite amount of milk
Precipitation of casein using 10% acetic acid
Filtration
Precipitation of lactalbumin by heating
Filtration
Volume measurement
Titration of Fehling's solution [1+2] against filtrate
Percentages of lactose

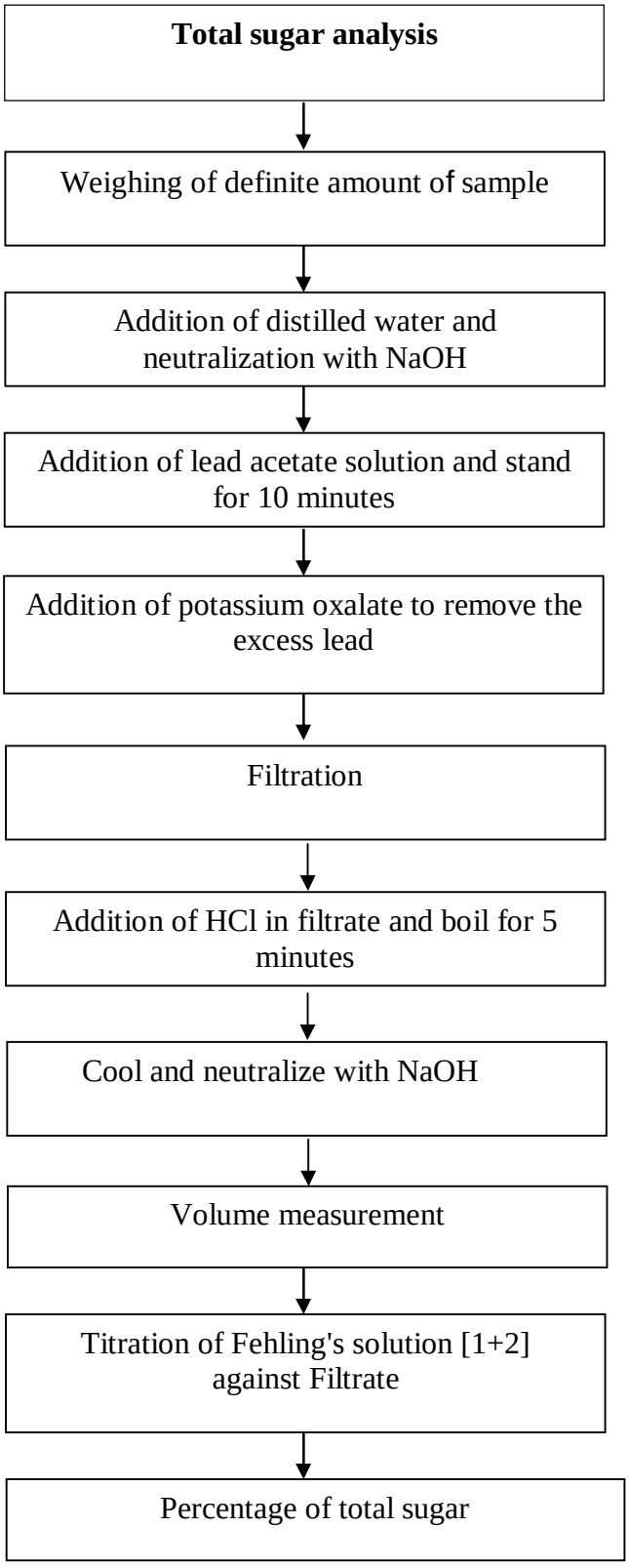

Metal Analysis
Collection of the sample
Preservation of the sample
Weighing of the sample
Digestion using conc. HNO_3 and conc. $HClO_4$
Volume reduction
Filtration on 50 mL volumetric flask using whatmann-40 filter paper
Stock solution
Absorbance and concentration
Co
Zn
Pb
Cd
Cr
As
Ni

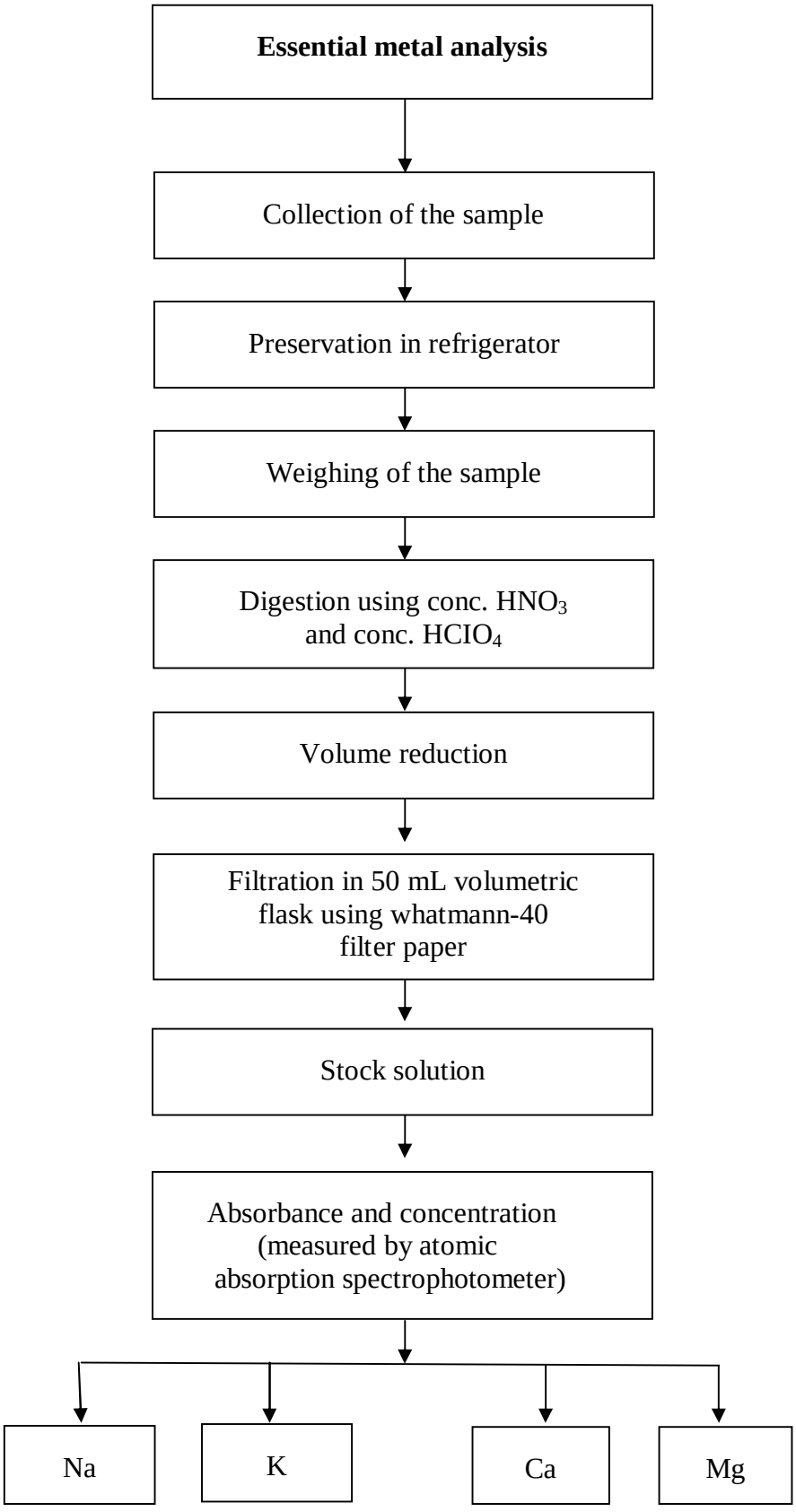
2.5.5 Research plan for jam, jelly and juice

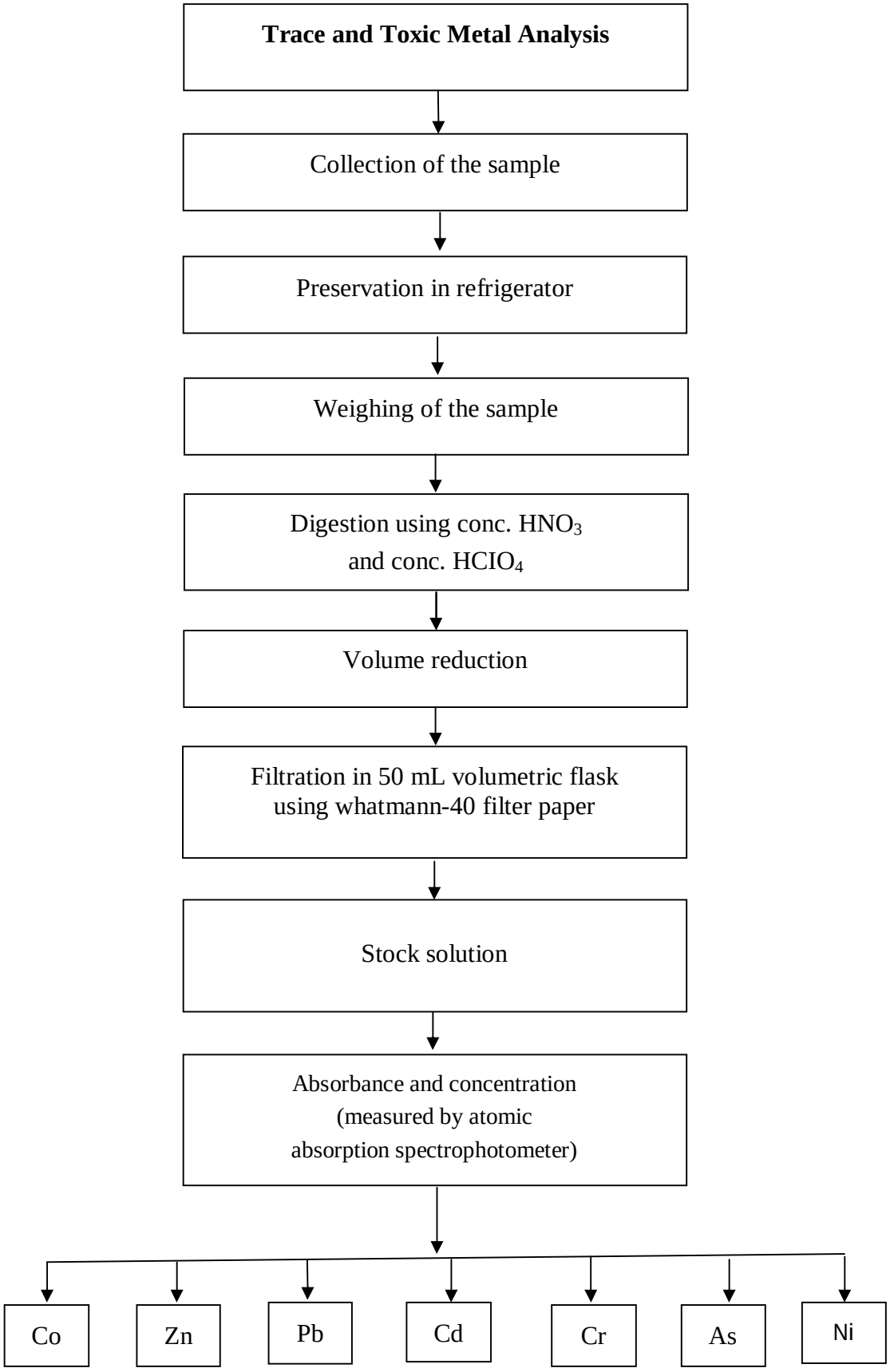





Reducing sugar analysis
Weighing of definite amount of sample
Addition of distilled Water and neutralization with NaOH
Addition of lead acetate solution and stand for 10 minutes.
Addition of potassium oxalate to remove the excess lead
Filtration
Volume measurement of filtrate
Titration of Fehling's solution [1+2] against filtrate
Percentage of reducing sugar


Total sugar analysis
Weighing of definite amount of sample
Addition of distilled water and neutralization with NaOH
Addition of lead acetate solution and stand for 10 minutes
Addition of potassium oxalate to remove the excess lead
Filtration
Addition of HCl in filtrate and boil for 5 minutes
Cool and neutralize with NaOH
Volume measurement
Titration of Fehling's solution [1+2] against Filtrate
Percentage of total sugar


Essential metal analysis
Collection of the sample
Preservation in refrigerator
Weighing of the sample
Digestion using conc. HNO_3 and conc. $HClO_4$
Volume reduction
Filtration in 50 mL volumetric flask using whatmann-40 filter paper
Stock solution
Absorbance and concentration (measured by atomic absorption spectrophotometer)
Na
K
Ca
Mg


Trace and Toxic Metal Analysis
Collection of the sample
Preservation in refrigerator
Weighing of the sample
Digestion using conc. HNO_3 and conc. $HCIO_4$
Volume reduction
Filtration in 50 mL volumetric flask using whatmann-40 filter paper
Stock solution
Absorbance and concentration (measured by atomic absorption spectrophotometer)
Co
Zn
Pb
Cd
Cr
As
Ni

2.6 Analytical Techniques

In present investigation the metal analysis (Na, K, Ca, Mg, Co, Zn, Pb, Cd, Cr, As and Ni) was done by Atomic Absorption Spectrophotometer (AAS). Micro Kjeldahl method [1] was used to carry out the protein analysis. Determination of acidity was carried out by tritratable method [2] and lactose was determined by volumetric method [3]. Determination of moisture and ash were carried out by AOAC (Official Methods of Analysis of the Association of Official Analytical Chemists) method [4] and sugar (reducing sugar, total sugar) was determined by Lane and Eyenon method [5].

2.7 Procedure of the Analysis

2.7.1 Protein analysis of baby powder milk

Percentages of protein of all milk samples were determined by Micro Kjeldahl method [1]. A definite amount of milk sample was digested into Kjeldahl flask using sulfuric acid and digestion mixture (copper sulfate, potassium sulfate and selenium powder). The contents of the flask were digested until the color of the solution was made alkaline by adding excess amount of 40% NaOH. During distillation ammonia was liberated and absorbed by boric acid (H_3BO_3) that was previously taken in conical flask with appropriate indicator. After complete absorption the solution was titrated against standard 0.1 N HCl. From the titration value the percentage of protein of the sample was calculated.

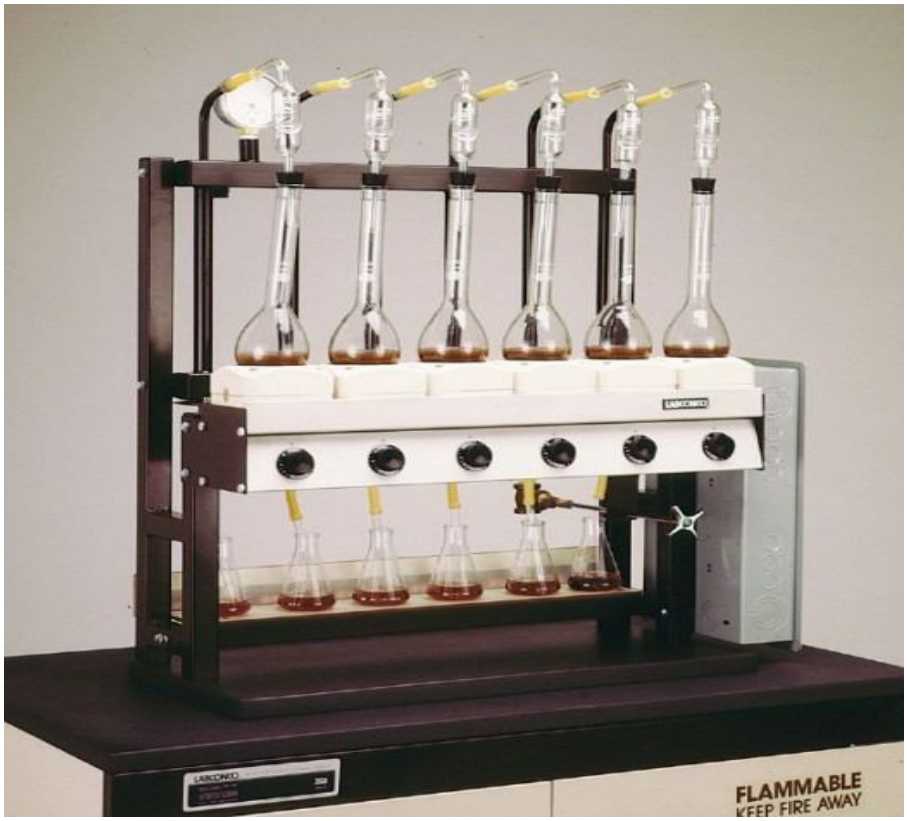


Figure 2.7: A view of the protein analysis of the sample under present study (Digestion Process)

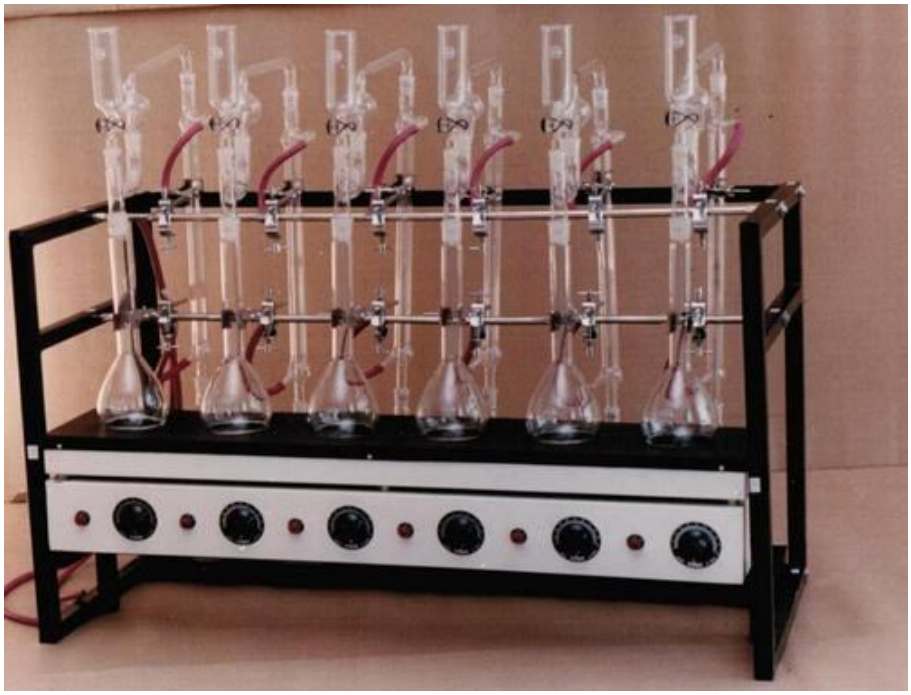


Figure 2.8: A view of the protein analysis of the sample under present study (Distillation Process**)**

2.7.2 Determination of titratable acidity of baby powder milk

Titratable acidity was determined according to AOAC method [2]. Milk sample was diluted with boiled and cooled distilled water and 2 mL of phenolphthalein (prepared by adding 1% in 95% ethanol). The mixture was titrated with standard 0.1 N NaOH and the final volumme of 0.1 N NaOH added was noted. Distilled water was added to powder milk in order to dilute them to fluid milk basis before titrating for acidity [6].

2.7.3 Determination of lactose (milk sugar) of baby powder milk

Lactose content was determined by volumetric method [3]. To determine lactose protein (casein) of the milk was precipitated using 10% acetic acid solution. The precipitate was allowed to settle down. After filtration the filtrate was heated and the protein, lactalbumin was precipitated out. The solution was filtered again. The volume of the filtrate was measured accurately. This filtrate was used to titrate the Fehling's solution. The percentage of milk sugar was calculated from this titration value.

2.8 Metal Analysis of Baby Powder Milk

2.8.1 Digestion of the sample

After weighing the sample was taken in a 3-necked digestion flask. About 150−200 mL of concentrated HNO_3 and 30−50 mL $HClO_4$ were added. The mixture was then refluxed by heating in a hot plate at 120 −140 °C for about 29 hrs with stirring. After completion of digestion, the volume of the solution was reduced to about 10 mL. This solution was allowed to cool and was diluted with 15−20 mL of redistilled water. Then the solution was filtered through whatmann-40 filter paper and makes the filtrate 50 mL in a 50 mL volumetric flask with redistilled water. All the sample solutions of different sets were prepared by allowing this wet digestion method. For each set a blank solution of 50 mL was also prepared. These sample solution were preserved for analysis.

2.8.2 Preparation of standard solution

The standard solution of the metals Co, Zn, Pb, Cd, Cr, As and Ni were prepared by pipetting the required amount of the solution form the stock solution manufactured by BDH laboratory supplies, Poole's BH 151 Td England. The standard solution of a metal of different concentrations was prepared before spectrophotometric determination.

2.8.3 Preparation of calibration curve

A calibration curve of the respective metal was prepared before each metal determination by plotting the absorbance against concentration of the standard solutions. Metal concentration of the sample was measured with the help of these calibration curves. Solutions were prepared with the concentrations covering the optimum linear absorbance range. In all absorbance measurements, the reading was taken after the instrumental zero has been adjusted. By measuring the absorbance of standard solutions of a metal, a calibration curve was automatically constructed and displayed in the monitor of spectrophotometer. The calibration curve was cheeked occasionally by measuring absorbance and concentration of the standard solutions according to the sensitivity of the instrument.

2.8.4 Atomic absorption spectrophotometric measurement

Atomic absorption spectrophotometric measurement depends on Beer-Lambert law. The electrons of the atoms in the atomizer can be promoted to higher orbitals for a short time by absorbing a set quantity of energy i.e. light for a given wavelength. This amount of energy or wavelength is specific to a particular electron transition in a particular element and in general, each wavelength corresponds to only one element. This gives the technique its elemental selectivity. As the quantity of energy put into the flame is known and the quantity remaining at the other side (at the detector) can be measured, it is possible, with Beer-Lambert law, to calculate how many of these transitions took place, and thus get a signal that is proportional to the concentration of the element being measured [7]. All the digested solutions were analyzed by using air acetylene flame and single element hollow cathode lamps into an atomic absorption spectrophotometer. Maximum absorbance was obtained by adjusting the cathode lamps at specific slit and wavelengths. The absorbance and concentration data of the solutions were automatically printed out and displayed. Standard solutions were made for each metal and bracketed the expected metal concentration. Flame conditions for analyses of metals in collected samples are shown in Table 2.5.

Table 2.5: Flame condition for metal analyses

Metal	Wavelength (nm)	Slit width
Pb	217.0	0.5
Cd	228.8	0.5
As	193.7	0.5
Cr	357.9	0.5
Ni	232.0	0.2

2.8.5 Preparation of equivalent milk

Milk of several mothers (with different lactation period) were collected and mixed together in a beaker. This milk was taken in a measuring cylinder. A cleaned lactometer poured into the milk. When the lactometer reached the stationary position the reading was taken. The reading was found to be 24 and this value was taken as standard. The determination was carried out at room temperature. A definite amount of milk sample was taken in a measuring cylinder and dissolved in distilled water. Then the milk was cooled to room temperature. Distilled water was added gradually till the lactometer reading becomes 24. The prepared milk was termed as equivalent milk. The equivalent milk was to ascertain how much powdered milk should be added per liter of serving milk.

2.9 Protein, Reducing Sugar, Total Sugar, Moisture and Ash Analysis of Jam, Jelly and Juice

2.9.1 Protein analysis of jam, jelly and juice

Percentages of protein of all samples were determined by micro kjeldahl method [8]. A definite amount of sample was digested into kjeldahl flask using sulfuric acid and digestion mixture (copper sulfate, potassium sulfate and selenium powder). The contents of the flask were digested until the color of the solution became greenish. After cooling, distilled water added and the solution was made alkaline by adding excess amount of 40% NaOH. During distillation ammonia was liberated and absorbed by boric acid (H_3BO_3) that was previously taken in conical flask with appropriate indication. After complete absorption the solution was titrated against standard 0.1N HC1. From the titration value, the percentage of protein of the sample was calculated.

2.9.2 Determination of reducing sugar in jam, jelly and juice

Reducing sugar content was determined by Lane and Eyenon method [5]. 100 mL of distilled water was added to the sample and neutralized with NaOH. 2 mL of lead acetate solution was added and stood for 10 min. The necessary amount of potassium oxalate solution was added to remove the excess lead. The solution was filtered and the volume of the filtrate was measured accurately. The filtrate was used to titrate the Fehling's solution [1+2]. The percentage of reducing sugar was calculated from the titration value.

2.9.3 Determination of total sugar in jam, jelly and juice

50 mL of the clarified solution was pipetted into a conical flask. Added 10 mL of dilute HCI and boiled gently for 5 min. to complete the inversion of sucrose, then cooled. The solution was neutralized with NaOH using phenolphthalein as indicator. The volume was measured accurately. The filtrate was used to titrate the Fehling's solution. The percentage of total sugar was calculated from this titration value.

2.9.4 Determination of moisture in jam, jelly and juice

Small amount of sample was taken in weighed crucible and again the weight was taken. The sample was dried in a drying oven at 105 °C for few hrs. Then the crucible was cooled in desiccators to room temperature and the weight was taken. This process was repeated until a constant weight was achieved. Moisture content was determined from loss in weight of sample [9].

2.9.5 Determination of ash in jam, jelly and juice

For the determination of ash in each of the sample, method of AOAC (Official Methods of Analysis of the Association of Official Analytical Chemists) (2000) was followed. According to the method, sample was weighed in silica crucible. The crucible was heated in a muffle furnace for about 3−5 hrs at 600 °C. It was cooled in desiccators and weighed to completion of ashing.

2.10 Metal Analysis of Jam, Jelly and Juice

2.10.1 Digestion of sample

After weighing, the sample was taken in a 3-nacked digestion flask. About 150−200 mL of concentrated HNO_3 and 30−50 mL $HCIO_4$ were added. The mixture was then refluxed by heating in a hot plate at 120−140 °C for about 8−16 hrs with stirring. After completion of digestion, the volume of the solution was reduced to about 10 mL. This solution was allowed to cool and was diluted with 15−20 mL of redistilled water. Then the solution was filtered through whatmann-40 filter paper and made the filtrate 100 mL in a 100 mL volumetric flask with redistilled water. All the sample solutions of different sets were prepared by allowing this wet digestion method. For each set a blank solution of 100 mL was also prepared. These sample solutions were preserved for analysis.

2.10.2 Preparation of standard solution

The standard solution of the metals Na, K, Ca, Mg, Co, Zn, Pb, Cd, Cr, As and Ni were prepared by pipetting the required amount of the solution form the stock solution manufactured by BDH Laboratory supplies, Poole's BH 151 Td England. The standard solution of a metal of different concentrations was prepared before spectrophotometric determination.

2.10.3 Preparation of calibration curve

A calibration curve of the respective metal was prepared before each metal determination by plotting the absorbance against concentration of the standard solutions. Metal concentration of the samples was measured with the help of these calibration curves. Solutions were prepared with the concentrations covering the optimum linear absorbance range. In all absorbance measurements, the reading was taken after the instrumental zero had been adjusted. By measuring the absorbance of standard solutions of a metal a calibration curve was automatically constructed and displayed in the monitor of the spectrophotometer. The calibration curve was checked after five measurements of the sample solutions by measuring absorbance and concentration of the standard solution according to the sensitivity of the instrument. Thus the accuracy and precision of the analytical data of the sample solutions were ensured.

2.10.4 Atomic absorption spectrophotometric measurement

Same technique was applied for estimation the amount of trace, toxic and essential metals in jam, jellies and juices by atomic absorption spectrophotometer which has been discussed in the Section 2.8.4 for baby powder milk. Flame conditions for analyses of metals in collected samples are shown in Table 2.6 and 2.7.

Table 2.6: Flame condition for trace and toxic metals analyses

Metal	Wavelength (nm)	Slit width
Pb	217.0	0.5
Cd	228.8	0.5
As	193.7	0.5
Cr	357.9	0.5
Ni	232.0	0.2

Table 2.7: Flame condition for essential metals analyses

Metal	Wave length (nm)	Slit width
Na	588.3	0.5
K	765.1	01
Ca	421.9	0.5
Mg	285.2	0.5

2.11 Experimental Techniques

2.11.1 Theory of atomic absorption spectroscopy

Atomic absorption spectrophotometer is a technique designed to determine the amount (concentration) of a specific metal element in a sample [10], utilizing the phenomenon that the atoms in the ground state absorb light of characteristic wavelength passing through an atomic vapor layer of the element. Trace metal determination by atomic absorption spectrometry in liquid is interference free and independent of the molecular form of the metal in the sample. It is established as widely used method for estimation of most of the elements. The technique can be used to analyze the concentration of over 70 different metals in a solution in almost any matrix such as, heavy metals in body fluid, polluted waters, foodstuff, soft drinks, analysis of metallurgical and geochemical samples and the determination of

many metals in soils, crude oils, petroleum products and plastic (Munoz, 1969). Although atomic absorption spectroscopy dates back to the nineteenth century, the modern form was largely developed during the 1950s by a team of Australian chemists. They were led by Alan Walsh and worked at the CSIRO (Commonwealth Science and Industry Research Organization) division of chemical physics in Melbourne, Australia [11]. Detection limits are generally in the range of 0.01 to 1.00 ppm but these can be improved by preconcentration procedures involving solvent extraction or ion exchange etc.

Atomic absorption spectroscopy is the study of the absorption of radiant energy by atoms. In this process, an electron of an atom transits from lower energy state to higher energy state. Since there are no vibration levels associated with atoms, these electronic energy changes are such that for most elements the principal line falls in the near ultraviolet or visible region of the electromagnetic spectrum. For analytical spectroscopy, the lines of greatest interest are the resonance lines of the elements, which are those lines, which arise from transition state in which particular energy state of the atom in involved. The absorption experiment, radiation from a high temperature source (usually an electrical discharge) is passed through the atomic vapor, and the attenuation of the source intensity due to absorption of photons by the atom is measured. For absorption to occur, the photon energy must match the energy difference between the lower energy state in which an atom finds itself and final higher energy state.

Figure 2.9: Atomic absorption spectrophotometer. Model AAS 240 VARIAN, Australia.

Since the ground state is almost invariably the state of highest population, resonance absorption is the most important process. Some fraction of the absorbed radiation back to the lower energy state, but since this radiation is emitted topicall, the fraction collected by the optical system is so small that it is insignificant for the instrument. The frequency of the absorption line is derived from

$$\Delta E = h\nu$$

Where E is the energy of excitation of the atom $E_2 - E_1$, (E_1 is the energy of lower level and E_2 is the energy of higher level), ν is the frequency.

2.11.2 Absorption of characteristic radiation

The extent to which radiation of particular frequency is absorbed by an atomic vapor is related to the length of the path traveled and the concentration of absorbing vapor. This obeys the Beer-Lambert law relating to the sample in solution. Thus for a collimated monochromatic bean of radiation of incident intensity I_o passing through an atomic vapor of thickness I,

$$I_\nu = I_o\, e^{-k\nu} \quad \text{..... (I)}$$

Where I is the intensity of the transmitted radiation at the frequency v and kv is the corresponding absorption coefficient. The value of kv is determined by the concentration of atom, which can absorb at the frequency and is given by expression.

$$\int K\nu \, dv = Be^2/me \, N_v f \ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots \text{(II)}$$

Where m and e represent the mass and charge of the electron, Nv is the number of atoms per cm^3 capable of absorbing radiation of frequency v (i.e., ground state atom) and f is the oscillator strength, defined as the number of electrons per atom capable of being excited by the incident radiation. Therefore, transition from the ground state, the integrated absorption is proportional to Nv, which approximates to the concentration of the element in the sample. In flame only Doppler Effect and to some extent, coalitional broadening contributes significantly to the overall line width. To make accurate measurement of the integrated absorption associated with such narrow line requires that the line width of the radiations source be appreciably smaller than that of the absorption line. In practice this could be achieved with a continuous source only if expensive instruments of extremely high resolving power were used. An alternate arrangement is to measure kv, at the center of the absorption line, where it reaches a maximum value of Nv, is given:

$$K_{max} = \frac{2\lambda^2 (In^2)^{1/2} \pi e^2}{\Delta\lambda \, \pi \, mc^2} \quad N\nu \, f \ldots\ldots\ldots\ldots\ldots \text{(III)}$$

Where the $\Delta\lambda$ is the Doppler line width at a wavelength. Thus K_{max} is directly proportional to Nv, the sample concentration, the radiation from the sharp line source is absorbed at the center of the absorption line and the amount absorbed represents a substantial proportion of the total radiated intensity. By contrast, radiation passed by monochromatic (Bandwidth 1−20 nm) is absorbed over the entire width of the absorption line, which invalidates eq. III and the fraction absorbed is exactly extremely small.

2.11.3 Principle

Atomic absorption spectroscopy (AAS) is an absorption method where radiation is absorbed by an excite atoms in vapor state. The technique involves the study of the absorption of radiation (usually in the ultraviolet, visible region) by neutral atoms in

the gaseous state. Thus in atomic absorption spectroscopy, the sample is competed into an atomic vapor and then the absorption by atomic vapor is measured at a selected wavelength, which is characteristic of each individual element. The measured absorption is directly proportion to the concentration and analysis are made by comparing third absorbable with that given under the sample experimental conditions by reference sample of known composition. Almost all analytical applications of the atomic absorption method at the present time involve spraying a solution of the sample into the flame. For this reason, the technique is also known as absorption flame photometry.

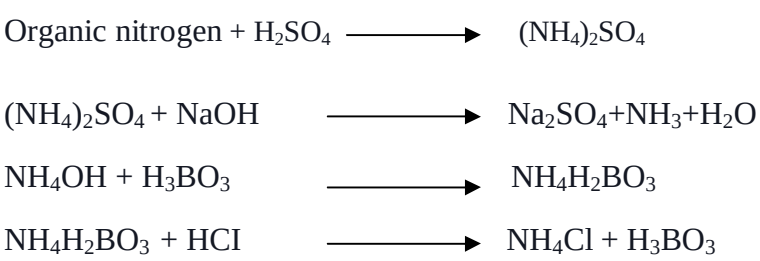
2.12 Determination of the Bio-Chemical Parameters in Baby Powder Milk

The Kjeldahl procedure for the determination of nitrogen in biological materials is characterized by the use of boiling, concentrated sulfuric acid to effect the oxidative destruction of the organic matter of the sample. The acid also reduces organic nitrogen to ammonia. The process is facilitated by the use of a catalyst. The ammonia is retained in the acid digest as ammonium bisulphate [12]. The digest is made alkaline using sodium hydroxide and the ammonia is distilled off. The ammonia is measured by titration [13].

The traditional method of estimating the protein content of food is to multiply its content of nitrogen by a suitable conversion factor. This factor will vary according to the nitrogen content of the particular proteins and can vary from 12 − 30%. The factor of 6.25 is generally used and it would apply to a protein containing 16% nitrogen [13]. For dairy products, a factor of 6.38 is used [14].

% Protein = % of nitrogen × 6.38 (Conversion factor)

Chemical reaction:



2.12.1 Determination of protein

2.12.1. a *Apparatus*

- Nitrogen Digesting apparatus (Model-OSK 6937)
- Nitrogen Distillation apparatus (Model - OSK 6936)
- Kjeldahl flask (250 mL capacity)
- Conical flask (250 mL)
- Analytical balance
- Measuring cylinder
- Burette

2.12.1.b *Reagents*

- Concentrated sulfuric acid (H_2SO_4)
- Digestion mixture
- Sodium hydroxide (40%)
- Pumice stone
- Boric acid (4%)
- Mixed indicator
- Hydrochloric acid (HCl, 0.1N)

2.12.1.c *Preparation of reagents*

1) Catalyst mixture: Copper sulfate and potassium sulfate in the ratio of 1:4 with a pinch addition of selenium dioxide as catalyst promoter were mixed well and preserved in stoppered bottle.
2) 40% sodium hydroxide: 200 g of sodium hydroxide was dissolved in distilled water and volume was made 500 mL with distilled water.
3) 4% boric acid: 40 g boric acid was dissolved in distilled water and volume of the solution was made 1000 mL with distilled water.
5) Mixed indicator: Mixed indicator was prepared by dissolving 100 mg of methyl red and 25 mg methylene blue in 100 mL of 95% ethanol.
6) Phenolphthalein indicator: 1% phenolphthalein was dissolved in 100 mL alcohol.

2.12.1.d *Procedure*

i) Digestion phase

1) About 0.5 g of sample was taken on a paper sheet.

2. The paper with sample was carefully inserted into the previously cleaned and dried Kjeldahl flask. About 2 g of catalyst mixture and 10 mL of conc. H_2SO_4 were added into the flask.
3) Then the sample was digested for 2−3 hours by heating at 350−400 °C in the digestion chamber until the color changes from black to light bottle green.

4) It was then cooled under running tap water.

5) 50 mL distilled water was added carefully and slowly.

ii) Distillation phase

1) 50 mL boric acid was taken in a 250 mL conical flask and about 5 drops of mixed indicator solution was added.

2) After cooling the Kjeldahl flask, pinch of pumice stone was taken in the flask to prevent bumping. Then about 33 mL 40% sodium hydroxide was added slowly.

3) Kjeldahl flask was adjusted into the distillation unit.

4) Receiving tube was dipped under 4% boric acid solution previously taken in a conical flask and distilled for about five hrs.

5. After complete distillation, ammonia absorbed boric acid was taken and titrated against standard 0.1N HCI until the color of the indicator pointed at an acidic condition (from blue to brown color)

Calculation:

The percentage of protein present in the sample was calculated by the following equation [1, 9]:

$$\%\ of\ Nitrogen = \frac{0.014 \times Normality\ of\ HCI \times Volume\ of\ HCI\ required \times 100}{Weight\ of\ sample}$$

% of Protein = % of nitrogen × 6.38

The percentages of protein in baby powder milk of different brands are given in Table-3.1 and 3.5.

2.12.2 Determination of acidity

Measuring milk acidity is an important test used to determine milk quality and usually known acidity is the result of titration [15]. The titratable acidity is the capacity of combination with a base [16]. The measurement principle is unique, and is based on adding to a given volume of milk, the necessary volume of alkaline solution (sodium

hydroxide) of an exact concentration until the neutralization point is reached, which is determined by the presence of an indicator, generally phenolphthalein, which turns from colorless to pink at pH 8.4.

2.12.2.a *Apparatus*

- Burette
- Conical flask
- Beaker
- Pipette
- Volumetric flask
- Measuring cylinder

2.12.2.b *Reagents*

- 0.1N sodium hydroxide
- Phenolphthalein
- Distilled water

2.12.2.c *Procedure*

Distilled water was added to powder milk in order to dilute them to fluid milk basis before titrating for acidity [6]. About 8−10 g of powder milk was dissolved in small amount of warmed distilled water and cooled. Then titration was carried out with NaOH using phenolphthalein indicator.

Calculation: Titratabe acidity of powder milk was measured by the following formula [6].

$$\%\ of\ \ lactic\ \ \ acid\ \ = \frac{(0.1N)\,alkali\ \ \ mL\ \times 0.009}{Grams\ \ \ of\ \ sample} \times 100$$

The acidity of baby (0−6 months and 6−24 months) powder milk of different brands is given in Table 3.3 and 3.7 respectively.

2.12.3 Determination of lactose (milk sugar)

Lactose is reducing sugar i.e., it is capable of reducing appropriate reducing agents, one of which is alkaline copper sulphate ($CuSO_4$ in sodium potassium tartrate; Fehling's solution.). For analysis by titration with Fehling's solution, the sample is treated to precipitate protein and fat, filtered and the filtrate titrated with alkaline $CuSO_4$, while heating [17]. 10 mL Fehling's solution corresponds to 0.0675 g lactose (milk sugar) [18].

2.12.3.a *Apparatus*

- Burette
- Conical flask
- Pipette
- Measuring cylinder
- Volumetric flask
- Funnel
- Whatmann-40 filter paper
- Beaker
- Glass rod
- Burner
- Fehling solution-1

2.12.3.b *Reagents*

- Fehling solution-2
- 0.1N sodium hydroxide
- Distilled water

2.12.3.c *Preparation of reagents*

1) Fehling solution-1: 34.6 g of pure $CuSO_4.5H_2O$ was dissolved in distilled water and the solution was made up to 500 mL.
2) Fehling solution-2: 70 g NaOH with 173 g Rochelle salt (sodium potassium tartrate) were taken and dissolved in distilled water and made the solution up to 500 mL with distilled water.
3) N/10 NaOH: 1 g NaOH pelette was dissolved in distilled water and the solution was made up to 250 mL with distilled water.

2.12.3.d *Procedure*

Lactose of the milk samples was determined by volumetric method. To estimate the lactose content in powder milk about 2 g milk sample was taken in a small beaker and dissolved with 10 mL warm distilled water. 10 mL of liquid milk was diluted with distilled water to a volume of 200 mL and about 8 drops of 10 percent acetic acid solution was added. After the precipitate was settled, it was filtered off and washed with cold water. The filtrate was boiled in a flask and the albumin

precipitated. This solution was cooled and filtered again. The precipitate was washed with cold water. The filtrate and washed water were mixed and measured accurately in a measuring cylinder. A portion of the filtrate was placed in a burette and this was run into a boiling mixture of 10 mL Fehling's solution (5 mL Fehling solution-1 and 5 mL Fehling solution-2) and 40 mL water. After the copper was completely precipitated the number of milliliters used was recorded.

Calculation:

The percentages of lactose of powder milk were measured by the following formula [3].

10 mL Fehling's solution corresponds to 0.0675 g milk sugar

The percentages of lactose of baby (0−6 months and 6−24 months) powder milk of different brands are given in Table3.2 and 3.6 respectively.

2.13 Estimation of the Amount of Trace and Toxic Metals in Baby Powder Milk and Baby Cereals.

2.13.1 Estimation of the amount of trace (Co and Zn) and toxic metals (pb, Cd, Cr, As and Ni) in baby (0−6 months) powder milk

2.13.1.a *Reagents and chemicals*

Analytical reagent grade $HClO_4$ and HNO_3 were procured from E. Merck, Germany. Certified standard stock solutions of Co, Zn, Pb, Cd, Cr, As and Ni were obtained from BDH, England for calibration purpose. All working solutions were also prepared in redistilled water.

2.13.1.b *Apparatus*

- Hot plate with magnetic stirrer
- Heating mantle
- Three necked digestion flask (500 mL)
- Condenser
- Volumetric flask of different volumes
- Pipette of different volumes
- Atomic Absorption Flame Emission Spectrophotometer (Model AA-6401 F, Shimadzu, Japan).

2.13.1.c *Procedure*

About 15 g of a sample was taken in a three necked flask. 180 mL HNO_3 and 30 mL $HClO_4$ were added. The mixture was then refluxed by heating with the help of hot plate with magnetic stirrer at 120 −140 ºC for about 20−30 hours. During digestion the color of the solution turned yellowish. The completion of the oxidation of organic materials in the sample was indicated by the disappearance of the brown color and the appearance of dense white fume. After complete digestion the volume of the solution was reduced to about 15−20 mL. 15 mL redistilled water was added an again volume was reduced to about 10−20 mL. This solution was allowed to cool and diluted with 10−15 mL redistilled water. Then the solution was filtered through whatmann-40 filter paper and made the filtrate to 50 mL in a 50 mL volumetric flask with redistilled water. All the sample solutions were prepared by allowing this wet digestion process. Similarly a blank solution of 50 mL was also prepared. These solutions were preserved for analysis.

The amount of trace and toxic metals in baby (0−6 months) powder milk were measured by atomic absorption spectrophotometric method. Preparation of calibration curves for estimation of the amount of trace and toxic metals in baby (0−6 months) powder milk [shown in Figure 3.1−3.7] are described in Chapter three, Section 3.2.4.a.

2.13.2 Estimation of the amount of trace and toxic metals in baby (6−24 months) cereals.

2.13.2.a *Reagents and chemicals*

Analytical reagent grade $HClO_4$ and HNO_3 were procured from E. Merck, Germany. certified standard stock solutions of Co, Zn, Pb, Cd, Cr, As and Ni were obtained from BDH, England for calibration purpose. All working solutions were also prepared in redistilled water.

2.13.2.b *Apparatus*

- Hot plate with magnetic stirrer
- Heating mantle
- Three necked digestion flask (500 mL)
- Condenser

- Volumetric flask of different volumes
- Pipette of different volumes
- Atomic absorption flame emission spectrophotometer (Model AA-6401 F, Shimadzu, Japan).

2.13.2.c *Procedure*

About 15 g of a sample was taken in a three necked flask. 180 mL HNO_3 and 30 mL $HClO_4$ were added. The mixture was then refluxed by heating with the help of hot plate with magnetic stirrer at 120−140 ºC for about 20−30 hrs. During digestion the color of the solution turned yellowish. The completion of the oxidation of organic materials in the sample was indicated by the disappearance of the brown color and the appearance of dense white fume. After complete digestion the volume of the solution was reduced to about 15−20 mL. 15 mL redistilled water was added and again volume was reduced to about 10−20 mL. This solution was allowed to cool and diluted with 10−15 mL redistilled water. Then the solution was filtered through whatmann-40 filter paper and made the filtrate to 50 mL in a 50 mL volumetric flask with redistilled water. All the sample solutions were prepared by allowing this wet digestion process. Similarly a blank solution of 50 mL was also prepared. These solutions were preserved for analysis.

The amount of trace and toxic metals in baby (6−24 months) Cereals were measured by atomic absorption spectrophotometric method. Preparation of calibration curves for estimation of the amount of trace and toxic metals in baby (6−24 months) cereals [Shown in Figure 3.1−3.7] are described in Chapter three, Section 3.2.4.a.

2.14 Estimation of Moisture, Total Solid, Ash, Protein and Sugar (Reducing Sugar and Total Sugar) in Jams, Jellies and Juices of Different Brands.

2.14.1 Estimation of moisture in jam, jelly and juice

A commonly used procedure for determining the moisture content of a food product is based on the separation of water from the solids and its measurements as the resulting loss in weight or by measurement of the amount of water lost. The accurate determination of moisture is difficult because of the problem of completely separating all the water from the food product without completely causing its decomposition with concomitant production of water, which would be included in the determination [18].Various drying oven methods (Official methods of Analysis of the Association of

Official Analytical Chemists) were approved for many food products. So, moisture content of collected samples was determined by drying the samples at 105 °C in a drying oven till a constant weight was attained [19]. Moisture of jams, jellies and juices was determined by oven drying method [19].

2.14.1.a *Apparatus*

- Crucible
- Electric balance
- Oven
- Desiccators

2.14.1.b *Procedure*

Crucible was cleaned, dried and weight was taken. Small amount of sample was placed in the dish and weight was taken. The crucible was kept in a drying oven and dried at 105 °C for three hours. Then the crucible cooled in desiccators to room temperature and the weight was taken. This process was continued until a constant weight was achieved. Moisture content of samples is calculated as follows:

$$\% \text{ of moisture} = \frac{W_1 - W_2}{W_1 - W} \times 100$$

Where,

W = Weight of the empty crucible

W_1 = Weight of the crucible + sample

W_2 = Weight of the crucible + dried sample.

Four replicate measurements were taken for the precision of the analytical data. The moisture content of samples is given in Table 4.5, 4.6 and 4.7 with their standard deviations.

2.14.2 Estimation of total solids

Total solids were estimated by deducting present moisture form hundred as described by James [19].

% Total solid = 100 − % Moisture

The total solid content of samples is given in Table 4.8, 4.9 and 4.10.

2.14.3 Estimation of ash

Ash is the residue of the inorganic matter (mineral) of the sample after burning. If the sample is heated in a muffle furnace at 600 °C, the organic matter is evaporated and residues are called ash. For determination of ash content, method of AOAC was followed. According to this method sample was heated in a muffle furnace for about 5 hours at 600 °C [4].

2.14.3.a *Apparatus*

- Crucible
- Electric balance
- Desiccators
- Muffle Furnace
- Drying Oven

2.14.3.b *Procedure*

Crucible was cleaned, dried and weight was taken. Small amount of sample was placed in the dish and weight was taken. The crucible containing the sample was placed to a Muffle furnace at 550−600 °C for 4 to 6 hrs. Decreased the temperature to 105 °C and remained it for 20 minutes. Cooled the porcelain crucible in desiccators and recorded the final weight of the crucible with ash.

Calculation:

$$\% \text{ Ash} = \frac{\text{Weight of ashed sample}}{\text{Weight of sample}} \times 100$$

The ash content of samples is given in Table 4.11, 4.12 and 4.13

2.14.4 Estimation of protein in jam, jelly and juice

The Kjeldahl procedure for the determination of nitrogen in biological materials is characterized by the use of boiling concentrated sulfuric acid to effect the oxidative destruction of the organic matter of the sample. The acid also reduces organic nitrogen to ammonia. The process is facilitated by the use of a catalyst. The ammonia is retained in the acid digest as ammonium bisulphate [12]. The digest is made alkaline using the ammonia is disgust is made alkaline using sodium hydroxide and the ammonia is distilled off. The ammonia is measured by titration [13].

The traditional method of estimating the protein content of food is to multiply its content of nitrogen by a suitable conversion factor. This factor will vary according to the nitrogen content of the particular proteins and can vary from 12 to 30%. The factor of 6.25 is generally used and it would apply to a protein containing 16% nitrogen [13].

% Protein = % of nitrogen × 0 6.25 (Conversion factor)

Chemical reaction

Organic nitrogen + H_2SO_4 ⟶ $(NH_4)_2SO_4$

$(NH_4)_2SO_4$ + NaOH ⟶ Na_2SO_4+NH_3+H_2O

NH_4OH + H_3BO_3 ⟶ $NH_4H_2BO_3$

$NH_4H_2BO_3$ + HCI ⟶ NH_4Cl + H_3BO_3

2.14.4.a *Apparatus*

- Nitrogen digesting apparatus (Model-OSK 6937)
- Nitrogen distillation apparatus (Model-OSK 6936)
- Kjeldahl flask (250 mL capacity)
- Conical flask (250 mL)
- Analytical balance
- Measuring cylinder
- Burette

2.14.4.b *Reagents*

- Concentrated sulfuric acid (H_2SO_4)
- Digestion mixture
- Sodium hydrochloric (40%)
- Pumice stone
- Boric acid (4%)
- Mixed indicator
- Hydrochloric acid (HC1, 0.1N)

2.14.4.c *Preparation of reagents*

1) Catalyst mixture: Copper sulfate and potassium sulfate in the ratio of 1: 4 with a pinch addition of selenium dioxide as catalyst promoter were mixed well and preserved in stoppered bottle.

2) 40% sodium hydroxide: 200 g of sodium hydroxide was dissolved in distilled water and volume was made 500 mL with distilled water.

3) 4% boric acid: 40 g boric acid was dissolved in distilled water and volume of the solution was made 1000 mL with distilled water.

5) Mixed indicator: Mixed indicator was prepared by dissolving 100 mg of methyl red and 25 mg ethylene blue in 100 mL of 95% ethanol.

6) Phenolphthalein indicator: 1% Phenolphthalein was dissolved in 100 mL alcohol.

2.14.4.d *Procedure*

i) Digestion phase

About 0.5 g of sample was taken on a paper. The paper with sample was carefully inserted into the previously cleaned and dried Kjeldahl flask. About 2 g of catalyst mixture and 10 mL of conc. H_2SO_4 were added into the flask. Then the sample was digested for 2−3 hrs by hating at 350−400 °C in the digestion chamber until the color changes from black to light bottle green. It was then cooled under running tap water. 50 mL distilled water was added carefully and slowly.

ii) Distillation phase

50 mL boric acid was taken in a 250 mL conical flask and about 5 drops of mixed indicator solution was added. After cooling Kjeldahl flask, pinch of pumice was taken in the flask to prevent bumping. Then about 33 mL 40% sodium hydroxide was added slowly. Kjeldahl flask was adjusted into the distillation unit. Receiving tube was dipped under 4% boric acid solution previously taken in a conical flask and distilled for about five hrs. After complete distillation, ammonia absorbed boric acid was taken and titrated against standard 0.1N HCl until the color of the indicator pointed at an acidic condition (from blue to brown color).

iii) Calculation

The percentage of protein present in the sample was calculated by the following equation [1, 19].

$$\%\ of\ Nitrogen = \frac{0.014 \times Normality\ of\ HCI \times Volume\ of\ HCI\ required \times 100}{Weight\ of\ sample}$$

% of Protein = % of nitrogen × 6.25

The percentage of protein in jams, jellies and juices of different brands are given in Table 4.14, 4.15 and 4.16 respectively.

2.14.5 Estimation of sugar in jams, jellies and juices

Jams, jellies and juices are fruit products. Sugar is an important ingredient of fruit and fruit product. Fruit sugar is something known as fructose. Fructose is often recommended for and consumed by people with diabetes mellitus or hypoglycemia, because it has a very low glycemic index. glycemic index is a ranking system for carbohydrates based on their immediate effect on blood glucose levels. Sucrose (common name: table sugar, also called saccharose) is a disaccharide (glucose + fructose) with the molecular formula $C_{12}H_{22}O_{11}$. There are differences between the different sugars. When we eat sucrose, our body quickly breaks it down into roughly equal parts of glucose and fructose. Glucose is the sugar our bodies use for both physical and mental energy. When our bodies sense an increase of glucose in the blood, it immediately directs the pancreas to push insulin into the blood stream. With the insulin, the body is able to burn the glucose as energy. If there's too much glucose in the blood stream to be used as energy, the glucose can be changed to glycogen, the body's short-term storage energy supply. And if the glycogen pool is already full, the body will turn it into long term storage in the form of fat. When a person eats a lot of sucrose sweets at one time, the large amount of sugar dramatically raises the blood-sugar level but the results can be radically different if no medication is given. Eating a huge amount of sugar at once with its resultant spike in the blood-sugar level can cause stress to a weak pancreas as it struggles to deliver enough insulin to bring down the blood-sugar to acceptable levels. This cycle is especially hard on people who have an abnormal pancreas. If the pancreas doesn't produce enough insulin that person is considered diabetic.If it produces too much insulin they are considered hypoglycemic.

These people will generally suffer from an abnormal glucose level depending on what their condition is. For this reason, it is very important to know the amount or quantity of sugar in our food items. Lane and Eynon method is used to determine the amount of sugar in the samples. To determine the amount of reducing sugar, clarified sample solution is filtered and measured accurately. The filtrate is used to titration the feeling's solution [1+2]. The percentage of reducing sugar is calculated from this titrated value. In case of total sugar, HCI is added and boiled to the clarified sample solution to complete the inversion of sucrose. After neutralization, the filtrate is used to titration the Feeling's solution. The percentage of total sugar can be calculated from this titrated value.

2.14.5.a *Apparatus*

- Burette
- Conical flask
- Pipette
- Measuring cylinder
- Volumetric flask
- Funnel
- Whatmann-40 filter paper
- Beaker
- Glass rod
- Burner

2.14.5.b *Reagents*

- Fehling's solution-1
- Fehling's solution-2
- 10% (N) sodium hydroxide
- Distilled water
- 1 N Hydrochloric acid
- Phenolphtalein
- Methylene blue indicator
- 45% Neutral lead acetate solution
- 22% Potassium oxalate solution
- Standard invert sugar solution

2.14.5.c *Preparation of reagents*

1) Fehling's solution-1: 34.6 g of pure $CuSO_4.5H_2O$ was dissolved in distilled water and the solution was made up to 500 mL.

2) Fehling's solution-2: 70 g NaOH with 173 g Rochelle salt (sodium potassium tartrate) were taken and dissolved in distilled water and made the solution upto 500 mL with distilled water.

3) Methylene blue 1%: To make 1%, 0.5 g methylene blue was taken and volume was made 50 mL.

4) 45% neutral lead acetate solution: 225 g of neutral lead acetate was dissolved in distilled water and the solution was made up to 500 mL.

5) 22% Sodium oxalate solution: 22g of sodium oxalate ($Na_2C_2O_4.H_2O$) was dissolved in distilled water and the volume was made 100 mL.

6) Standard invert sugar solution: 9.5 g of AR sucrose was taken onto a 1 litre volumetric flask. 100 mL water and 5 mL conc. HCI was added. It was allowed to stand for 3 days 20−25 °C for inversion to take place, and then made up to mark with water.

2.14.5.d *Procedure for reducing sugar*

5 g of jam or jelly sample was taken in a 500 mL beaker. 100 mL warm water was added and neutralized with 10% NaOH. 2 mL of lead acetate solution was added and it was kept for 10 min. The necessary amount of sodium oxalate solution was added to remove the excess of lead. The volume was made up to 250 mL with distilled water and filtered. 10 mL of a mixed Fehling's solution (5 mL Fehling's solution-1 and 5 mL Fehling's solution-2) were pipetted into a conical flask. A burette was filled with the clarified sample solution and running the whole volume required to reduce the Fehling's solutions so that, 0.5−1.0 mL was still required to complete the titration. The content of the flask was mixed and then heated to boiling for 2 min. Three drops of methylene blue indicator were added. Then the titration was continued till color completely disappeared.

25 g of juice was taken to prepare the juice sample for sugar analysis. Then, the above procedure was followed.

Calculation:

The percentage of reducing sugar was calculated by the following formula:

$$\text{Reducing sugars (mg per 100 mL)} = \frac{\text{Factor x100}}{\textit{Titre}}$$

$$\text{\% of reducing sugar} = \frac{\text{mg/100 mL x Dilution x100}}{\text{Weight of the sample x 1000}}$$

The factor is obtained from the invert sugar Table by Pearson, [20].

2.14.5.e *Procedure for total sugar*

5 g of jam or jelly sample was taken in a 500 mL beaker. Then 100 mL warm water was added and neutralized with 10% NaOH. After that 2 mL of lead acetate solution was added and kept for 10 min. The necessary amount of sodium oxalate solution was added to remove the excess lead. The volume was made up to 250 mL with distilled water and filtered. 50 mL of the clarified and deleaded solution was transferred to a 250 mL flask. 10 mL 1N HCI was added into the flask. This solution was then boiled for 2 min. After cooling, 2−3 drops of phenolphthalein were added and the contents were neutralized with NaOH. The solution was filtered and the volume was made 250 mL. Thus the sample solution was prepared. 10 mL of a mixed Fehling's solution (5 mL Fehlng's solution-1 and 5 mL Fehling's solution-2) were pipetted into a conical flask. A burette was filled with the clarified sample solution and running the whole volume required to reduce the Fehling's solutions so that, 0.5−1.0 mL was still required to complete the titration. The content of the flask was mixed and then heated to boiling for 2 min. Three drops of methylene blue indicator were added. Then the titration was continued till color completely disappeared.

25 g of juice was taken to prepare the juice sample for sugar analysis. Then, the above procedure was followed.

Calculation:

The percentage of total sugar was calculated by the following formula:

$$\text{Reducing sugars (mg per 100 mL)} = \frac{\text{Factor x100}}{\text{Titre}}$$

$$\% \text{ Total sugars} = \frac{\text{mg/100 mL x Dilution x100}}{\text{Weight of the sample x 1000}}$$

The factor is obtained from the invert sugar Table by Pearson, [20].

c) % Non-reducing sugar = Total sugar – Reducing sugar

Four replicate measurements were taken for the precision of the analytical data. The percentage of reducing sugar, non-reducing sugar and total sugar of different jams, jellies and juices are given in Table 4.17. 4.18, 4.19, 4.20, 4.21, 4.22, 4.23, 4.24 and 4.25.

2.15 Estimation of the Amount of Trace and Toxic Metals in Jams, Jellies and Juices

2.15.1 Reagents and chemicals

Analytical reagent grade $HClO_4$ and HNO_3 were procured from E. Merck, Germany. certified standard stock solutions of Co, Zn, Pb, Cd, Cr, As and Ni were obtained from BDH, England for calibration purpose. All working solutions were also prepared in redistilled water.

2.15.2 Apparatus

- Hot plate with magnetic stirrer
- Heating mantle
- Three necked digestion flask (500 mL)
- Condenser
- Volumetric flask of different volumes
- Pipette of different volumes
- Atomic Absorption Flame Emission Spectrophotometer (Model AA-6401 F, Shimadzu, Japan).

2.15.3 Procedure

About 30 g of a sample was taken in a three necked flask. 100 mL HNO_3 and 20 mL $HClO_4$ were added. The mixture was then refluxed by heating on a hot plate with magnetic stirrer at 120−140 °C for about 12 hrs. After complete digestion the volume

of the solution was reduced to about 8−10 mL. 100 mL redistilled water was added and again volume was reduced to about 15−20 mL. This solution was allowed to cool. Then the solution was transferred into a 100 mL volumetric flask. The total volume was made just 100 mL with redistilled water. All the sample solutions were prepared by allowing this wet digestion process. Similarly a blank solution of 100 mL was also prepared. These solutions were preserved for analysis. About 75 mL of juice sample was taken and same technique was applied for digestion. Estimation of the amount of trace and toxic metals in jams, jellies and juices have been discussed in Chapter four and Section 4.2.6.

2.16 Estimation of Essential Metals (Na, K, Ca and Mg) in Jams, Jellies and Juices of Different Brands.

Estimation of essential metals in jams, jellies and juices have been discussed in Chapter Four and Section 4.2.7.

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CHAPTER THREE

Estimation of the Bio-chemical Parameters and the Amount of Trace and Toxic Metals in Baby (0−6 Months and 6−24 Months) Milk Powder and (6−24 Months) Baby Cereals

CHAPTER THREE

Estimation of the Bio-chemical Parameters and the Amount of Trace and Toxic Metals in Baby (0−6 Months and 6−24 Mon ths) Milk Powder and (6−24 Months) Baby Cereals

3.1 Introduction

During early life, infants usually consume a diet predominantly made up of milk which is the main source of their protein, energy and minerals. Milk is a complex biological fluid including water (87.3%), proteins (3.2%), carbohydrates especially lactose (4.6%), fat (3.9%), and a mineral fraction (0.7%). Milk is considered as a rich source of some essential trace metals like zinc, but deficient in copper [1].

Mothers are consistently being reminded about the necessity of breast feeding to their infants. However, some instances, like the presence of hypogalactia, inverted nipple, nipple tenderness and other medical conditions prohibiting breast feeding are inevitable, thus it needs the formula milk for infant. Infants, particularly in the 6−12 month's age group are vulnerable to infection due to their immature immune system. This is also the time when they are weaned from a pure breast milk diet to one with solid food. Infant formula milk, when in liquid form, may be used either directly or diluted appropriately with water before feeding. In powder form it requires water for preparation [2].

Milk may be defined as the normal secretion of the mammary glands of mammals. For centuries, milk has been recognized as an almost indispensable food for mankind. Milk contains all the food constituents required in the human diet and in essentially the proper proportions. Cow, goat, buffalo, sheep and the camel are usually used as a source of milk for man. The term milk will always be understood as referring to the milk of the cow, because the cow supplies such a large proportion of the product used that little attention need to be directed towards other sources [3]. As a food, milk serves the following broad purposes: (a) growth, (b) reproduction, (c) supply of energy, (d) maintenance and repair, and (e) appetite satisfaction. Nutritionally, milk has been defined as the most nearly perfect food. It provides more essential nutrients in significant amounts than any other single food [4].

Protein in an extremely important class of naturally occurring compound that is essential to all life processes. It performs a variety of functions in living organisms ranging from providing structure to reproduction. Milk proteins represent one of the greatest contributions to human nutrition [4]. Proteins are polymers of amino acids. Proteins are among the most complex of organic substances. They contain carbon, hydrogen, oxygen, nitrogen, sulphur and sometimes phosphorus. They are characterized chiefly by the fact that they contain nitrogen. Protein is indispensable part of the food of animals as it is the chief constituent of the protoplasm which forms the vital part of every living cell. Familiar examples of protein are the white part of an egg and the curd of milk. In milk the average composition of fat is about 3.4 percent, the protein content is approximately 3 percent. Nearly all samples of normal milk are between 2.80 and 4.00 percent. The protein of milk is not a single compound but includes two major proteins and other in small quantitiy. Of these casein constitutes about 80 percent of the total milk and lactalbumin is 18 percent. A third protein recognized as present in milk is lactoglobulin. It is present in very small amount, probably about 0.05 to 0.07 percent. Milk contains a group of nitrogenous substances in addition to the proteins. These substances do not occur in large quantities but they are known to be present. The non-protein nitrogenous substances of milk may be as follows; urea nitrogen, amino nitrogen, creatinine, uric acid, adenine and guanine. Their presence contributes slightly to the protein analysis of milk or milk products. As a result the protein value becomes slightly higher [3].

When milk is freshly drawn from the cow, it has shown an amphoteric reaction. Normal fresh milk has a hydrogen-ion concentration of approximate P^H 6.5 to 6.6, which indicates that the milk is slightly acidic. When normal fresh milk is titrated with an alkali solution using phenolphthalein as an indicator, it appears acid and showing 0.10 to 0.26 percent acid. It should be pointed out that perfectly fresh milk contains no lactic acid. The acidity of fresh milk is due to certain constituents of milk some of which give an acid reaction and some of which actually combine with the alkali. The acidity of fresh milk is known to be due to phosphates of milk, the proteins (casein and albumin) and the presence of slight carbon dioxide and citrates. The albumin accounts regularly for a little less than 0.01 percent [5]. It should be clear that the true acidity of milk actually cannot be measured by titrating with a standard alkali

solution. The practice of titrating milk with an alkali is followed because it is known that the acidity of milk reaches a point near 0.18 to 0.20 percent. A large proportion of this acidity is due to lactic acid formed by the action of bacteria on the lactose [3]. Milk sugar, commonly designated by the chemist as lactose, is found only in milk. It is a reducing disaccharide which upon hydrolysis yields one molecule of glucose and one molecule of galactose. The carbohydrate lactose gives milk its sweet taste and contributes about 40% of calories [6]. It has the molecular formula $C_{12}H_{22}O_{11}$, same as ordinary cane sugar or sucrose. It differs from sucrose, however, in molecular configuration, relative sweetness, solubility and chemical reactivity. Lactose is made up of β-D-glucose and it is held together by β (1−4) glycoside bond. Lactose is known as reducing sugar. The reducing groups of glucose and fructose are involved in glycoside bond and it is known as non-reducing sugar [7].

Sucrose is about six times as sweet as lactose. Lactose is true solution in the milk serum. Prolonged heating of aqueous solution of lactose at temperatures 100−130 ℃ results in a decomposition which is indicated by a light-brown or caramel color. In heat-treated milk, in the presence of proteins and certain mineral salts, brown color develops readily and gives rise to the browning of the sterilized milk and certain condensed and dried milk products during storage. The greater reactivity of lactose as compared with sucrose is due to the presence of a potentially free aldehyde group in the glucose portion of the molecule. Milk contains on the average about 4.8 percent of lactose [3]. Processing treatments, with the aim of extending shelf-life, have direct influences on the nutritional, biological and functional properties of milk nutrients [8, 9].

Milk is a complex colloidal system in which the dispersion medium is water that contains salts and sugar. Therefore milk is heavier than water. The specific gravity of milk is influenced by the relation of its constituents, each of which has a different specific gravity, e.g. fat, 0.93; lactose, 1.666; proteins, 1.346; casein, 1.31; salts, 4.12. The specific gravity of milk is usually determined by a lactometer. The lactometer is a hydrometer with a scale adapted to the limits of the specific gravity of milk.

The objective of this study was to determine the amount of Co, Zn, Pb, Cd, Cr, As and Ni in baby (0−6 months and 6−24 months) powder milk and baby (6−24 months) cereals of different brands available in the market. The present study also deals with

some important biochemical parameters such as percentage of protein, lactose and acidity of the studied milk samples.

3.2 Experimental

3.2.1 Determination of protein

Discussed In Chapter Two, Section 2.12.1

3.2.2 Determination of titratable acidity

Discussed In Chapter Two, Section 2.12.2

3.2.3 Determination of lactose (Milk sugar)

Discussed In Chapter Two, Section 2.12.3

3.2.4 Estimation of the amount of trace (Co and Zn) and toxic metals (Pb, Cd, Cr, As and Ni) in baby powder milk and (6−24 months) baby cereals.

Reagents, chemicals, apparatus and digestion of sample are discussed in Chapter Two, Section 2.13.1. In this Chapter, preparation of calibration curves for the estimation of the amount of trace and toxic metals in baby powder milk and cereals have been discussed.

3.2.4.a *Preparation of calibration curves for estimation of the amount of Co, Zn, Pb, Cd, Cr, As and Ni in baby powder milk and baby cereals.*

0.5, 1, 2 and 4 ppm solutions of cobalt in deionized water are prepared from the stock solution (1000 ppm standard Co solution). The absorbances of these solutions are measured by AAS. The data obtained from AAS the following calibration curve for Co is prepared. The unknown concentrations of Co of the investigated samples are determined form the calibration curve and those are tabulated in Table 3.10, 3.13 and 3.15.

Similarly the calibration curves for the determination of the amount of Zn, Pb, Cd, Cr, As and Ni are prepared. Then the unknown concentrations of these metals are determined with the help of these calibration curves and the obtained results are given in Table 3.10, 3.13 and 3.15.

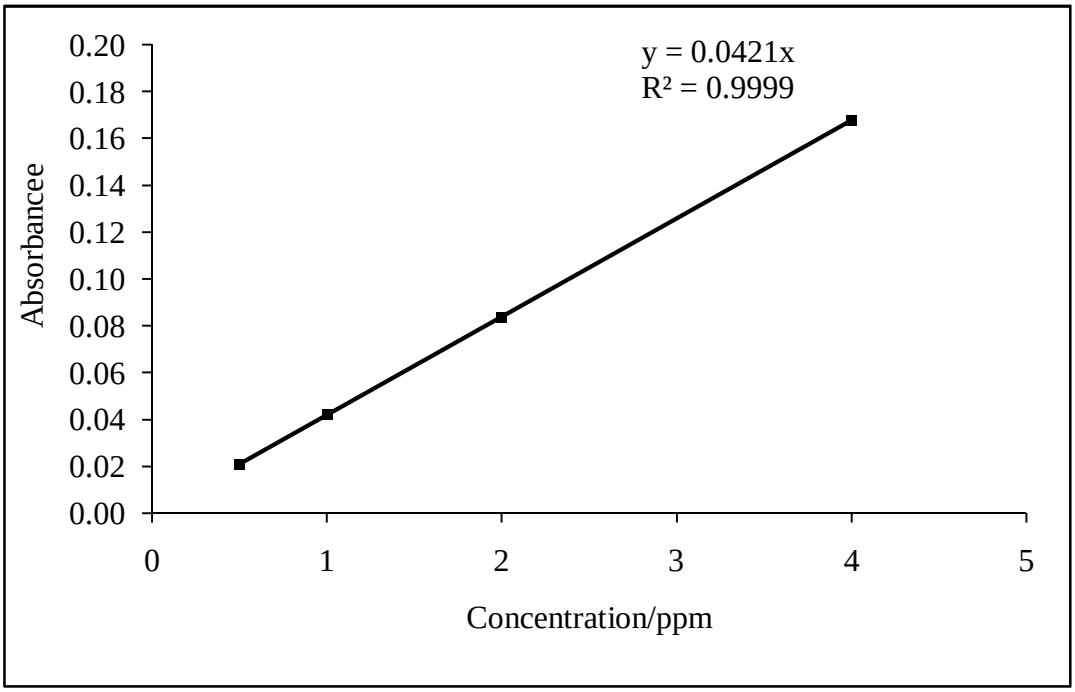

Figure 3.1: Calibration curve for the determination of cobalt

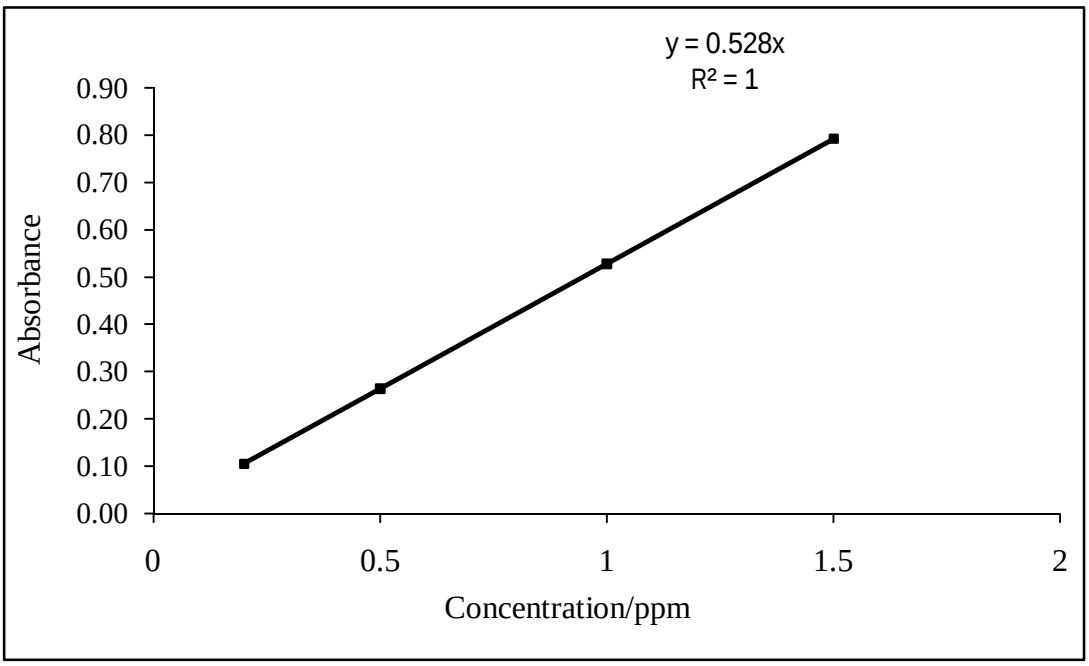

Figure 3.2: Calibration curve for the determination of zinc

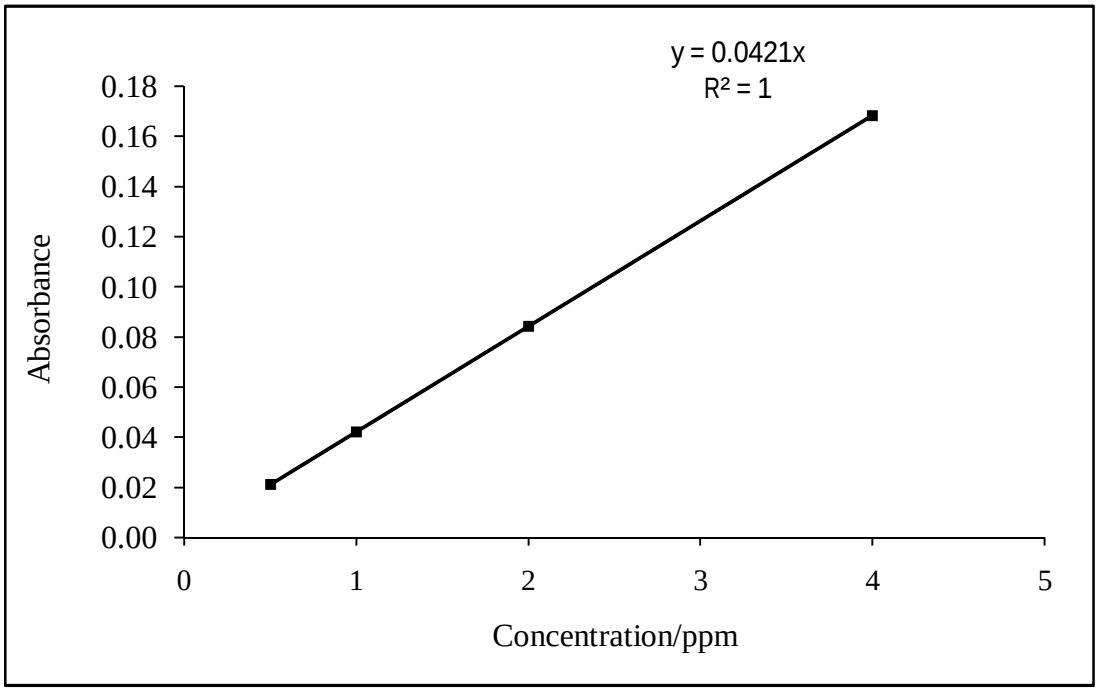

Figure 3.3: Calibration curve for the determination of lead

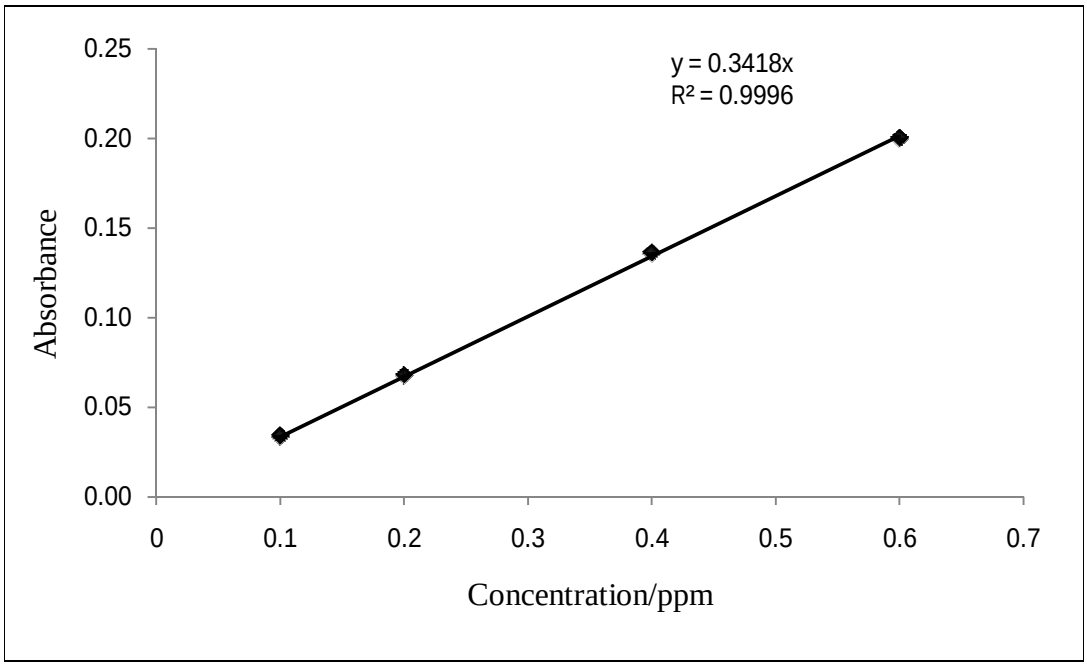

Figure 3.4: Calibration curve for the determination of cadmium

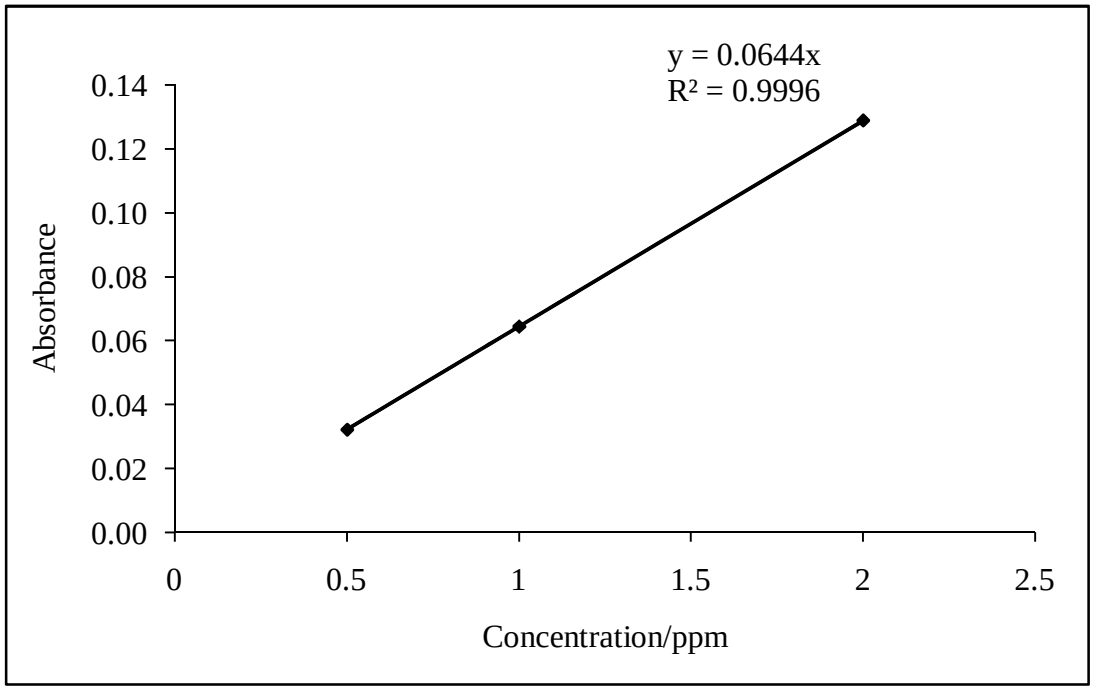

Figure 3.5: Calibration curve for the determination of chromium

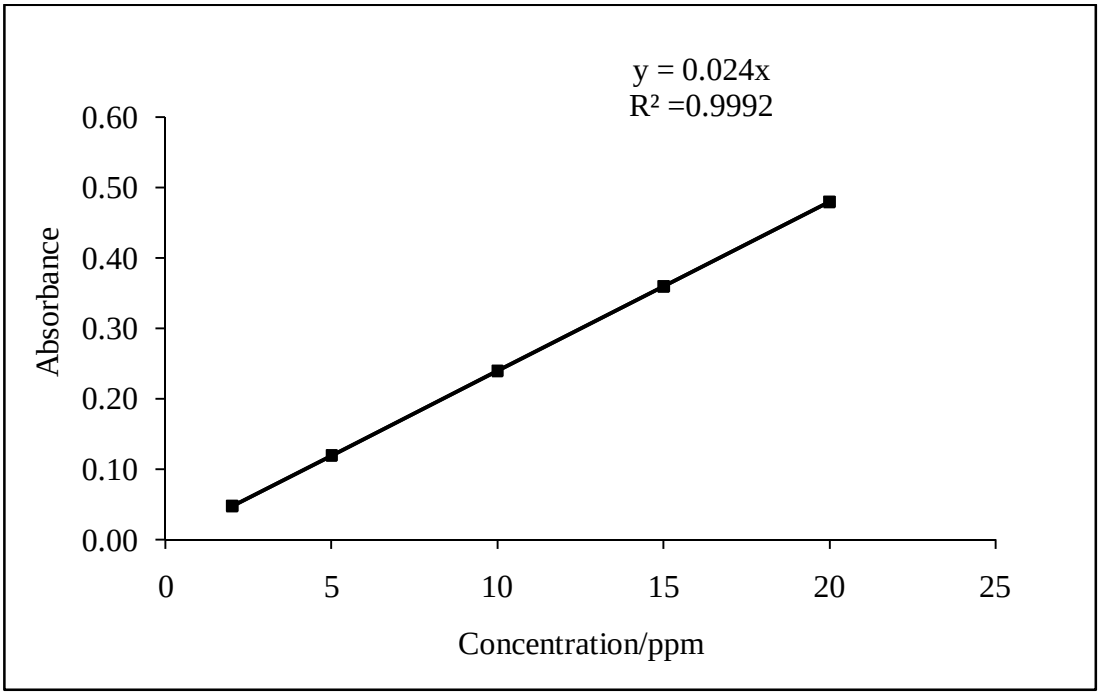

Figure 3.6: Calibration curve for the determination of arsenic

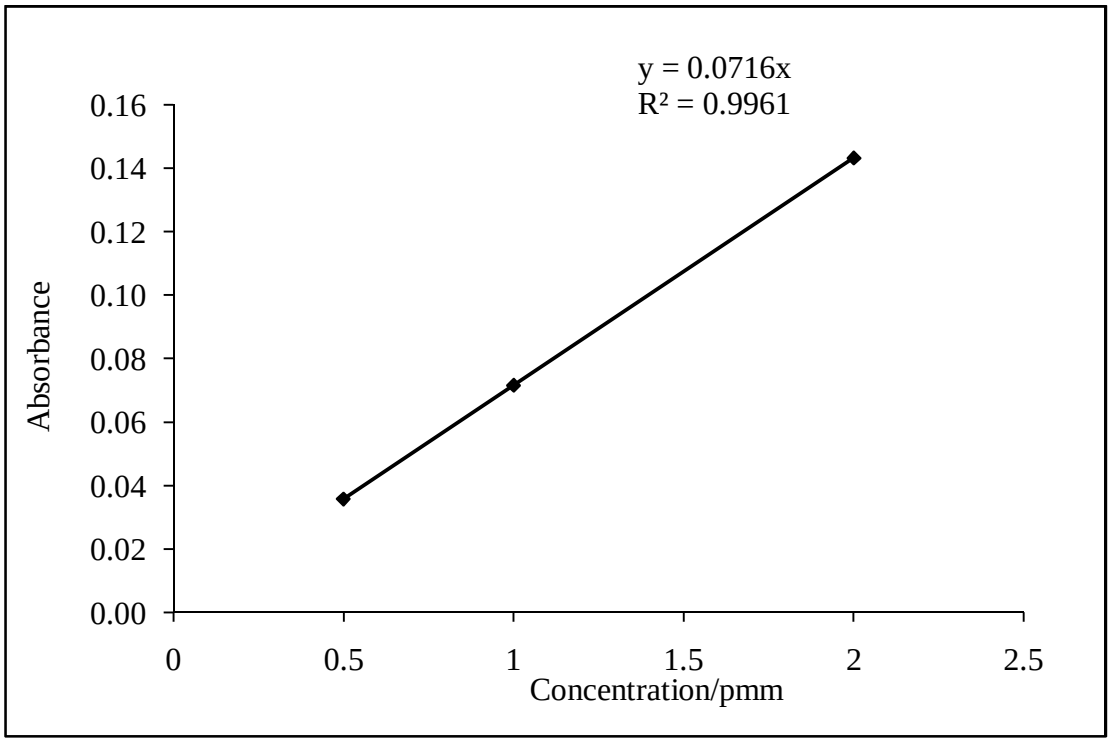

Figure 3.7: Calibration curve for the determination of nickel

3.3 Results and Discussion

3.3.1 Measurements of the percentage of protein, lactose and acidity of 0−6 months baby powder milk

Percentage of protein, lactose and acidity of (0−6 months) baby powder milk and equivalent milk of same product of different brands are shown in Table 3.1 to 3.3.

Table 3.1: Bio-chemical composition (protein) of baby powder milk and equivalent milk **(**g /L**)** of different brands

Name of brand	Country of origin	Equivalent powder milk	Percentage of protein	
			Experimental value (Mean± SD)	Given value*
Biomil-1	Belgium	107.69	17.73 ± 0.48	12.5
Lactogen-1	Switzerland	109.28	15.42 ± 0.71	10.5
M smile-1	Australia	108.90	16.97 ± 0.33	12.0
Eldorin-1	Netherland	108.40	17.75 ± 0.58	10.08
Mamilag-1	Poland	109.28	15.94 ± 0.41	12.5
Baby care-1	Korea	108.30	17.74 ± 0.00	12.9
Biomil soy	Belgium	111.60	18.50 ± 0.36	12.5

* Values given in the level of the container/packet. M smile = Mother's smile.

Table 3.1(a): Descriptive statistics of protein of different milk powder

Name of brand	Mean	SD	SE	95% CI for mean		Min	Max
				lower bound	upper bound		
Biomil-1	17.73	0.48	0.28	16.53	18.93	17.28	18.24
Lactogen-1	15.42	0.71	0.41	13.65	17.19	14.60	15.88
M smile-1	16.97	0.33	0.19	16.16	17.78	16.65	17.30
Eldorin-1	17.75	0.58	0.33	16.32	19.18	17.30	18.40
Mamilag-1	15.94	0.41	0.24	14.91	16.97	15.50	16.32
B care-1	17.74	0.00	0.00	17.74	17.74	17.74	17.74
Biomil soy	18.50	0.36	0.21	17.60	19.40	18.20	18.90
Overall	17.15	1.12	0.24	16.64	17.66	14.60	18.90

SD = Standard Deviation, SE = Standard Error, CI = Confidence Interval, Min = Minimum, Max = Maximum, M smile-1=Mother's smile-1, B care-1 =Baby care-1.

We want to test H_0 (Null hypothesis) and H_1 (Alternative hypothesis). Here H_0 = the mean value of protein of each milk powder is same and H_1= the mean value of protein of each milk powder is different.

Table 3.1(b): Analysis of variance (ANOVA) for the variation study of protein of different milk powder.

	Source of variation	SS	DF	MS	F	P
% of protein	Between groups	22.07	6	3.68	17.41	0.00
	Within group	2.96	14	0.21	–	–
	Total	25.03	20	–	–	–

SS = Sum of Square, DF = Degrees of Freedom, MS = Mean of Square, F =Variance comparison test, P =Level of Significant.

Table 3.1(b) distinguishes the statistical information of protein value of the experimental milk powder and indicates a significant difference of the intake level of protein of the selected milk powder. Also the Table indicates the level of significant,

P = 0.00 < 0.01, so the variance of the protein value of each milk powder is statistically significant at 1% level and we may conclude that the mean variation of the protein of each milk powder is significantly different.

Table 3.1(c): Duncan's multiple range test of protein for mean comparison of different milk powder.

Duncan[a]

Brand	**Subset for alpha = 0.05**		
	1	**2**	**3**
Lactogen-1	15.42	–	–
Mamilag-1	15.94	–	–
M smile-1	–	16.97	–
Biomil-1	–	17.73	17.73
Babycare-1	–	17.74	17.74
Eldorin-1	–	17.75	17.75
Biomil soy	–	–	18.50
Sig	0.19	0.08	0.08

Mean for group in homogeneous subsets are displayed. a Uses harmonic mean sample size = 3.00.

The above Table 3.1(c) shows that within the group the mean value of protein is insignificantly different and between the groups is significantly different and group-2 and group-3 have interaction effect between and within the groups.

From the Table 3.1(c), it is seen that the studied milk powder are divided into three groups. So the significant differences of protein values are observed between the group-1 and group-2, group-1 and group-3, group-2 and group-3. On the other hand insignificant differences of protein values are observed within the group-1, group-2 and group-3. The variation of percentage of protein in baby (0−6 months) powder milk of different brands is described below and shown in Figure 3.8 prepared from the Table 3.1(a)

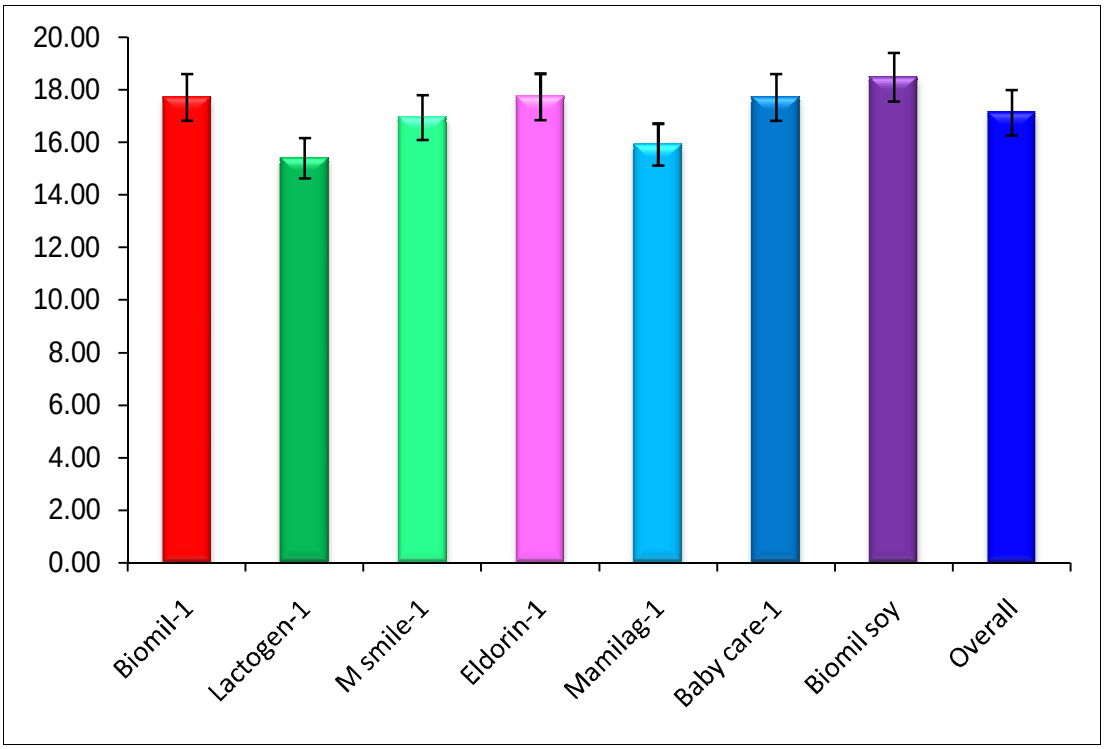

Figure 3.8: The variation of percentage of protein in baby (0−6 months) powder milk of different brands.

Table 3.1 indicates that the highest percentage of protein is present in Biomil soy (18.50%) and the lowest amount in Lactogen-1 (15.42%). The protein content in each milk is given on the packet by the respective company based on the method of production. The percentage of protein of the investigated milk exceeds the given value. A significant difference is observed in Lactogen-1, Eldorin-1, Mother's smile-1 and Baby care-1. Small differences are observed for other brands. Only in Mamilag-1 the experimental value is closer to the given value. The reason of getting excess protein in all of this milk may be due to the presence of non-protein nitrogenous substances such as urea nitrogen, amino nitrogen, creatinine, uric acid, adenine and guanine [3].

According to the American College of Sports Medicine protein requires for infants is about 12−15% of total calories and 2.2 g/kg of body weight per day. The recommended protein intake for infants from birth to 6 months is 9.1 g per day (1.52 g/kg of body weight per day). United Nations University Centre reported that the

protein content in mother milk is 1.1 g/100 mL. In the nutrition oedema group the protein level of the breast milk is 1.13% (range 0.5−1.8 %) [10].

Table 3.2: Bio-chemical composition (lactose) of baby powder milk and equivalent milk (g/L) of different brands

Name of brand	Country of origin	Equivalent powder milk	Percentage of lactose	
			Experimental value (Mean ± SD)	Given value*
Biomil-1	Belgium	107.69	52.83 ± 0.94	54.0
Lactogen-1	Switzerland	109.28	56.20 ± 0.08	55.7
M smile-1	Australia	108.90	51.60 ±0. 22	55.0
Eldorin-1	Netherland	108.40	62.49 ± 0.13	57.0
Mamilag-1	Poland	109.28	53.40 ± 0.00	59.0
Baby care-1	Korea	108.30	51.02 ± 0.16	58.2
Biomil soy	Belgium	111.60	61.25 ± 0.11	60.0

* Values given in the level of the container/packet. M smile = Mother's smile.

Table 3.2(a): Descriptive statistics of lactose of different milk powder

Name of brand	Mean	SD	SE	95% CI for mean		Min	Max
				lower bound	upper bound		
Biomil-1	52.83	0.94	0.54	50.51	55.15	51.88	53.75
Lactogen-1	56.20	0.08	0.05	56.00	56.40	56.14	56.29
M smile-1	51.60	0.22	0.13	51.06	52.14	51.45	51.85
Eldorin-1	62.49	0.13	0.08	62.17	62.81	62.40	62.64
Mamilag-1	53.40	0.00	0.00	53.40	53.40	53.40	53.40
Babycare-1	51.02	0.16	0.09	50.63	51.41	50.90	51.20
Biomil soy	61.25	0.11	0.07	60.97	61.53	61.18	61.38
Overall	55.54	4.41	0.96	53.53	57.55	50.90	62.64

SD = Standard Deviation, SE = Standard Error, CI = Confidence Interval, Min = Minimum, Max = Maximum.

We want to test H_0 (Null hypothesis) and H_1 (Alternative hypothesis). Here H_0 = the mean value of lactose of each milk powder is same and H_1= the mean value of lactose of each milk powder is different.

Table 3.2(b): Analysis of variance for the variation study of lactose **o**f different milk powder.

	Source of variation	SS	DF	MS	F	P
Amount of lactose (g)	Between groups	4509.88	6	751.65	34254.75	0.00
	Within group	0.31	14	0.02	–	–
	Total	4510.19	20	–	–	–

SS = Sum of Square, DF = Degrees of Freedom, MS = Mean of Square, F =Variance comparison test, P =Level of Significant.

Table 3.2(b) provides the statistical information about lactose value of the experimental milk powder and indicates a significant difference of the intake level of lactose of the selected milk powder. Also Table 3.2(b) indicates that the level of

significant, P = 0.00 < 0.01, so the variance of the lactose value of each milk powder is statistically significant at 1% level and we may conclude that the mean variation of the lactose of each milk powder is significantly different.

Table 3.2(c): Duncan's multiple range test (DMRT) for mean comparison of lactose of different milk powder.

Duncan[a]

Brand	Subset for alpha = 0.05				
	1	**2**	**3**	**4**	**5**
Baby care-1	51.02	–	–	–	–
M smile-1	51.60	–	–	–	–
Biomil-1	–	52.83	–	–	–
Mamilag-1	–	53.40	–	–	–
Lactogen-1	–	–	56.20	–	–
Biomil soy	–	–	–	61.25	–
Eldorin-1	–	–	–	–	62.49
Sig	0.08	0.08	1.00	1.00	1.00

Mean for group in homogeneous subsets are displayed. a Uses harmonic mean sample size = 3.00.

From the above Table it is observed that within the group the mean value of lactose is insignificantly different and between the groups it is significantly different, and group-1 and group-2 have interaction effect between and within the groups.

From the Table 3.2(c), it is found that the investigated powder milk samples are divided into 5 groups [Table 3.2(c)]. A significant difference is observed between the group-1 and group-2, group-1 and group-3, group-1 and group-4, group-1 and group-5, group-2 and group-3, group-2 and group-4, group-2 and group-4, group-2 and group-5, group-3 and group-4, group-3 and group-5 and group-4 and group-5. The insignificant difference is also observed within the group-1 and group-2. The variation of the percentage of lactose in baby (0−6 months) powder milk is given below and shown in Figure 3.9 plotted from the Table 3.2(a).

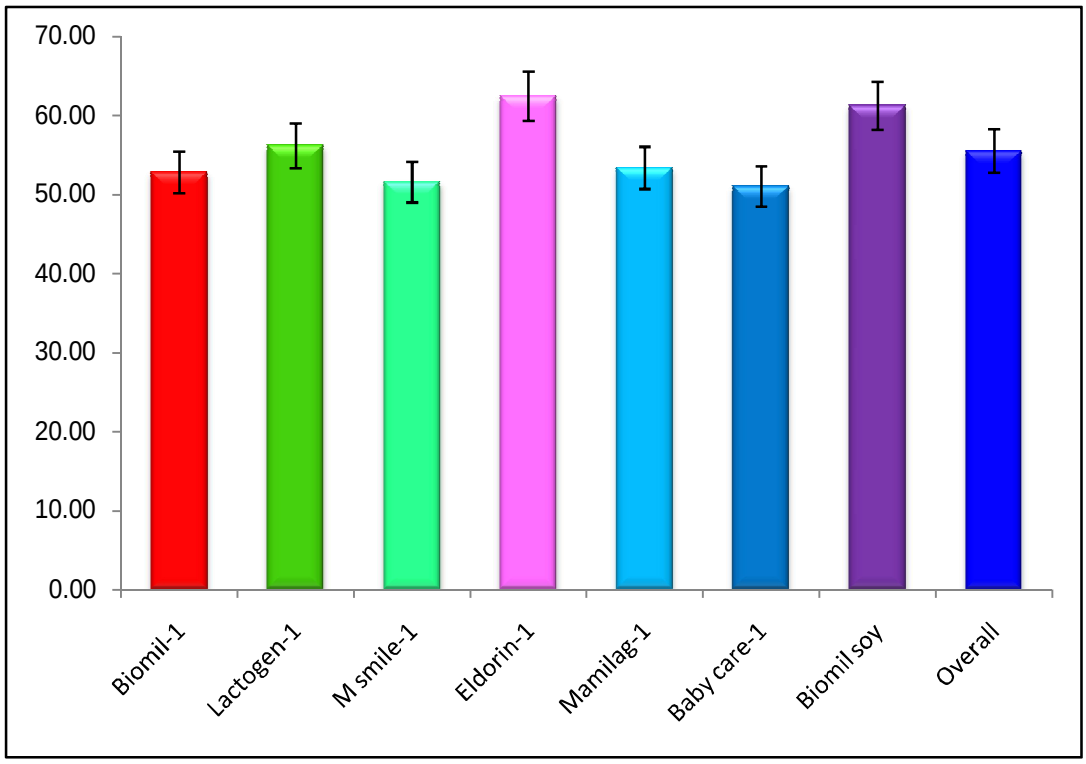

Figure 3.9: The variation of percentage of lactose in baby (0−6 months) powder milk of different brands.

From Table 3.2 it is observed that the percentage of lactose in milk samples varies from 51.02% to 62.49%. The lactose found in Lactogen-1, Eldorin-1 and Biomil soy exceeds their given values. Among these, Biomil soy contains the highest amount of lactose (62.49%) and Baby care-1 contains the lowest amount (51.02%). Biomil-1 and Lactogen-1 show negligible difference. But Mother's smile-1, Mamilag-1 and Baby care-1 contain comparatively lesser amount of lactose than their given values. The lower value indicates that some lactose might be lost during processing of the raw milk or other carbohydrate such as sucrose (non-reducing) may be added which gives the total carbohydrate value higher. The reason of decrease of the lactose content may be due to either decomposition of lactose during processing of milk or some conversion of lactose to lactic acid by bacteria. United Nations University Centre reported that the lactose content in mother milk is 7.0 g/100 mL.

Table 3.3 Bio-chemical composition (acidity) of baby powder milk and equivalent milk (g /L) of different brands

Name of brand	**Country of origin**	**Equivalent powder milk**	**Acidity of powder milk (Mean ± SD)**
Biomil-1	Belgium	107.69	1.09 ± 0.07
Lactogen-1	Switzerland	109.28	0.67 ± 0.13
M smile-1	Australia	108.90	0.66 ± 0.08
Eldorin-1	Netherland	108.40	0.69 ± 0.14
Mamilag-1	Poland	109.28	0.68 ± 0.08
Baby care-1	Korea	108.30	0.71 ± 0.00
Biomil soy	Belgium	111.60	1.28 ± 0.77

Table 3.3 (a): Descriptive statistics of acidity of different milk powder

Name of brand	Mean	SD	SE	95% CI for mean		Min	Max
				lower bound	upper bound		
Biomil-1	1.09	0.07	0.04	0.91	1.27	1.01	1.15
Lactogen-1	0.67	0.13	0.08	0.35	0.99	0.58	0.82
M smile-1	0.66	0.08	0.05	0.47	0.85	0.61	0.75
Eldorin-1	0.69	0.14	0.08	0.34	1.04	0.60	0.85
Mamilag-1	0.68	0.08	0.05	0.49	0.87	0.63	0.77
Baby care-1	0.71	0.00	0.00	0.71	0.71	0.71	0.71
Biomil soy	1.28	0.77	0.45	0.63	3.19	0.83	2.17
Overall	0.83	0.35	0.08	0.67	0.98	0.58	2.17

In this case the same parameters were tested.

Table 3.3(b): Analysis of variance for the variation study of acidity of different milk powder.

	Source of variation	SS	DF	MS	F	P
% of acidity	Between groups	1.14	6	0.19	2.06	0.13
	Within group	1.30	14	0.09	–	–
	Total	2.44	20	–	–	–

Table 3.3(b) gives statistical information of the result of acidity of the experimental milk powder as earlier. Also Table 3.3 (b) is indicating that the level of significant, $P = 0.125 > 0.01$, so the variance of acidity of each milk powder is statistically insignificant at 1% level and we may conclude that the mean variation of the results of acidity of each milk powder is insignificantly different.

Table 3.3(c): Duncan's multiple range test for mean comparison of acidity of different milk powder.

Duncan[a]

Brand	Subset for alpha = 0.05	
	1	2
M Smile-1	0.66	–
Lactogen-1	0.67	–
Mamilag-1	0.68	–
Eldorin-1	0.69	–
Baby care-1	0.71	–
Biomil-1	1.09	1.09
Biomil soy	–	1.28
Sig	0.142	0.457

Mean for group in homogeneous subsets are displayed. a Uses harmonic mean sample size = 3.00.

The above Table 3.3(c) informs about Duncan's multiple range test for mean comparison of acidity of different milk powder, and group-1 and group-2 have interaction effect between and within the groups.

From the above Table 3.3(c), it is found that the seven milk powder of different brands are divided into 2 groups. A significant difference of the amount of acidity is found in group-1 and group-2. Insignificant difference of acidity is also observed within the group-1 and group-2. The variation of percentage of acidity in baby (0−6 months) powder milk of different brands is described below and shown in Figure 3.10 plotted from the Table 3.3(a).

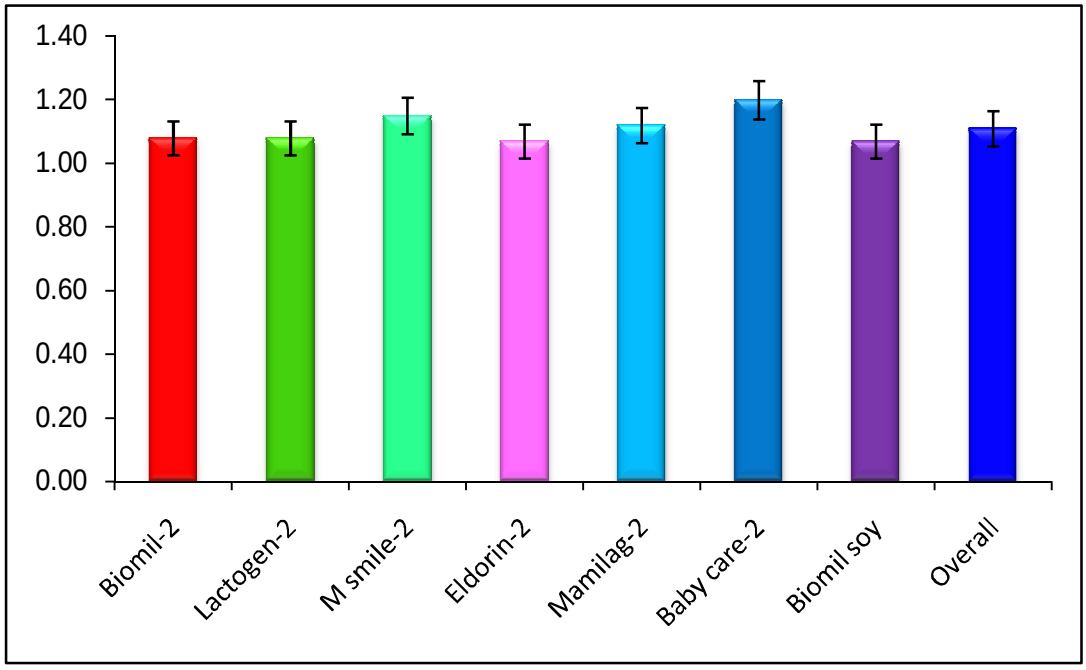

Figure 3.10: The variation of percentage of acidity in baby (0−6 months) powder milk of different brands.

Table 3.3 represents the acidity of baby (0−6 months) powder milk ranges from 0.66−1.28%. Biomil soy has the highest acidity (1.28 %) and Mother's smile-1 has the lowest acidity (0.66%).The acidity of powder milk meets the requirement of the standards of USA which is less than 1.50% [11].

3.3.2 Comparison between experimental value and standard value of protein and lactose.

The daily intake of the protein and lactose depends on the amount of food consumed. The mean of 24 hours output of breast milk of an Indian mother is 550 mL for first 3 months, 680 mL for 4 to 6 months. From the Table 3.1 it is seen that 107.69, 109.28, 108.90, 108.40, 109.28, 108.30 and 110.60 g powder of Biomil-1, Lactogen-1, Mother’s smile-1, Eldorin-1, Mamilag-1, Baby care-1 and Biomil soy respectively are required for preparing per liter of milk equivalent to that amount of breast milk.

Now if an infant is given the same amount of powder milk instead of mother milk daily it is seen that the infant consumes 53.85, 54.64, 54.45, 54.20, 54.64, 54.15 and 55.8 g powder of Biomil-1, Lactogen-1, Mother’s smile-1, Eldorin-1, Mamilag-1,

Baby care-1 and Biomil soy respectively. The daily intake of protein and lactose by infants from baby (0−6 months) powder milk are shown in the Table 3.4.

Table 3.4: Amount of protein and lactose from baby powder milk consumed by infants (g/day)

Name of brand	Country of origin	Protein	Lactose
Biomil-1	Belgium	8.87	26.41
Lactogen-1	Switzerland	7.71	28.10
M smile-1	Australia	8.49	25.8
Eldorin-1	Netherland	8.88	31.25
Mamilag-1	Poland	7.97	26.7
Baby care-1	Korea	8.87	25.51
Biomil soy	Belgium	9.25	30.63

From the Table 3.4 it is found that the highest amount of protein is present in Biomil soy (9.25 g) and the lowest amount in Lactogen-1 (7.71 g). Protein also present in Biomil-1 is 8.87 g, Mother's smile-l is 8.49 g, Eldorin-1 is 8.88 g, Mamilag-1 is 7.97 g and Baby care-l is 8.87 g. The amount of protein present in mother milk is 1.13 g/100 mL. The daily intake of protein is 9.1 g. It is seen that studied samples contain the amount of protein close to daily intake. It shows that studied samples are free from protein deficiency and excessive intake of protein.

The amount of lactose present in baby powder milk ranges from 25.0 l to 30.75 g. The highest amount of lactose is found in Eldorin-l (30.75 g) and the lowest amount in Baby care-1(25.0 l g). The contents of lactose in Biomil-1, Lactogen-1, Mother's smile-1, Mamilag-1 and Biomil soy are 26.66 g, 27.92 g, 26.19 g, 26.80 g and 30.14g respectively. The amount of lactose present in mother milk is 7.0 g/100 mL or 35.0 g/500 mL. From the Table 3.4 it is seen that Eldorin-1 and Biomil soy contain lactose close to mother milk.

The data on the third column (from the left) of the Table 3.1 gives the idea about the amount of powder milk of each brand to prepare one liter equivalent milk which is

equals to mother milk. A variation is observed in the amount of powder milk of different brands to prepare equivalent milk. It is seen that 111.60 g powder of Biomil soy is necessary to make one liter equivalent milk whereas only 107.69 g of Biomil-1 is needed for the same purpose. This information will help the user to select the economic brand and also to know how much powder milk of each brand requires preparing equivalent milk. Considering the above results we can say Biomil-1 is more economic than any other brands.

3.3.3 Determination of the percentage of protein, lactose and acidity of baby (6−24 months) powder milk

Table 3.5: Bio-chemical composition (protein) of baby powder milk and equivalent milk (g/L) of different brands

Name of brand	Country of origin	Equivalent powder milk	Percentage of protein	
			Experimental value (Mean ± SD)	Given value*
Lactogen-2	Switzerland	112.30	17.27±0.10	14.25
Biomil-2	Belgium	110.50	21.14±0.27	17.00
Baby care-2	Korea	112.90	22.98±0.63	19.00
Eldorin-2	Netherland	110.60	18.04±0.47	15.00
Lailac-2	France	113.50	18.82±0.72	15.00
M smile-2	Australia	113.20	19.17±0.1.08	16.30
Mamilag-2	Poland	111.90	21.32±0.72	16.00

* Values given in the level of the container/packet.

Table 3.5(a): Descriptive statistics of protein of different milk powder

Name of brand	Mean	SD	SE	95% CI for Mean		Min	Max
				lower bound	upper bound		
Lactogen-2	17.27	0.10	0.06	17.01	17.53	17.15	17.33
Biomil-2	21.14	0.27	0.16	20.46	21.82	20.90	21.44
Baby care-2	22.98	0.63	0.36	21.43	24.54	22.33	23.58
Eldorin-2	18.04	0.47	0.27	16.87	19.21	17.51	18.40
Lailac-2	18.82	0.72	0.42	17.02	20.61	18.04	19.47
M smile-2	19.17	1.08	0.62	16.49	21.86	18.04	20.19
Mamilag-2	21.32	0.72	0.42	19.52	23.11	20.90	22.15
Overall	19.82	2.01	0.44	18.90	20.74	17.15	23.58

Description of H_0 and H_1 is almost same as earlier.

Table 3.5(b): Analysis of variance for the variation study of protein of different milk powder

	Source of variation	SS	DF	MS	F	P
% of protein	Between groups	75.22	6	12.54	30.16	0.00
	Within group	5.82	14	0.42	–	–
	Total	81.04	20	–	–	–

Statistical explanation of protein values is same as earlier. Information obtained from the Table 3.5(b) shows the level of significant, $P = 0.00 < 0.01$, so the variance of the protein value of each milk powder is statistically significant at 1% level and we may conclude that the mean variation of the protein of each milk powder is significantly different.

Table 3.5(c): Duncan's multiple range test for mean comparison of protein of different milk powder.

Duncan[a]

Brand	Subset for alpha = 0.05			
	1	**2**	**3**	**4**
Lactogen-2	17.27	–	–	–
Eldorin-2	18.04	18.04	–	–
Lailac-2	–	18.82	–	–
M smile-2	–	19.17	–	–
Biomil-2	–	–	21.14	–
Mamilag-2	–	–	21.32	–
Baby care-2	–	–	–	22.98
Sig	0.16	0.06	0.74	1.00

Table 3.5(c) indicates that within the group the mean value of protein is insignificantly different and between the groups it is significantly different. Also interaction effect is found between and within the group-1 and group-2.

According to Table 3.5(c), Duncan's multiple range test for protein shows that the experimental powder milk of different brands are divided into four groups. Here, a major difference is found in Lactogen-2 and Baby care-2, Lactogen-2 and Lailac-2, Lactogen-2 and Mother's smile-2, Eldorin-2 and Biomil-2, Mother's smile-2 and Biomil-2 due to the mean value of protein is significantly different between the groups. Protein values show small difference within the group of the selected powder milk because the mean value of the protein is insignificantly different of those groups.

The variation of percentage of protein in baby (6−24 months) powder milk of different brands is illustrated below and shown in Figure 3.11 plotted from the Table 3.5(a).

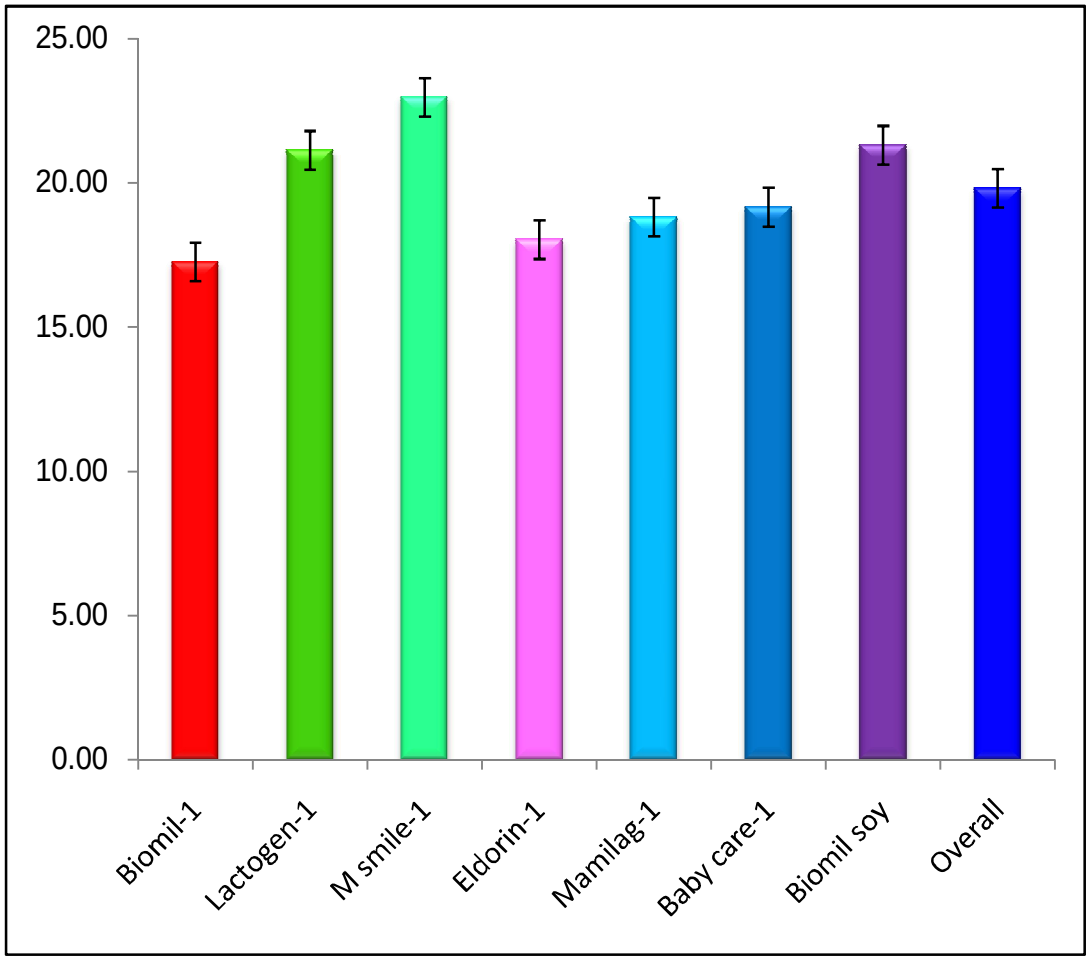

Figure 3.11: The variation of percentage of protein in baby (6−24 months) powder milk of different brands.

From the Table 3.5, it is seen that the protein content ranges from 17.27 to 22.98%. Lactogen-2 has the lowest amount of protein (17.27%) and Baby care-2 has the highest amount of protein (22.98%). Biomil-2, Eldorin-2, Lailac-2, Mother's smile-2 and Mamilag-2 have 21.14%, 18.04%, 18.82%., 19.17% and 21.32% protein respectively. The protein content of each of the powder milk is given on the packet by the respective company based on the method of production.

The percentage of protein of the investigated powder milk exceeds the given value on the packet. A significant difference is observed in Mamilag-2. Other brands show a small difference and Mother's smile-2 has comparatively quite closer.

Table 3.6: Bio-chemical composition (lactose) of baby powder milk and equivalent milk (g/L) of different brands

Name of brand	Country of origin	Equivalent powder milk	Percentage of lactose	
			Experimental value (Mean ± SD)	Given value*
Lactogen-2	Switzerland	112.30	56.95 ± 0.30	55.34
Biomil-2	Belgium	110.50	56.03 ± 0.21	56.50
Baby care-2	Korea	112.90	52.73 ± 0.08	51.00
Eldorin-2	Netherland	110.60	58.69 ± 0.10	57.30
Lailac-2	France	113.50	53.38 ± 0.52	56.00
M smile-2	Australia	113.20	50.87 ± 0.06	53.60
Mamilag-2	Poland	111.90	55.16 ± 0.05	57.50

* Values given in the level of the container/packet.

Table 3.6(a): Descriptive statistics of lactose of different milk powder

Name of brand	Mean	SD	SE	95% CI for Mean		Min	Max
				lower bound	upper bound		
Lactogen-2	56.95	0.30	0.18	56.19	57.71	56.60	57.15
Biomil-2	56.03	0.21	0.12	55.51	56.55	55.88	56.27
Baby care-2	52.73	0.08	0.05	52.53	52.93	52.64	52.79
Eldorin-2	58.69	0.10	0.06	58.45	58.93	58.61	58.80
Lailac-2	53.38	0.52	0.30	52.09	54.67	53.04	53.98
M smile-2	50.87	0.06	0.04	50.71	51.03	50.82	50.94
Mamilag-2	55.16	0.05	0.03	55.03	55.29	55.10	55.20
Overall	54.83	2.55	0.56	53.67	55.99	50.82	58.80

Description of H_0 and H_1 is almost same as earlier.

Table 3.6(b): Analysis of variance for the variation study of lactose of different milk powder

	Source of variation	SS	DF	MS	F	P
Amount of lactose (g)	Between groups	129.38	6	21.56	352.40	0.00
	Within group	0.86	14	0.06	–	–
	Total	130.24	20	–	–	–

Statistical information about the value of lactose of the experimental milk powder obtained from the Table 3.6(b) is almost same as earlier.

Table 3.6(c): Duncan's multiple range test for mean comparison of lactose of different milk powder

Duncan[a]

Brand		Subset for alpha = 0.05					
	1	2	3	4	5	6	7
M smile-2	50.87		–	–	–	–	–
Baby care-2	–	52.73	–	–	–	–	–
Lailac-2	–	–	53.38	–	–	–	–
Mamilag-2	–	–	–	55.16	–	–	–
Biomil-2	–	–	–	–	56.03	–	–
Lactogen-2	–	–	–	–	–	56.95	–
Eldorin-2	–	–	–	–	–	–	58.69
Sig	1.00	1.00	1.00	1.00	1.00	1.00	1.00

Mean for group in homogeneous subsets are displayed. a. Uses harmonic mean sample size = 3.00.

Table 3.6(c) informs that between the groups the mean value of lactose is significantly different and found no interaction effect within the group.

In the Table 3.6(c), Duncan's multiple range test for lactose shows that the experimental powder milk of different brands are divided into seven groups [Table 3.6(c)]. From the above Table it is concluded that Mother's smile-2 of group-1 contains the lowest amount of lactose and Eldorin-2 of group-7 contains the highest amount of lactose because statistically significant different is found in Mother's smile-2 and Eldorin-2. A major difference is also observed between the groups for lactose of different milk powder due to same reason.

The variation of percentage of lactose in baby (6−24 months) powder milk of different brands is described below and shown in Figure 3.12 obtained from the Table 3.6(a).

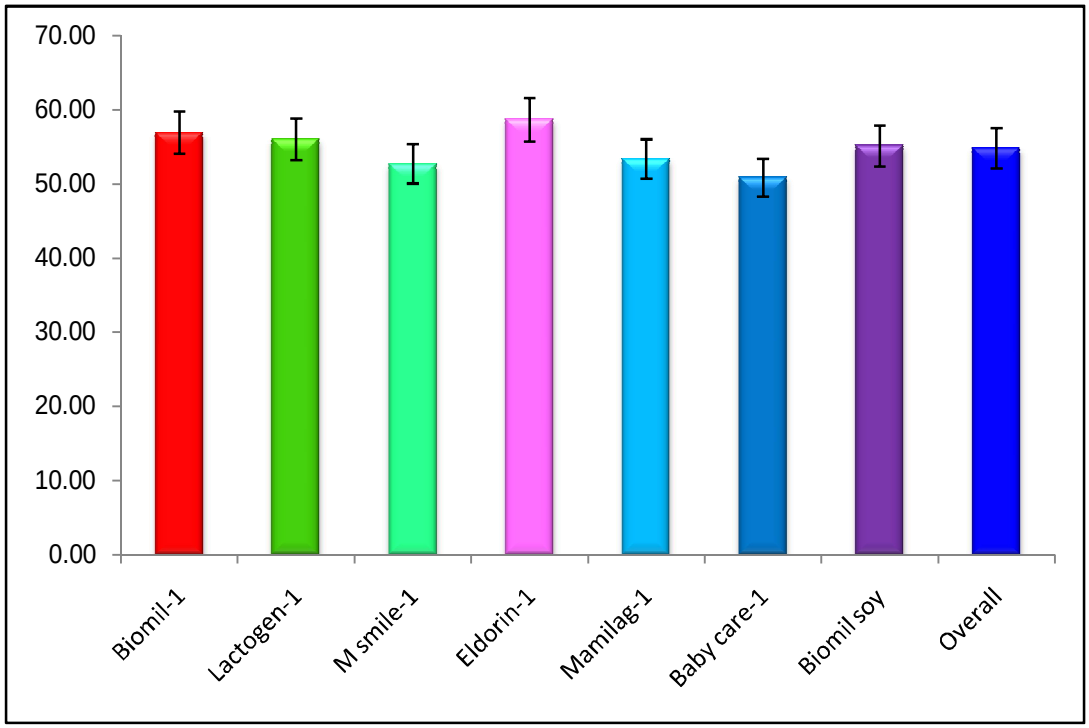

Figure 3.12: The variation of percentage of lactose in baby (6−24 months) powder milk of different brands.

In the Table 3.6 it is seen that the lactose content of baby (6−24 months) powder milk of seven brands varies from 58.68% to 50.87%. The highest amount of lactose is found in Eldorin-2 (58.69%) and the lowest amount in Mother's smile-2 (50.87%). Biomil-2 shows a negligible difference compared to the given value. Lailac-2, Mother's smile-2 and Mamilag-2 contain the lower amount of lactose than their given values.

Table 3.7: Bio-chemical composition (acidity) of baby powder milk and equivalent milk (g /L) of different brands

Name of brand	Country of origin	Equivalent powder milk	Acidity of powder milk (Mean ± SD)
Lactogen-2	Switzerland	112.30	1.08 ± 0.07
Biomil-2	Belgium	110.50	1.08 ± 0.03
Baby care-2	Korea	112.90	1.15 ± 0.04
Eldorin-2	Netherland	110.60	1.07 ± 0.04
Lailac-2	France	113.50	1.12 ± 0.04
M smile-2	Australia	113.20	1.20 ± 0.05
Mamilag-2	Poland	111.90	1.07 ± 0.04

Table 3.7(a): Descriptive statistics of acidity of different milk powder.

Name of brand	Mean	SD	SE	95% CI for mean		Min	Max
				lower bound	upper bound		
Lactogen-2	1.08	0.07	0.04	0.92	1.24	1.02	1.15
Biomil-2	1.08	0.03	0.02	1.01	1.15	1.06	1.11
Baby care-2	1.15	0.04	0.02	1.06	1.24	1.11	1.18
Eldorin-2	1.07	0.04	0.02	0.98	1.16	1.04	1.11
Lailac-2	1.12	0.04	0.02	1.03	1.21	1.08	1.15
M smile-2	1.20	0.05	0.03	1.07	1.33	1.14	1.24
Mamilag-2	1.07	0.04	0.03	0.96	1.18	1.04	1.12
Overall	1.11	0.06	0.01	1.08	1.14	1.02	1.24

Description of H_0 and H_1 is almost same as earlier.

Table 3.7(b): Analysis of variance for the variation study of acidity of different milk powder.

	Source of variation	SS	DF	MS	F	P
% of acidity	Between groups	0.04	6	0.01	3.81	0.02
	Within group	0.03	14	0.00	–	–
	Total	0.07	20	–	–	–

Table 3.7(b) provides the statistical information about the result of acidity of the experimental milk powder and informs a significant difference of the intake level of acidity of the selected milk powder. Table 3.7(b) also indicates that the level of significant, P = 0.02 > 0.01, so the variance of acidity of each milk powder is statistically insignificant at 1% level and we may conclude that the mean variation of the results of acidity of each milk powder is insignificantly different.

Table 3.7(c): Duncan's multiple range test for mean comparison of acidity of different milk powder.

Duncan[a]

Brand	Subset for alpha = 0.05	
	1	2
Eldorin-2	1.07	–
Mamilag-2	1.07	–
Lactogen-2	1.08	–
Biomil-2	1.08	–
Lailac-2	1.12	1.12
Baby care-2	1.15	1.15
M smile-2	–	1.20
Sig	0.07	0.05

Mean for group in homogeneous subsets are displayed. a. Uses harmonic mean sample size = 3.00.

Table 3.7(c) informs that within the group the mean value of acidity is insignificantly different and between the groups it is significantly different and interaction effect is found between and within the groups.

Duncan's multiple range test for acidity indicates that the milk powder are divided into two groups. From the above Table It is concluded that M Smile-2 of group-2 contains the highest amount of acidity and Eldorin-2 and Mamilag-2 of group-1 contains the lower amount and statistically a significant difference is found in M Smile-2 and Eldorin-2. A lower difference is also observed between the groups of different milk powder. The variation of percentage of acidity in baby (6−24 months) powder milk of different brands is illustrated below and shown in Figure 3.13 plotted from the Table 3.7(a).

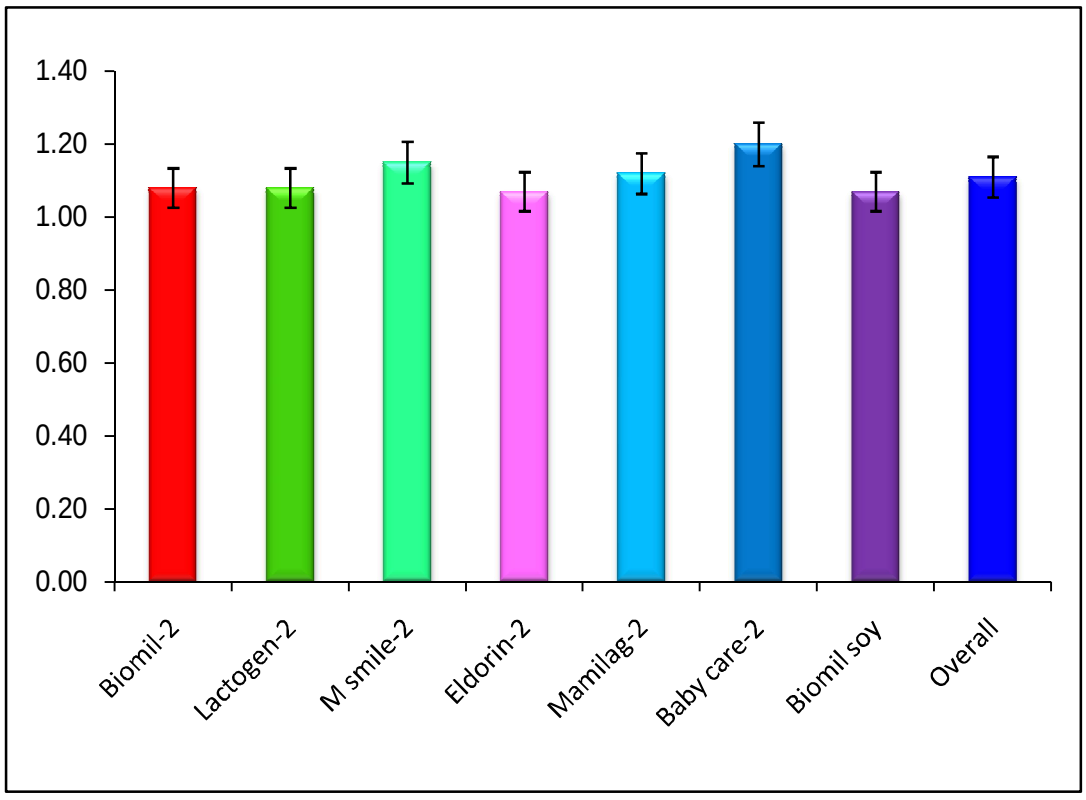

Figure 3.13: The variation of percentage of acidity in baby (6−24 months) powder milk of different brands.

It is observed from Table 3.7 that the acidity of baby (6−24 months) powder milk ranges from 1.05−1.20%. Mother's smile-2 has the highest acidity (1.20%) and Lactogen-2, Eldorin-2 and Mamilag-2 have the lower acidity.

3.3.4 Comparisons between experimental and standard value

From baby (6−24 months) powder milk the daily intake of protein and lactose by infants are shown in the Table 3.8.

Table 3.8: Amount of protein and lactose consumed by infants from baby powder milk (g/day)

Name of brand	Country of origin	Protein	Lactose
Biomil-2	Belgium	10.40	28.02
Lactogen-2	Switzerland	8.63	28.48
M smile-2	Australia	9.58	25.44
Eldorin-2	Netherland	9.02	29.35
Mamilag-2	Poland	10.68	27.58
Baby care-2	Korea	11.51	26.37
Lailac-2	Belgium	9.43	26.53

From the Table 3.8, it is found that the highest amount of protein is present in Baby care-2 (11.51g) and the lowest amount in Lactogen-2 (8.63 g). According to the American College of Sports Medicine, protein should make up 12−15% of the total calories consumed based on body weight (2.2 g per kg of body weight for infants). The recommended protein intake for infants from 6 to 24 months is about 11.0 g per day (1.52 g/kg of body weight per day). In 2002, the National Academy of Sciences (NAS) recommended dietary allowances (RDAs) of protein for baby of 6 −24 months age is 13.3 g. It is seen that the protein contain in Baby care-2 (11.51 g), Biomil-2 (10.40 g) and Mamilag-2 (10.68 g) which is close to the daily intake level of protein. Lactogen-2, Mother's smile-2 and Lailac-2 contains less amount of protein (Table 3.8) than required amount for daily intake. It shows that studied samples are free from excessive intake of protein.

The amount of lactose present in baby powder milk ranges from 25.44 to 29.35 g. The amount of lactose present in mother milk is 6.25 g/100 mL. Table 3.8 shows that Eldorin-2, Lactogen-2 and Biomil-2 contain lactose close to mother milk.

The data on the third column (from the left of the Table 3.7) gives the idea about the amount of powder milk of each brand. This information will also help the user to select the economic brand. So, we can say Biomil-2 is more economic than the other brands.

3.3.5 Comparative study between (0−6 months) and (6−24 months) baby powder milk of different brands

The experimental results of bio-chemical parameters in two categories 0−6 months and 6−24 months of baby powder milk of different brands are given in Table 3.9 (a) and 3.9(b) respectively.

Table 3.9(a): The experimental results of bio-chemical compositions of (0−6 months) baby powder milk

Brand name	Percentage of protein	Percentage of	Percentage of acidity
Biomil-1	17.73 ± 0.48	52.83 ± 0.94	1.09 ± 0.07
Lactogen-1	15.42 ± 0.71	56.20 ± 0.08	0.67 ± 0.13
M smile-1	16.97 ± 0.33	51.60 ±0. 22	0.66 ± 0.08
Eldorin-1	17.75 ± 0.58	62.49 ± 0.13	0.69 ± 0.14
Mamilag-1	15.94 ± 0.41	53.40 ± 0.00	0.68 ± 0.08
Baby care-1	17.74 ± 0.00	51.02 ± 0.16	0.71 ± 0.00
Biomil soy	18.50 ± 0.36	61.25 ± 0.11	1.28 ± 0.77

Table 3.9(b): The experimental results of bio-chemical compositions of (6−24 months) baby powder milk

Brand name	Percentage of protein	Percentage of lactose	Percentage of acidity
Biomil-2	21.14±0.27	56.03 ± 0.21	1.08 ± 0.07
Lactogen-2	17.27±0.10	56.95 ± 0.30	1.08± 0.03
M smile-2	19.17±0.1.08	50.87 ±0.06	1.20 ± 0.05
Eldorin-2	18.04±0.47	58.69 ± 0.10	1.07± 0.04
Mamilag-2	21.32±0.72	55.16 ± 0. 05	1.07 ± 0.04
Baby care-2	22.98±0.63	52.73 ± 0.08	1.15 ± 0.04
Lailac-2	18.82±0.72	53.38 ± 0.52	1.12 ± 0.04

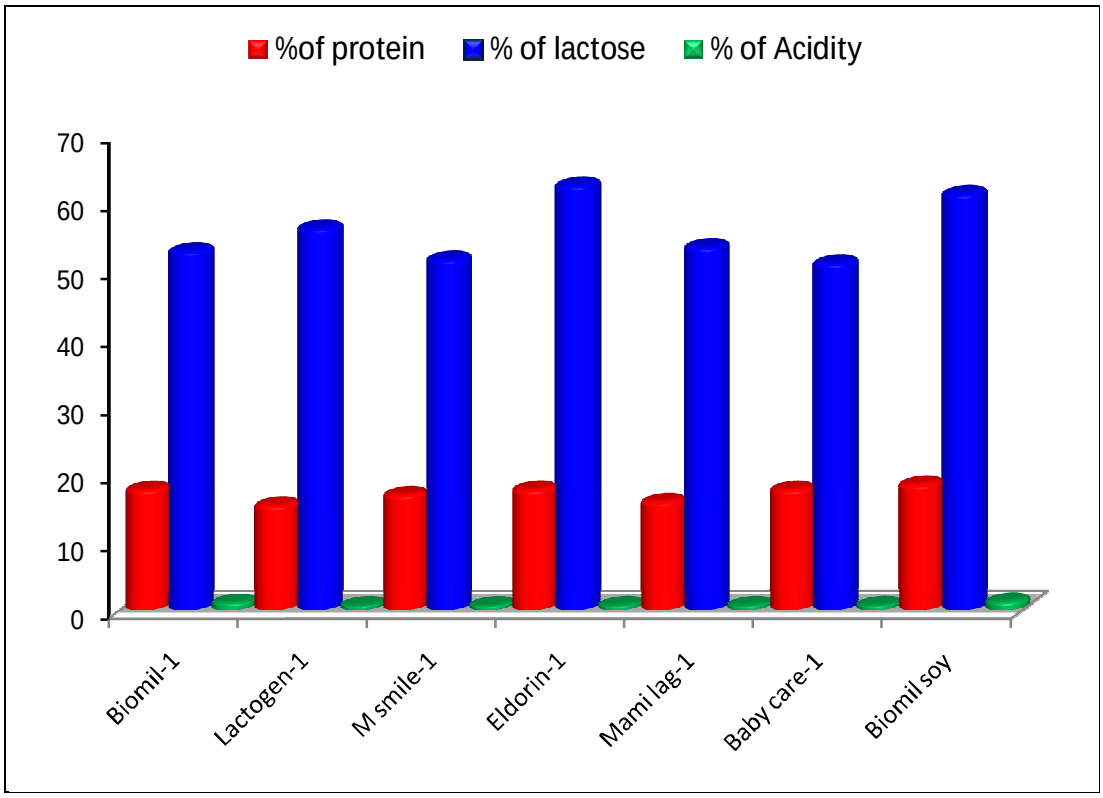

Figure 3.14: Comparison of protein, lactose and acidity status in baby (0−6 months) powder milk of different brands.

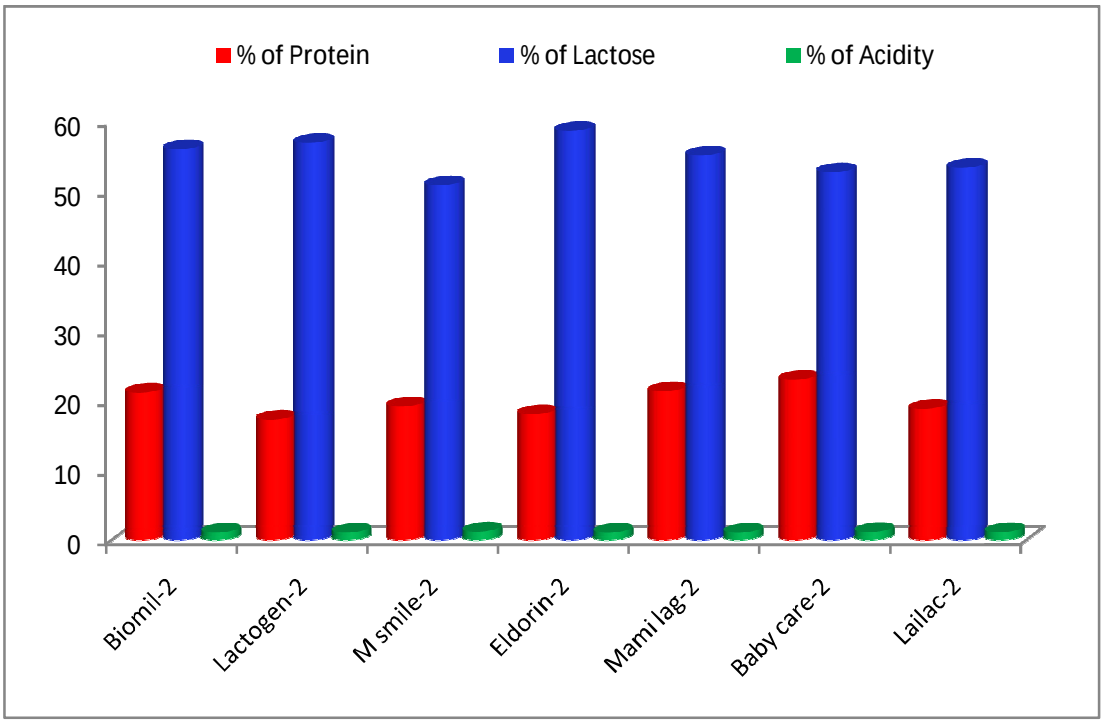

Figure 3.15: Comparison of protein, lactose and acidity status in baby (6−24 months) powder milk of different brands.

Table 3.9(a) and Table 3.9(b) indicate a significant difference of protein value between (0−6) and (6−24 months) baby powder milk of different brands. (6−24 months) baby powder milk contains higher protein value than that of (0−6 months) baby. The recommended protein intake for infants from birth to 6 months is about 9.1 g per day (1.52 g per kg of body weight per day). (0−6 months) powder milk product fulfills this requirement but (6−24 months) powder milk of different brands have higher value than that of recommended value for (0−6 months) baby. For this reason it is suggested that one should not feed the above (0−6 months) powder milk to the (6−24 months) baby. Because excessive intake of protein may lead to kidney problems, accelerated bone loss, developing diabetes, allergies, ear infections and diarrhoeal infection. Any type of anomalies in taking baby feed may decrease production of enzymes, hormone and creating new cells, consequently hamper in maintaining fluid balance, building and repairing body tissues. From the Table 3.9(a) and 3.9(b) it is found that the percentage of lactose and acidity in both categories of powder milk have little and irregular difference.

3.3.6 Estimation of the amount of trace and toxic metals in baby powder milk

Toxic metals enter into the human body through food chain and water. The metals of particular concern in relation to harmful effect on health are mercury, lead, cadmium and arsenic [12]. Toxic metals replace nutrient minerals in enzyme binding sites. When this occurs, the metals inhibit over stimulate and alter thousands of enzyme function. An affected enzyme may operate at 5% of normal activity. Toxic metals also replace other substances in tissue structure. The arteries, joints, bones and muscles are weakened by the replacement process. Toxic metals may also be simply deposited in many sites, causing local irritation and other toxic effects. They may also support the development of fungal, bacterial and viral infections that are difficult or impossible to eradicate until this cause is removed [13].The concentration of trace (Co and Zn) and toxic metals (Pb, Cd, Cr, As, and Ni) in baby milk samples of selected brands are estimated by atomic absorption spectrophotometer that are tabulated in the Tables 3.10, 3.13 and 3.15.

Table 3.10: Amount of trace and toxic metals in baby powder milk of selected brands (mg/kg)

Sample	Country	Co	Zn	Pb	Cd	Cr	As	Ni
Biomil-1	Belgium	BDL	29.15	0.42	0.01	0.03	0.39	BDL
Lactogen-1	Switzerland	BDL	30.20	0.38	0.01	0.06	0.38	BDL
M smile-1	Australia	0.02	28.90	0.24	0.02	0.16	0.26	BDL
Eldorin-1	Netherland	0.04	34.10	0.30	0.01	0.12	0.77	BDL
Mamilag-1	Poland	BDL	19.18	0.22	0.02	0.13	0.28	BDL
Baby care-1	Korea	0.02	16.95	0.15	0.01	0.14	0.61	BDL
Biomil soy	Belgium	BDL	52.86	0.12	0.02	0.15	0.42	BDL
Mean	–	0.027	30.19	0.26	0.014	0.11	0.39	BDL

M smile = Mother's smile

From the Table 3.10 it is seen that the mean concentration of Co in baby (0−6 months) powder milk of selected brands is 0.027 mg/kg. The highest concentration of Co is found in Eldorin-1 (0.04 mg/kg) and lower concentrations were found in Mother's smile-1 and Baby care-1(0.02 mg/kg). Co is found below detection limit in Biomil-1, Lactogen-1, Mamilag-1 and Biomil soy. The permissible limit of

Co for infant is 4×10^{-4} mg/day. Cobalt is necessary mineral for good human health. Without cobalt, vitamin B_{12} could not exist; the body uses this vitamin for numerous purposes. Additionally vitamin B_{12} prevents nerve damage by contributing to the formation of the protective sheath that insulates nerve cells. The vitamin B_{12} is greatly found in animal sources such as red meat, fish, eggs, cheese and milk [14].

The amount of zinc is found in baby powder milk ranges from 16.95−52.86 mg/kg. Biomil soy contains higher amount of Zn (52.86 mg/kg) and Baby care-1 contains less amount (16.95 mg/kg). Zinc is an essential trace element for human health which constitutes of a number of enzymes involved in major metabolic pathways and function. Significant amount of dietary zinc plays a role in growth and reproduction. Prasad et al. showed that dietary zinc supplementation causes improved growth and appearance of pubic hair. Zinc is necessary for wound healing. The deficiency can cause loss of appetite, growth retardation and immunological abnormalities [15, 16]. Recommended value of zinc is in the range of 3−5 mg/day [17].

Table 3.10 Indicates that the average concentration of Pb in baby powder milk of selected brands is 0.26 mg/kg. The highest concentration of Pb is found in Biomil-1 (0.42 mg/kg) and the lowest concentration was found in Biomill soy (0.12 mg/kg). Pb was also found in Lactogen-1 (0.38 mg/kg), Mother's Smile-1 (0.24 mg/kg). Milk is a major dietary source for infants and children. Recommended level of lead for infants is 12.5×10^{-3} -17.5×10^{-3} mg/day [17, 18]. The lead exposure is much more insidious in its presentation and can lead to a wide array of problems. Short-term to long-term exposure levels of lead can cause brain damage, paralysis, anemia and gastrointestinal symptoms. Long-term exposure can cause damage to the kidneys, reproductive and immune systems in addition to effects on the nervous system. Infants and young children are more vulnerable than adults to the toxic effects of lead, and they can also absorb lead more readily [12].

The concentrations of cadmium (Cd) in baby (0 to 6 months) powder milk are in the range of 0.01−0.02 mg/kg. It is seen that the comparatively lower concentration of Cd is found in Biomil-1, Lactogen-1, Eldorin-1 and Baby care-1 (0.01 mg/kg). The higher concentration was found in Mother's smile-1, Mamilag-1 and Biomil soy (0.02 mg/kg). Kidney may be damaged by the toxic effect of Cd, although it has also been

associated with lung damage and skeletal changes in occupationally exposed population. Recommended value of Cd for infants is 3×10^{-3}–4×10^{-3} mg/day [17, 18].

Chromium (Cr) particularly Cr(III) plays an important role in the body function but it becomes toxic if it exceeds permissible level. Cr(IV) is toxic and has no important role in body. Trivalent Cr is an essential element that involves in the metabolic function of carbohydrate, lipid, protein and nucleic acid. Cr is also a cofactor of insulin, promoting insulin activity and enhancing amino acid uptake into muscular cell for protein synthesis. It should be mentioned that Cr(III) and Cr(IV) remain in equilibrium in human body. Here the total amount of chromium [Cr(III) + Cr(IV)] is determined. So it is uncertain how much of Cr(III) and Cr(IV) are present separately in the studied samples. The concentration of chromium in baby 0 to 6 months powder milk of different brands available in market is on the average to 0.11 mg/kg. Least concentration of Cr is present in Biomil-1 (0.03 mg/kg) and highest amount of Cr is found in Mother's smile-1 (0.16 mg/kg). Recommended value of chromium for infants is 0.01−0.12 mg/day [19].

Arsenic (As) toxicity is a global health problem affecting many millions of people. The main source of exposure is drinking water contaminated by natural geological sources [20]. Current risk assessment is based on the recognized carcinogenicity of arsenic [21, 22], but neurotoxic risk has been overlooked. In 1955, an outbreak of arsenic poisoning occurred among Japanese infants, with more than 100 deaths. The concentration of As in baby powder milk of selected brands varies between 0.26 mg/kg and 0.77 mg/kg. The lowest amount of As is present in Mother's smile-1 (0.26 mg/kg) and the highest amount in Eldorin-1 (0.77 mg/kg). Arsenic in Biomil-1, Lactogen-1, Baby care-1, Mamilag-1 and Biomil soy was found 0.39, 0.38, 0.28, 0.61 and 0.42 mg/kg respectively. The recommended value of As is 0.61 mg/day for infant [23].

Nickel is required for normal growth and reproduction in animals and presumably in human being as well. It appears to have a role in the modulation of the immune system and in development of the brain. The danger of nickel toxicity from food appears to be very low, since large amount of nickel are required to produce any toxic effects through ingestion [14]. It is seen that the concentration of nickel in baby (0 to 6 months) powder milk is below the detection limit. So there is no toxic effect of Ni in

our studied samples but infants may suffer from nickel deficiency. Deficiency of nickel lead to anemia, deformation of leg bone, delayed puberty, depressed oxidative ability of the liver, high newborn mortality, poor growth and poor zinc absorption [14]. The concentration of some metals in mother's milk is listed in the Table 3.11.

Table 3.11: Metal concentration (Zn, Pb, Cd and Cr) in human milk samples of different countries (µg/L)

Country	Zn	Pb	Cd	Cr
Australia[24]	ND	0.55	–	–
Bangladesh [25]	1800	–	–	–
China [26]	ND	4.74	–	–
Sweden [27]	–	0.7	0.6	–
Sudan [28]	1300	2.6	–	–
India [17]	1772	1.9	0.09	–
Poland [29]	5017	5.4	6.2	14.2
Saudi Arabia [30]	1384.2	3.9	1.9	16.1
Spain [31]	–	–	–	22.3

ND - Not detected.

There is a wide range of variation of the concentration of element in human milk of different countries [Table 3.11]. The concentration of Zn is the highest in mother milk of Poland and lowest in Sudan. From this data the concentration level of Pb is highest in Poland (5.4 µg/L) and lowest in Sweden (0.7 µg/L). But Pb was not found within the detection limit for Spanish brand. The concentration level of Cd is the highest in Poland (6.2 µg/L) and the lowest in India (0.09 µg/L). Cd is not found within the detection limit in Australia, China, Sudan and Spain. Cr content is the highest in Spain (22.3 µg/L) and the lowest in Poland (14.2 µg/L) and is below detection limit in Australia, China, Sudan, Sweden and India. The daily intake of the metal depends on both the concentration and the amount of food consumed. The mean of 24 hours output of breast milk of an Indian mother is 550 mL for first 3 months, 680 mL for 4 to 6 months [17, 32]. From the published result it is found that the mean daily output

of breast milk of an American mother is 400 to 500 mL at the early transitional and mature stages of lactation [33]. From the Table 3.1, 3.2 and 3.3 it is seen that 107.69, 109.28, 108.90, 108.40, 109.28, 108.30 and 111.60 g powder of Biomil-1, Lactogen-1, Mother's smile-1, Eldoren-1, Mamilag-1, Baby care-1 and Biomil soy are required to prepare one liter equivalent milk against breast milk respectively. An infant can feed 500 mL breast milk per day. That's why she/he consumes 53.85, 54.64, 54.45, 54.20, 54.64, 54.15 and 55.8 g powder of Biomil-1, Lactogen-1, Mother's smile-1, Eldorin-1, Mamilag-1, Baby care-1 and Biomil soy respectively per day. From baby (0−6 months) powder milk the daily intake (mg/kg) of trace and toxic metals by infants is shown in the Table 3.12.

Table 3.12: Amount of trace and toxic metals from baby powder milk consumed by infants (mg/day)

Sample	Country	Co	Zn	Pb	Cd	Cr	As	Ni
Biomil-1	Belgium	BDL	1.57	0.022	0.0005	0.0016	0.021	BDL
Lactogen-1	Switzerland	BDL	1.65	0.021	0.0005	0.0032	0.021	BDL
M smile-1	Australia	0.001	1.57	0.013	0.001	0.0087	0.014	BDL
Eldorin-1	Netherland	0.002	1.85	0.016	0.0005	0.0065	0.041	BDL
Mamilag-1	Poland	BDL	1.05	0.012	0.001	0.0071	0.015	BDL
Baby care-1	Korea	0.001	0.88	0.008	0.0005	0.0076	0.033	BDL
Biomil soy	Belgium	BDL	2.95	0.007	0.001	0.0084	0.023	BDL

M smile = Mother's smile, BDL= Below Detection Limit.

From the Table 3.12, it is found that Eldorin-1 contains the highest amount of Co (2×10^{-3} mg/day). Mother's smile and Baby care-1 contain the lower amount (1×10^{-3} mg/day). Permissible limit of Co is 4×10^{-4} mg/day. Eldorin-1, Mother's smile-1 and Baby care-1 contain higher amount of Co than the permissible limit. Cobalt requires for good human health. Vitamin B_{12} could not exist without Co. Vitamin B_{12} prevents nerve damage. But excess intake of cobalt may causes

respiratory irritation, wheezing, asthma, decreased lung function, pneumonia, and fibrosis [34, 35]. So Eldorin-1, Mother's smile-1 and Baby care-1 are not safe for our babies.

The amount of Zn in baby powder milk ranges from 0.88−2.95 mg/day. Recommended level of zinc is 3−5 mg/day. It shows that the concentration of Zn in the studied baby (0−6 months) powder milk is lower compared to the recommended value. Prasad et al. showed that dietary zinc supplementation causes improved growth. Zinc is necessary for wound healing. The deficiency can cause loss of appetite, growth retardation and immunological abnormalities [15, 16]. Hence the milk samples are safe but more Zn is required to improve the quality of the milk samples for our baby.

It is seen that the highest amount of Pb is found in Biomil-1 (0.022 mg/day) and the lowest in Biomil soy (7×10^{-3} mg/day). The Recommended level of Pb is 12.5×10^{-3} – 17.5×10^{-3} mg/day. From our study it is found that consumption of Biomil-1 and Lactogen-1 by infant intake level of Pb is higher than the recommended value. But in Mother's smile-1, Mamilag-1, Baby care-1, Eldorin-1 and Biomil soy contain lower value of Pb (< recommended value) and these five brands are safe for our infants. Biomil-1 and Lactogen-1 are not safe and appear some adverse effect which causes brain damage, paralysis, anemia and damage to the kidneys also.

Comparatively lower amount of Cd is present in Mother's smile-1, Eldorin-1, Mamilag-1 and Baby care-1 (5×10^{-4} mg/day) and higher amount in Biomil-1, Lactogen-1 and Biomil soy (1×10^{-3} mg/day). The recommended value of Cd is 3×10^{-3} – 4×10^{-3} mg/day. Biomil-1, Lactogen-1 and Biomil soy (1×10^{-3} mg/day) contain Cd near to the recommended value. Mother's smile-1, Eldorin-1, Mamilag-1 and Baby care-1 (5×10^{-4} mg/day) contain lower amount of Cd than the recommended value. Excess intake of Cd may damage of kidney and lung. This is why Biomil-1. Lactogen-1 and Biomil soy are not safe for infants.

The highest concentration of Cr is found in Bioml soy (8.7×10^{-3} mg/day) and lowest in Biomil-1 (1.6×10^{-3} mg/day). The recommended value of Cr is 0.01−0.12 mg/day. It shows that the concentration of Cr in the studied baby (0−6 months) powder milk samples is lower compared to the recommended value and free from toxic effect.

Infant consumed As 0.021 mg/day from Biomil-1 and Lactogen-1. Mother's smile-1, Eldorin-1, Baby care-1 and Biomil soy contain arsenic 0.014, 0.041, 0.033 and 0.023 mg/day respectively. The recommended value of As is 0.61mg/day which is higher than the baby (0−6 months) powder milk samples and hence it is safe.

The amounts of Ni in all samples were below detection limit.

The variation of concentration (mg/kg) of metals and their comparison with maximum permissible limit in baby (0−6 months) powder milk are shown in Figures 3.16, 3.17, 3.18, 3.19, 3.20, 3.21, 3.22, 3.23, 3.24, 3.25, 3.26 and 3.27.

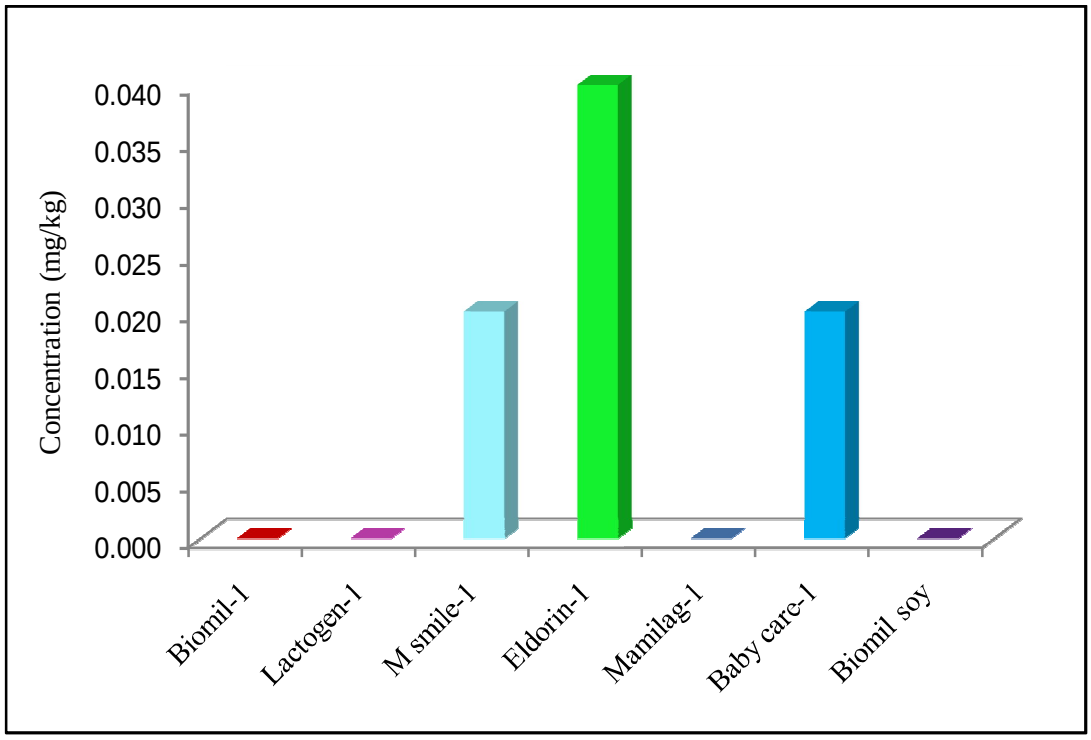

Figure 3.16: The variation of concentration of cobalt in baby (0−6 months) powder milk of different brands.

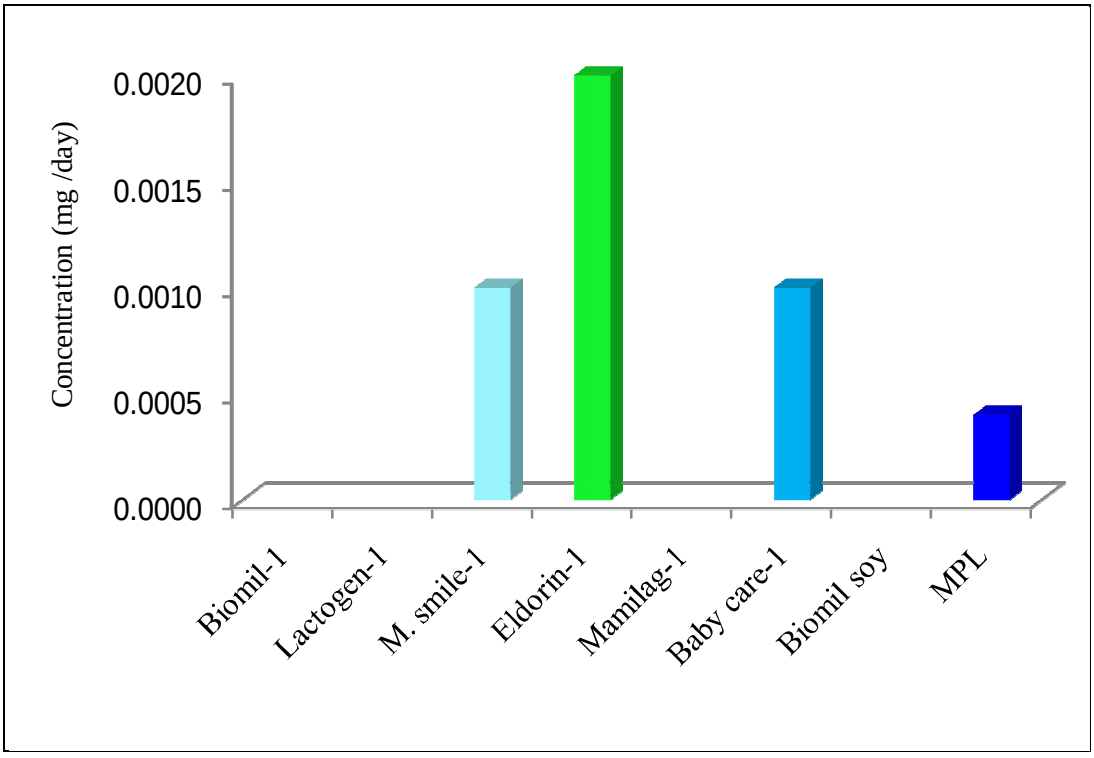

Figure 3.17: The comparison of concentration of cobalt in baby (0−6 months) powder milk of different brands with maximum permissible limit.

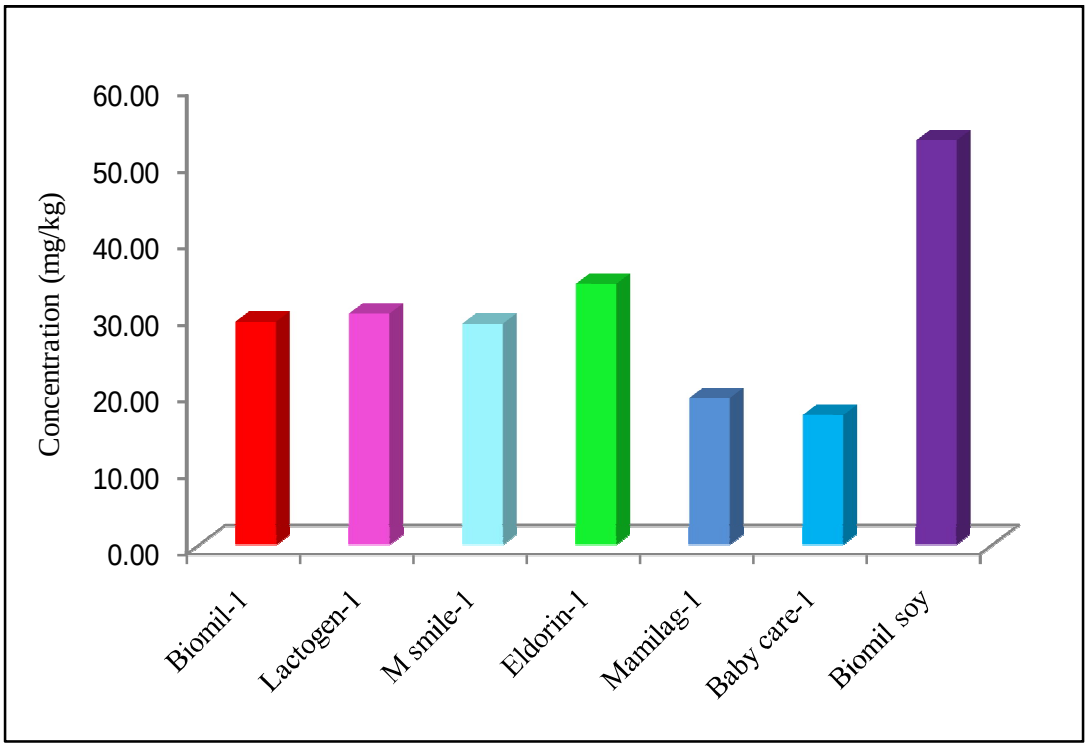

Figure 3.18: The variation of concentration of zinc in baby (0−6 months) powder milk of different brands.

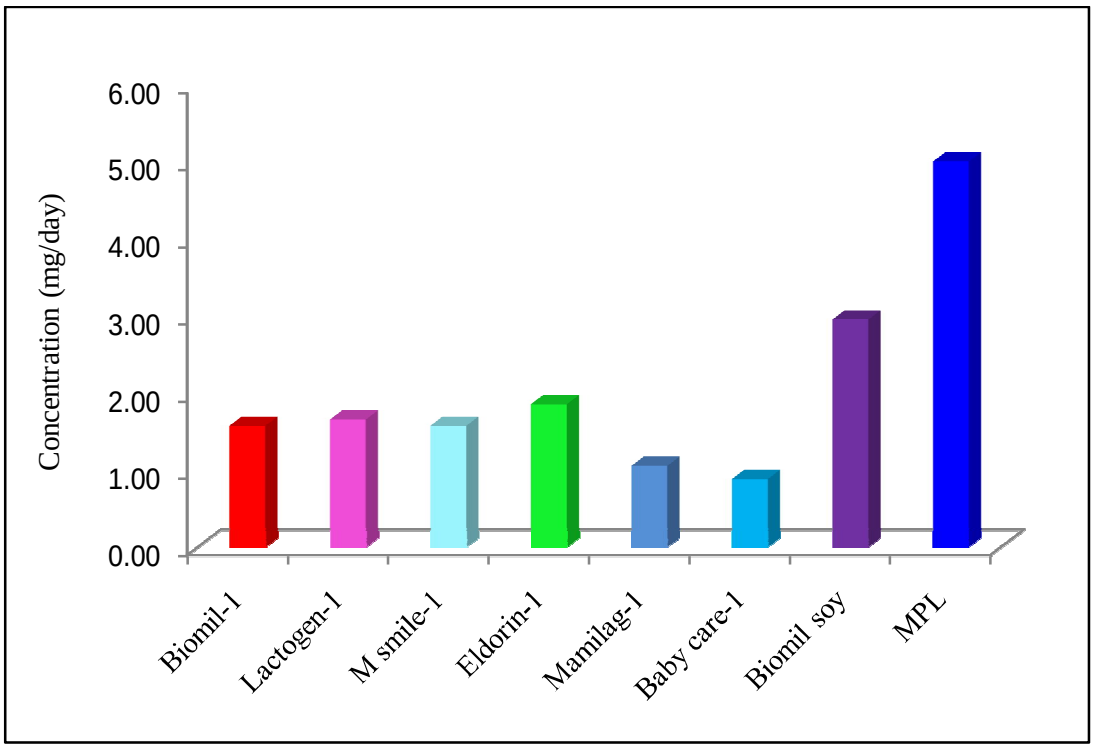

Figure 3.19: the comparison of concentration of zinc in baby (0−6 months) powder milk of different brands with maximum permissible limit.

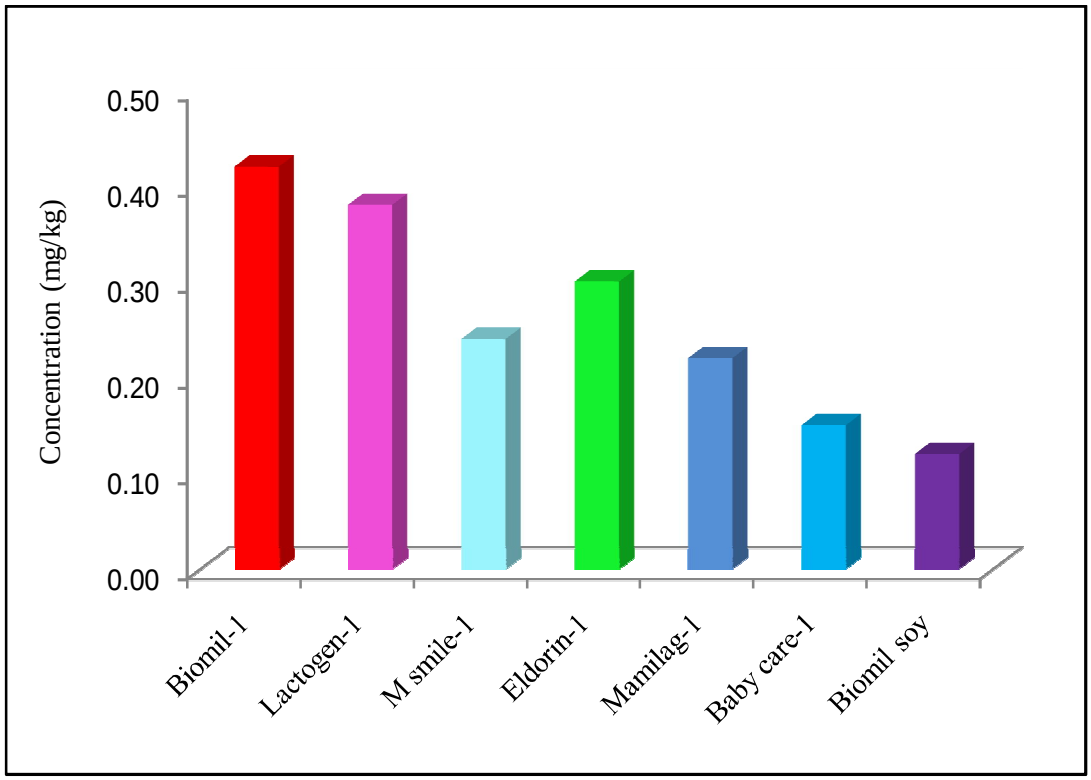

Figure 3.20: The variation of concentration of lead in baby (0−6 months) powder milk of different brands

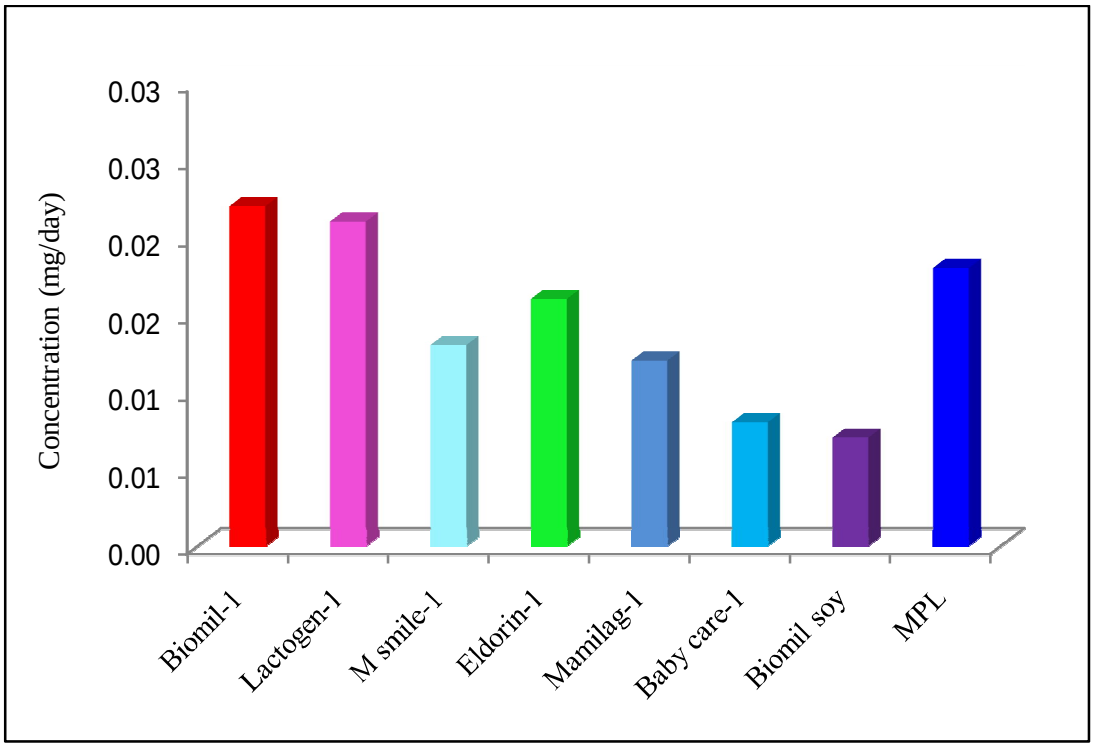

Figure 3.21: The comparison of concentration of lead in baby (0−6 months) powder milk of different brands with maximum permissible limit.

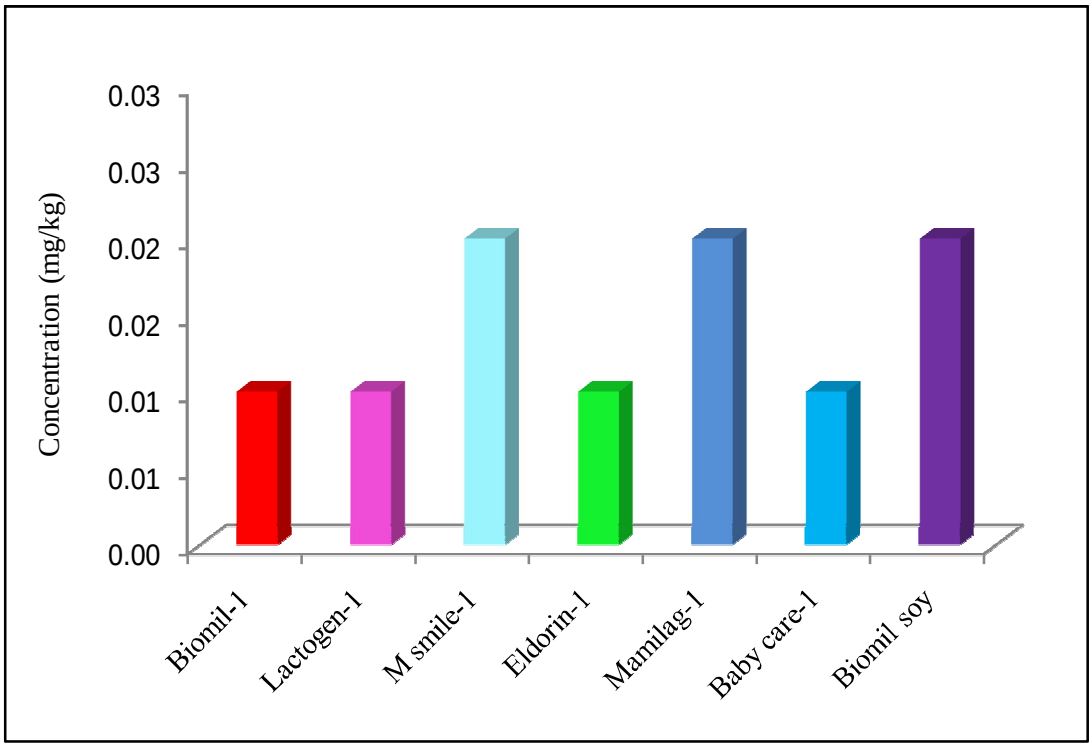

Figure 3.22: The variation of concentration of cadmium in baby (0−6 months) powder milk of different brands.

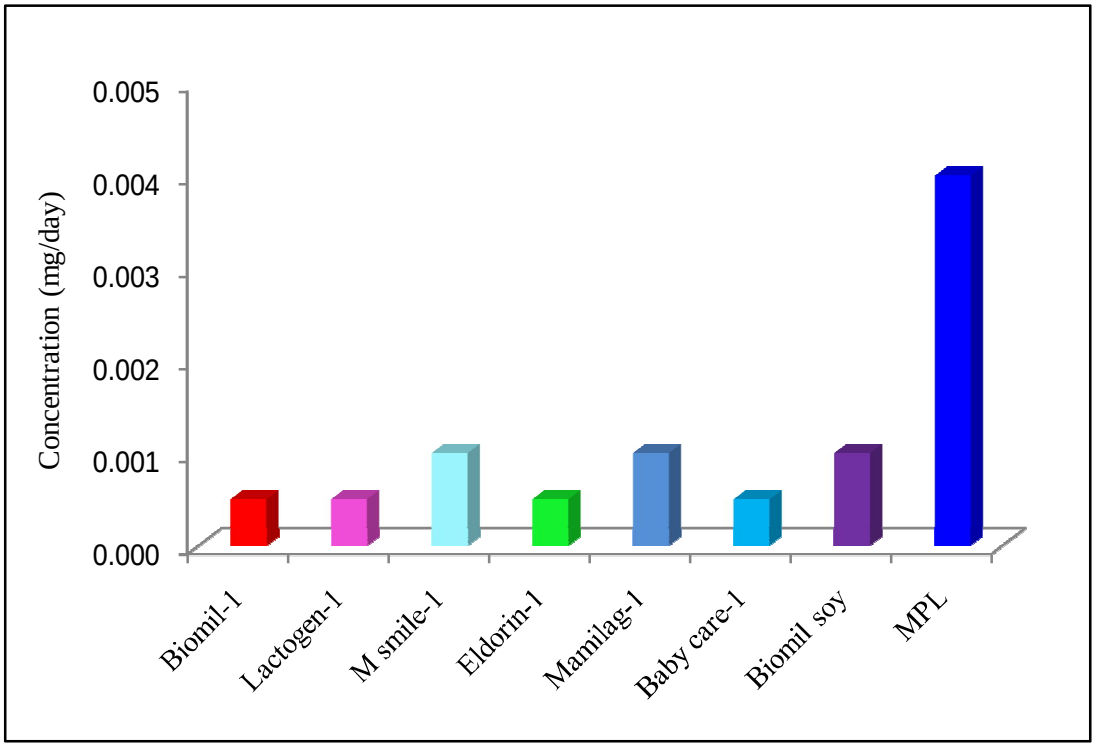

Figure 3.23: The comparison of concentration of cadmium in baby (0−6 months) powder milk of different brands with maximum permissible limit.

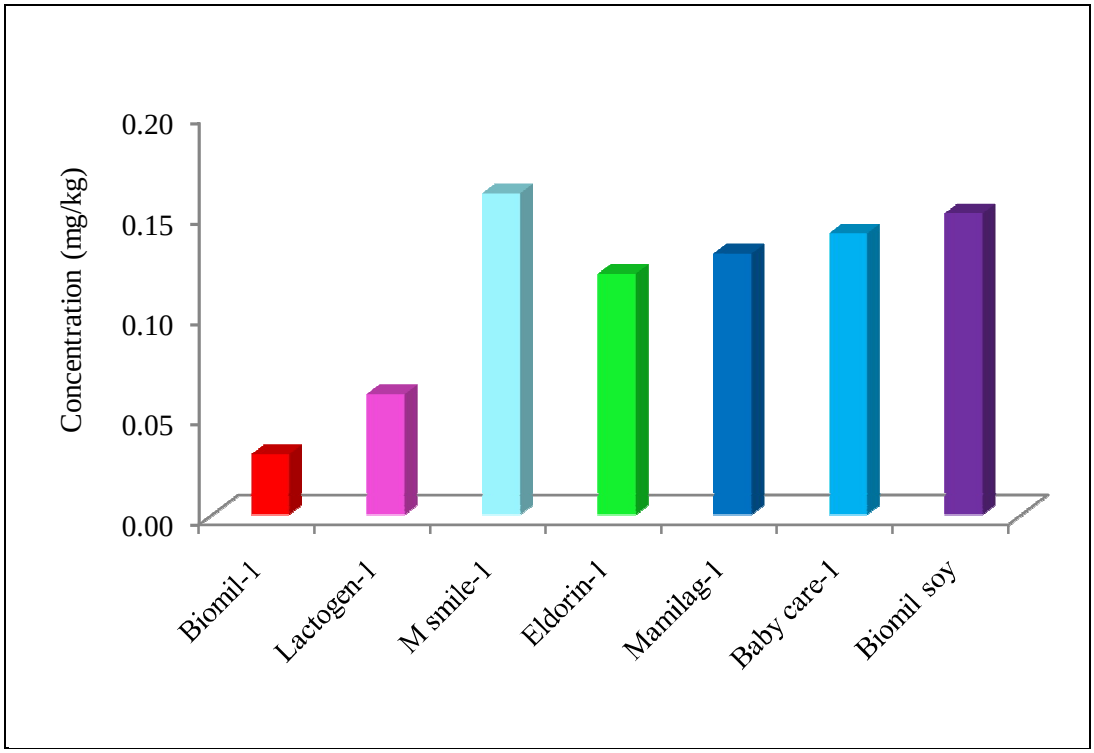

Figure 3.24: The variation of concentration of chromium in baby (0−6 months) powder milk of different brands.

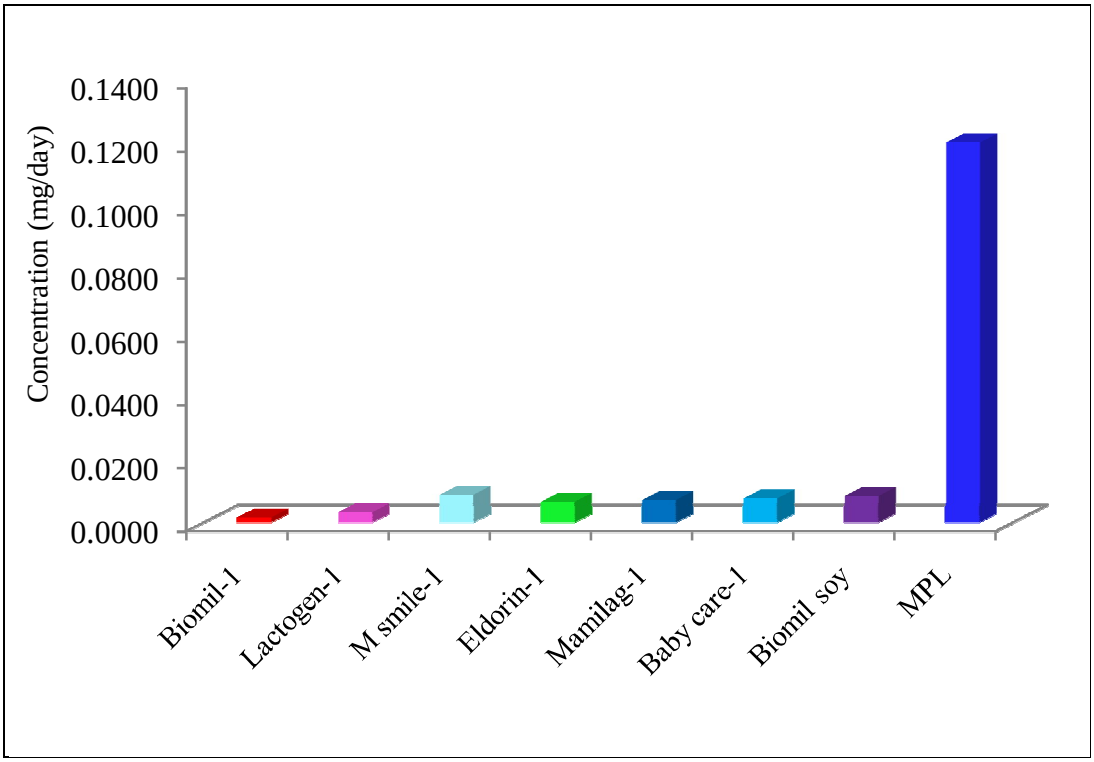

Figure 3.25: The comparison of concentration of chromium in baby (0−6 months) powder milk of different brands with maximum permissible limit.

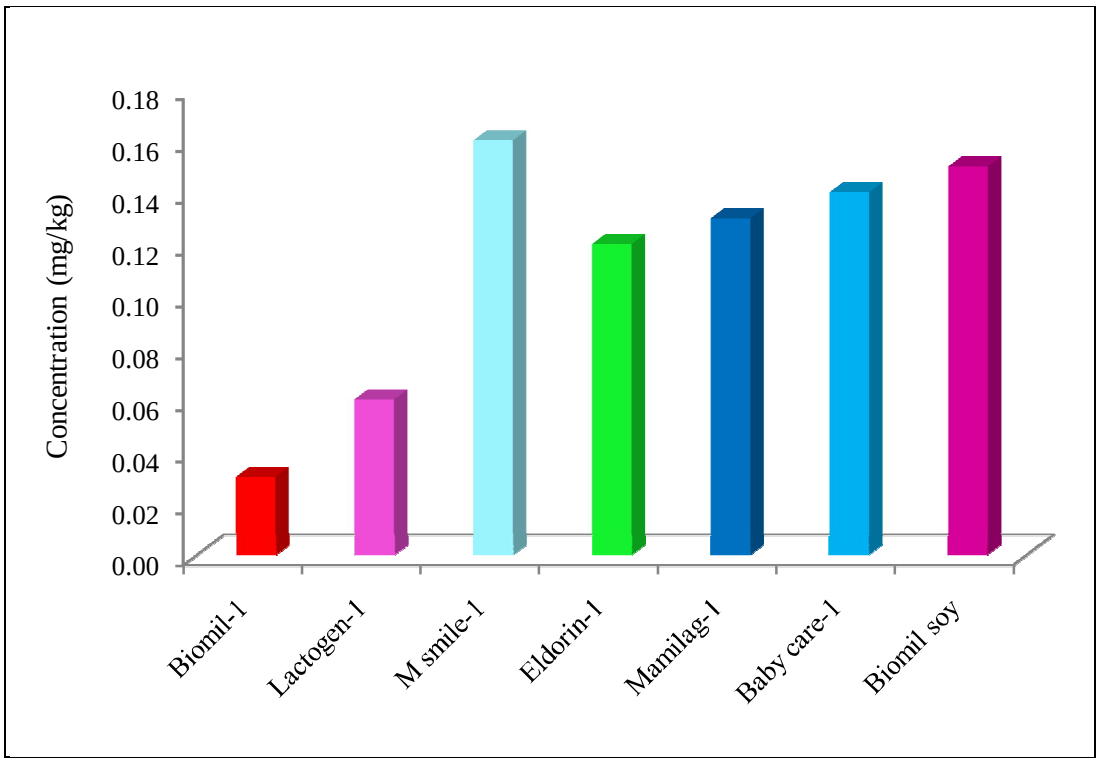

Figure 3.26: The variation of concentration of arsenic in baby (0−6 months) powder milk of different brands.

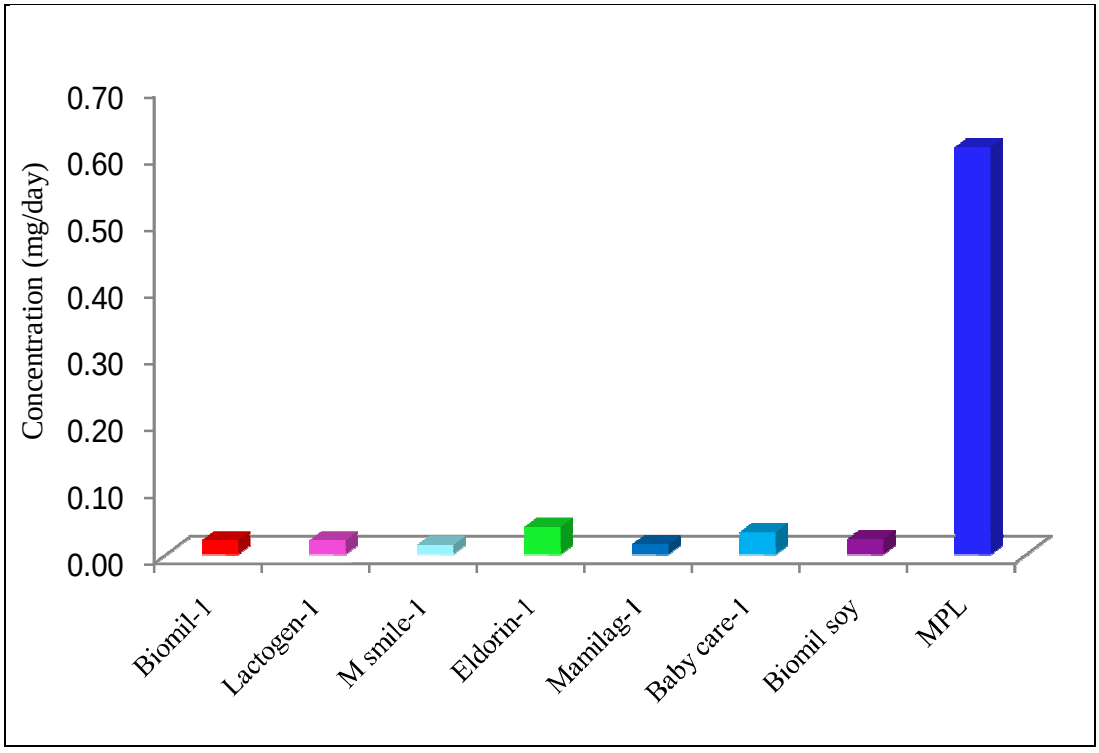

Figure 3.27: The comparison of concentration of arsenic in baby (0−6 months) powder milk of different brands with maximum permissible limit.

Table 3.13: Amount (mg/kg) of trace and toxic metals in baby (6−24 months) powder milk of selected brands

Sample	Country	Co	Zn	Pb	Cd	Cr	As	Ni
Lactogen -2	Switzerland	0.44	BDL	BDL	0.04	4.11	BDL	4.25
Biomil -2	Belgium	0.47	13.0	BDL	0.05	4.48	BDL	5.09
Biomil -2	Korea	0.47	5.4	BDL	0.01	4.67	0.24	8.12
Eldorin -2	Netherland	0.41	BDL	BDL	0.01	3.81	0.12	2.39
Lailac-2	France	0.44	42	BDL	0.02	4.06	BDL	9.20
M smile-2	Australia	0.40	41	BDL	0.01	3.58	BDL	0.65
Mamilag -2	Poland	0.38	BDL	BDL	0.01	3.84	BDL	0.76
Mean	−	0.43	25.35	BDL	0.021	4.08	0.18	4.35

BDL: Below Detection Limit.

It is seen from the Table 3.13 that the mean concentration of cobalt in baby (6−24 months) powder milk of selected brands is 0.43 mg/kg. The higher concentration of Co is found in Biomil-2 and Baby care-2 (0.47 mg/kg) and the lowest in Mamilag-2 (0.38 mg/kg). The permissible limit of Co is (4×10^{-4} mg/day). Consumption of Co by infants from Lactogen-2, Biomil -2, Baby care-2, Eldorin-2, Lailac-2, M smile-2 and Mamilag-2 is 0.025, 0.026, 0.027, 0.023, 0.025, 0.023 and 0.021 mg/day respectively [Table 3.14]. All of these brands show the higher value of Co than the permissible limit and hence are harmful to infants. So all of these milk samples are not safe for infants.

The amount of zinc is found in baby milk ranges from 5.4 to 42.0 mg/kg. Lailac-2 contains highest amount of Zn (42 mg/kg) and Baby care-2 contains the lowest amount (5.4 mg/kg). It is also found the below detection limit in Biomil-2 and Mamilag-2. Daily intake levels of Zn (mg/day) [Table 3.14] in the milk powder of different brands are lower than the permissible limit (3−5 mg/day). Hence Zn deficiency may cause growth retardation. So the customer should be conscious about these brands.

Table 3.13 indicates that the concentration of Pb in baby (6−24 months) powder milk of selected brands is below the detection limit. Hence it is safe for infants.

The concentration of cadmium in baby powder milk is in the range of 0.01−0.05 mg/kg. It is seen that the highest concentration of Cd is found in Biomil-2 (0.05 mg/kg) and the lower concentration in Baby care-2, Eldorin-2, Mami lag-2 and Mother's smile-2 (0.1 mg/kg). The recommended value of Cd is in the range of 3×10^{-3}–4×10^{-3} mg/day. From the Table 3.14 it is evident that the amount of Cd (mg/day) taken by infants from the powder milk samples has lower value than the recommended level. Hence it is not so harmful.

The mean concentration of chromium in baby (6−24 months)) powder milk of different brands is 4.08 mg/kg. The lowest concentration was present in Mother's smile-2 (3.58 mg/kg) and the highest amount in Baby care-2 (4.67 mg/kg) [Table 3.13]. Recommended level of Cr for infants is between 0.01 and 0.12 mg/day [19]. It is found that the amount of Cr (mg/day) taken by infants from the powder milk [Table 3.14] is higher than the recommended value. So, it is also harmful and these brands should be avoided.

The concentration of As in baby powder milk of selected brands varies between 0.12 and 0.24 mg/kg. The lowest amount of As is present in Eldorin-2 (0.12 mg/kg) and the highest amount in Baby care-2 (0.24 mg/kg). Arsenic found in Lactogen-2, Biomil-2, Lailac-2, Mother's smile-2 and Mamilag-2 is below the detection limit. The amount of As (mg/day) consumed by infants from milk samples is lower than the recommended value (0.61mg/day). This is why the milk powder of different brands have no toxic effects for infants.

The mean concentration of Ni in baby (6−24 months) powder milk of selected brands is found to be 4.35 mg/kg. The highest amount was found in Lailac-2 (9.20 mg/kg) and the lowest amount in Mother's smile-2 (0.65 mg/kg). Recommended value of Ni is 0.45 mg/day. Consumption of Ni (mg/day) from Babycare-2 and Lailac-2 by infants is higher than the recommended value [Table 3.14]. Chest pain, cough, fever with leukocytosis, pulmonary haemorrhage, cerebral oedema, toxic myocarditis etc. are caused by the excessive intake of Ni. For these reasons Babycare-2 and Lailac-2 are not safe.

From the Table 3.5, 3.6 and 3.7, it is seen that 112.30, 110.50, 112.90, 110.60, 113.50, 113.20 and 111.90 g powder of Lactogen-2, Biomil-2, Baby care-2, Eldorin-2, Lailac-2, Mother's smile-2 and Mamilag-2 respectively are required to prepare per litter equivalent milk of breast milk. An infant can take 500 mL breast milk per day. That's why she/he consumes 56.15, 55.25, 56.45, 55.30, 56.75, 56.60 and 55.95 g powder of Lactogen-2, Biomil-2, Baby care-2, Eldorin-2, Lailac-2, Mother's smile-2 and Mamilag-2 per day respectively.

From (6 −24 months) baby powder milk the daily intake (mg/day) of trace and toxic metals by infants is shown in the Table 3.14.

Table 3.14: Amount of trace and toxic metals of baby powder milk consumed by infants (mg/day)

Sample	Country	Co	Zn	Pb	Cd	Cr	As	Ni
Lactogen-2	Switzerland	0.025	BDL	BDL	0.0022	0.23	BDL	0.24
Biomil-2	Belgium	0.026	0.72	BDL	0.0027	0.24	BDL	0.28
Baby care-2	Korea	0.027	0.30	BDL	0.0006	0.26	0.014	0.46
Eldorin-2	Netherland	0.023	BDL	BDL	0.0006	0.21	0.007	0.13
Lailac-2	France	0.025	2.38	BDL	0.0011	0.23	BDL	0.52
M smile-2	Australia	0.023	2.32	BDL	0.0006	0.20	BDL	0.04
Mamilag-2	Poland	0.021	BDL	BDL	0.0006	0.21	BDL	0.04

BDL: Below Detection Limit.

The variation of concentration (mg/kg) of metals and their comparison with maximum permissible limit (mg/day) in baby (6−24 months) powder milk are shown in the Figures 3.28 3.29, 3.30, 3.31, 3.32, 3.33, 3.34, 3.35, 3.36, 3.37, 3.38 and 3.39.

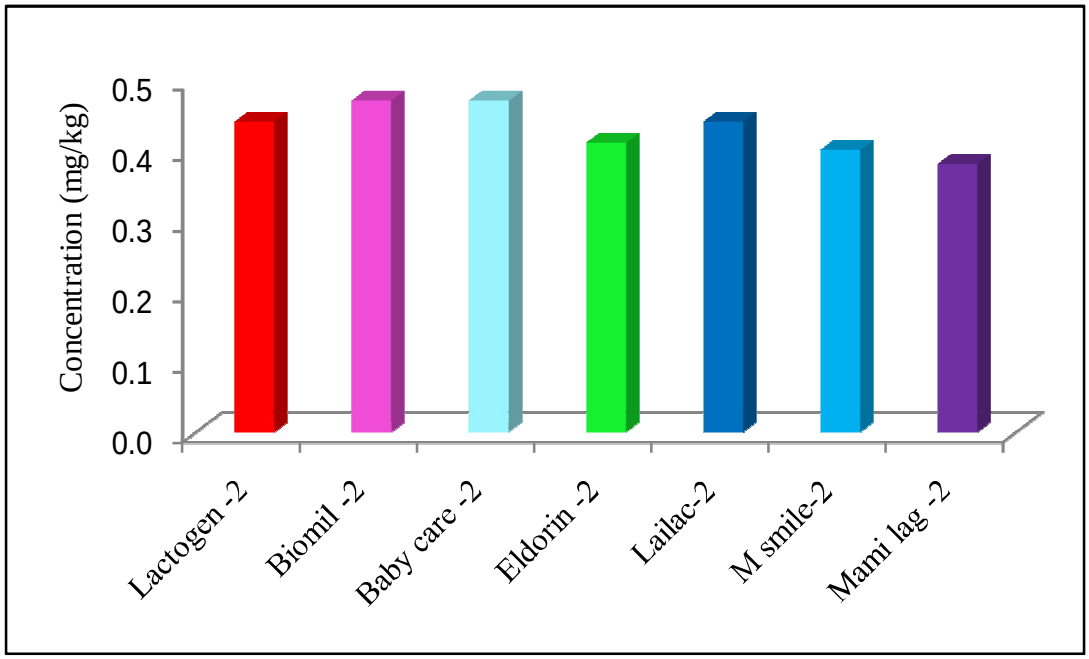

Figure 3.28: The variation of concentration of cobalt in baby (6−24 months) powder milk of different brands.

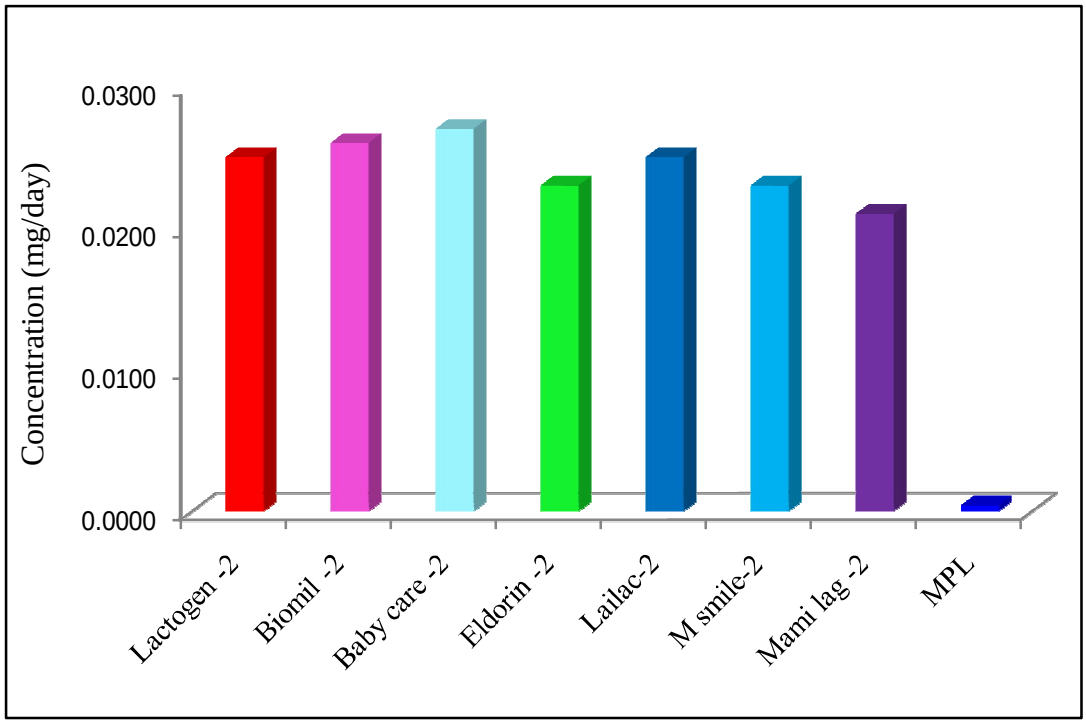

Figure 3.29: The comparison of concentration of cobalt in baby (6−24 months) powder milk of different brands with maximum permissible limit.

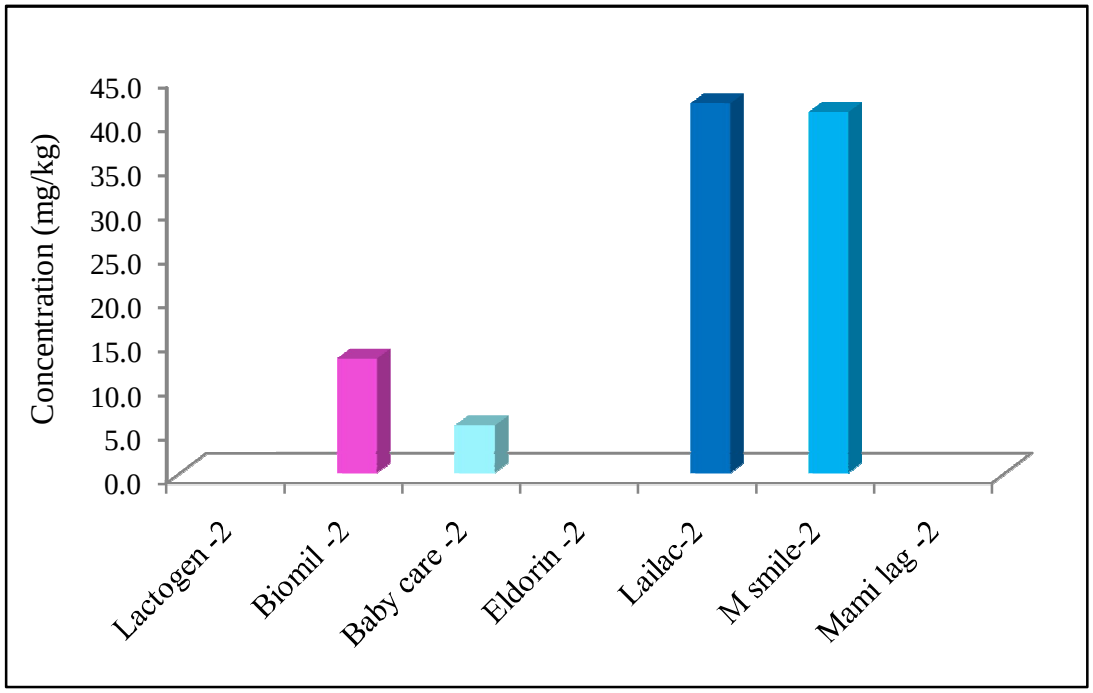

Figure 3.30: The variation of concentration of zinc in baby (6−24 months) powder milk of different brands.

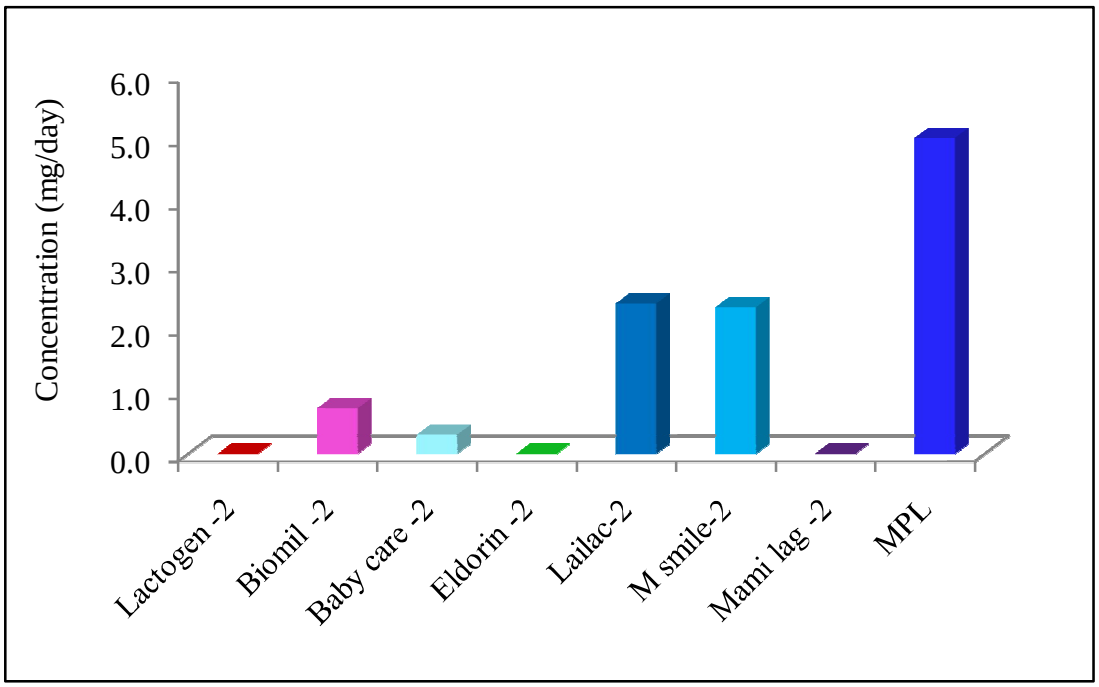

Figure 3.31: The comparison of concentration of zinc in baby (6−24 months) powder milk of different brands with maximum permissible limit.

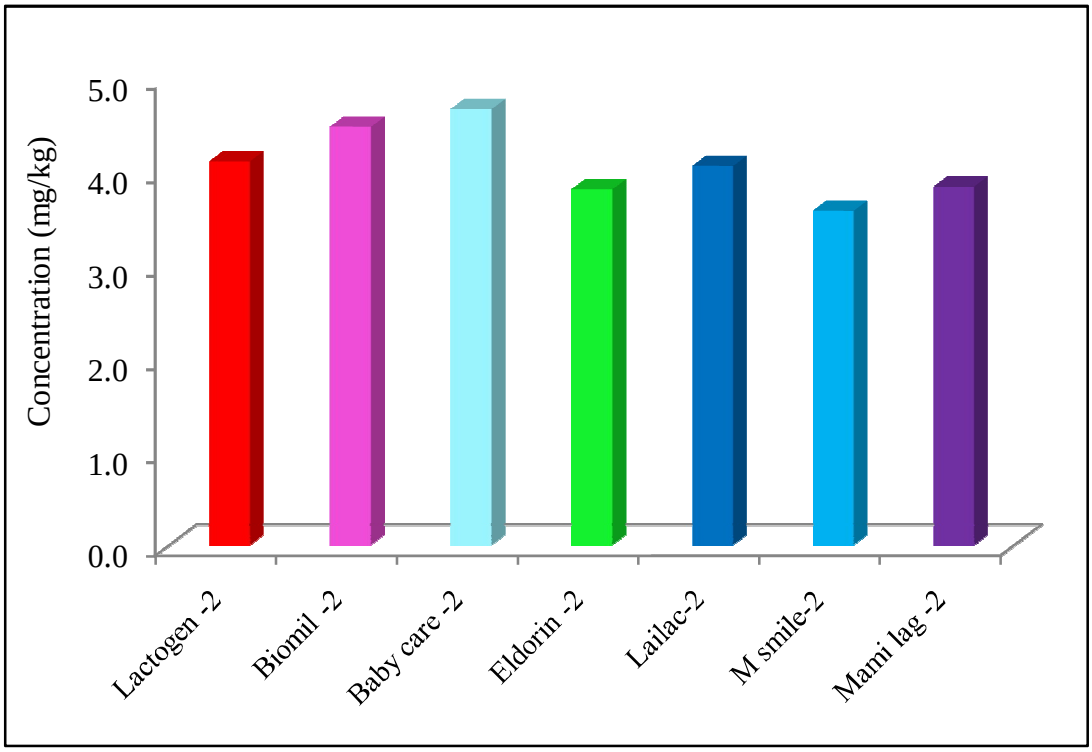

Figure 3.32: The variation of concentration of cadmium in baby (6−24 months) powder milk of different brands.

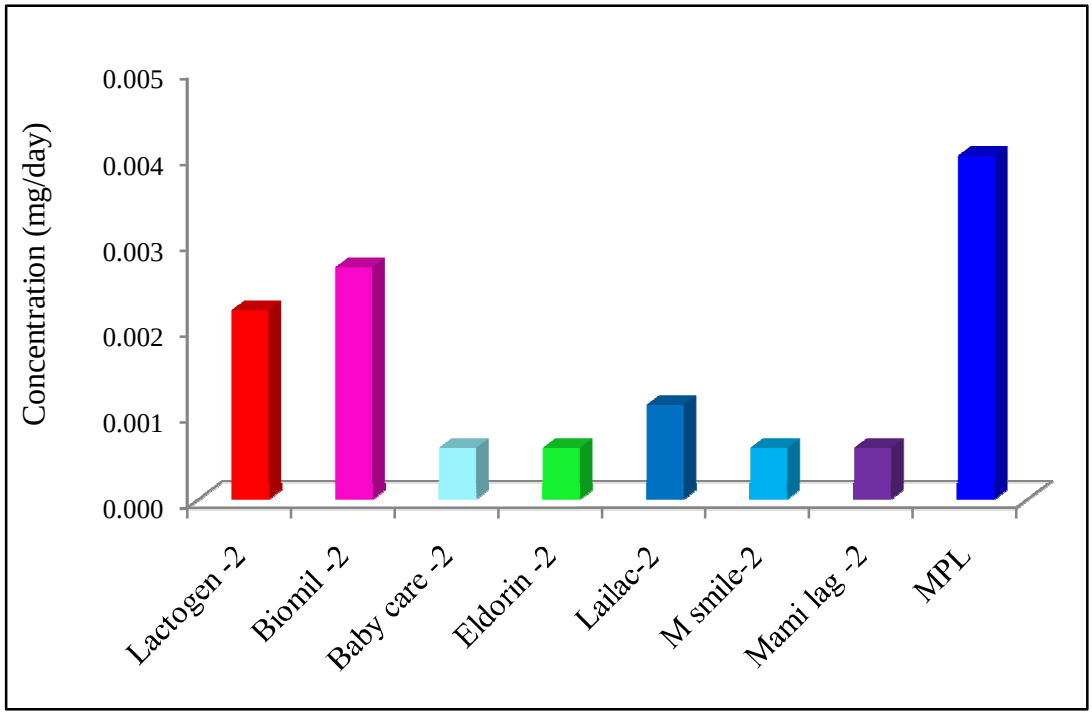

Figure 3.33: The comparison of concentration of cadmium in baby (6−24 months) powder milk of different brands with maximum permissible limit.

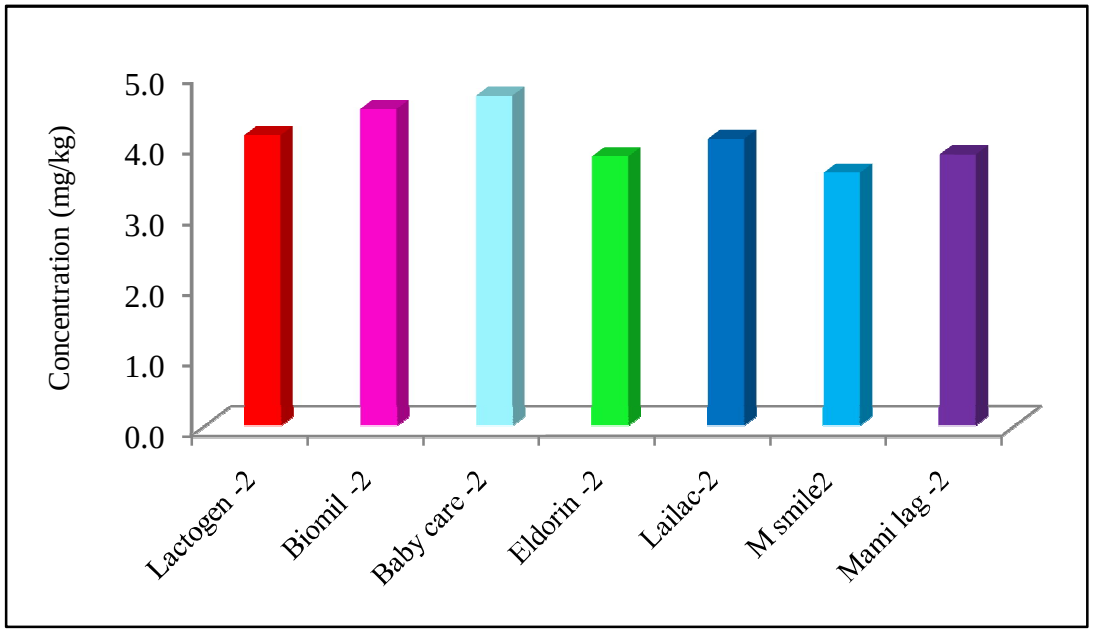

Figure 3.34: The variation of concentration of chromium in baby (6−24 months) powder milk of different brands.

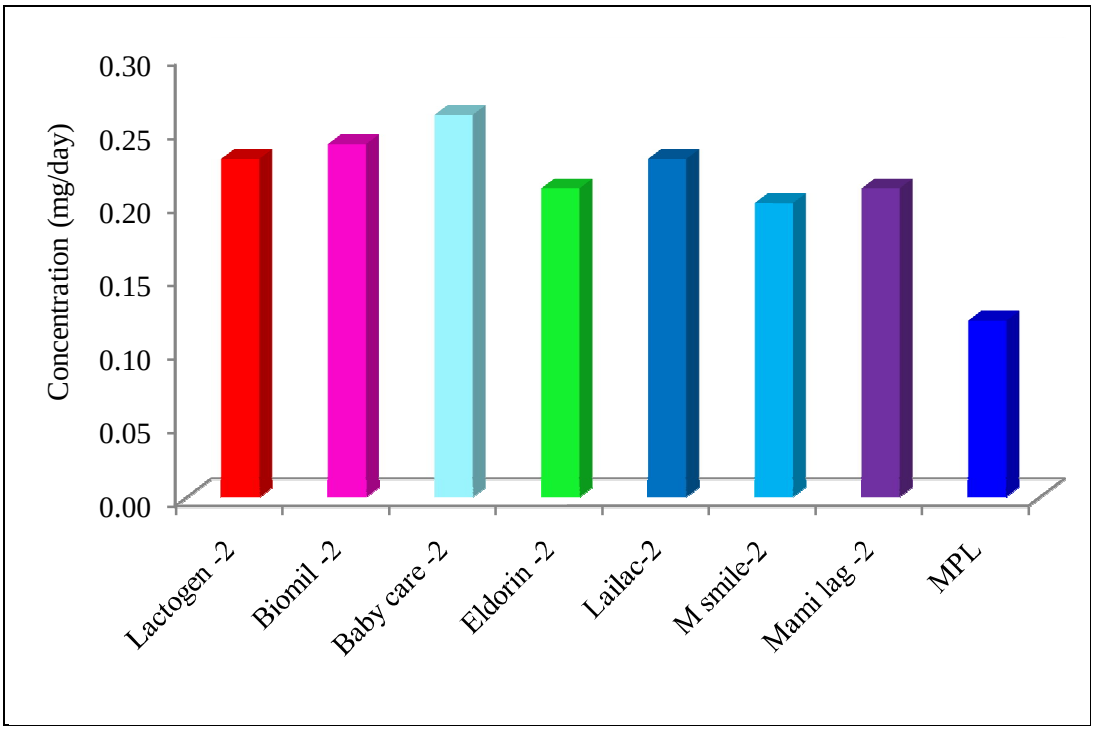

Figure 3.35: The comparison of concentration of chromium in baby (6−24 months) powder milk of different brands with maximum permissible limit.

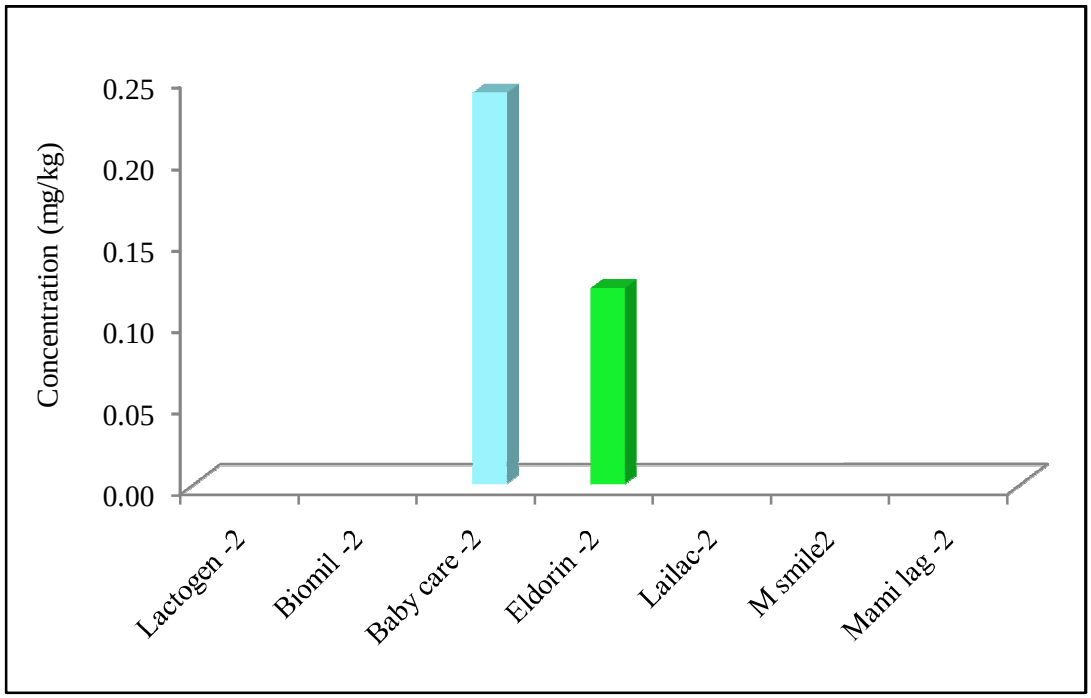

Figure 3.36: The variation of concentration of arsenic in baby (6−24 months) powder milk of different brands.

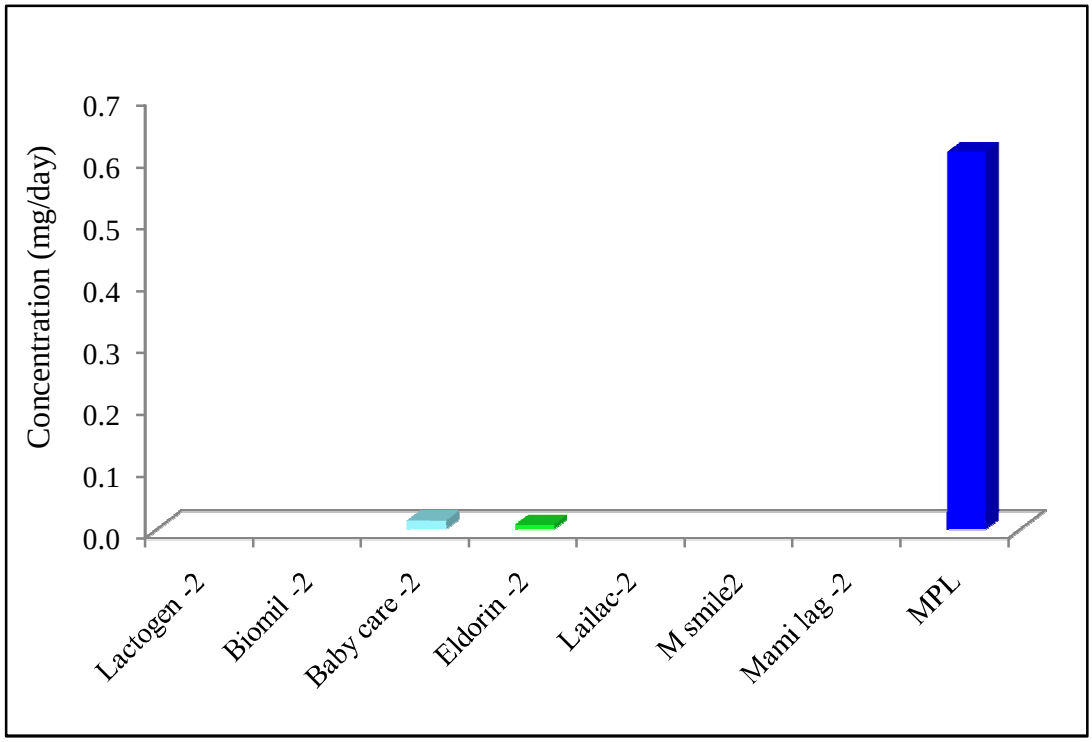

Figure 3.37: The comparison of concentration of arsenic in baby (6−24 months) powder milk of different brands with maximum permissible limit.

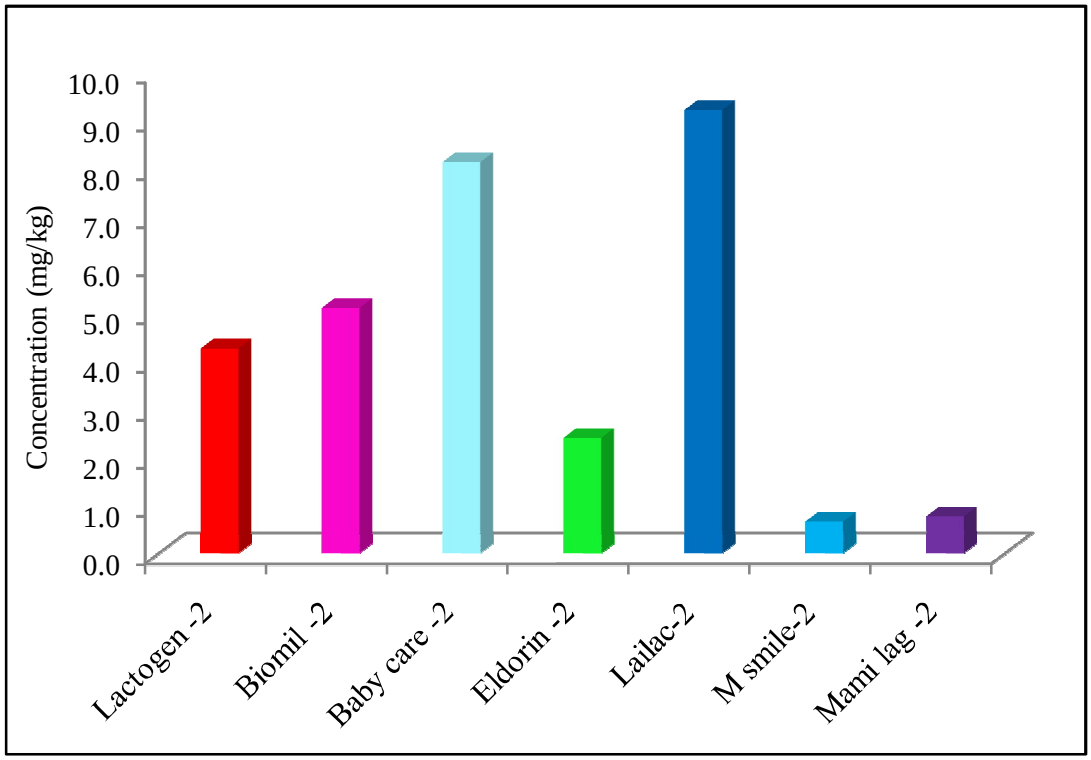

Figure 3.38: The variation of concentration of nickel in baby (6−24 months) powder milk of different brands.

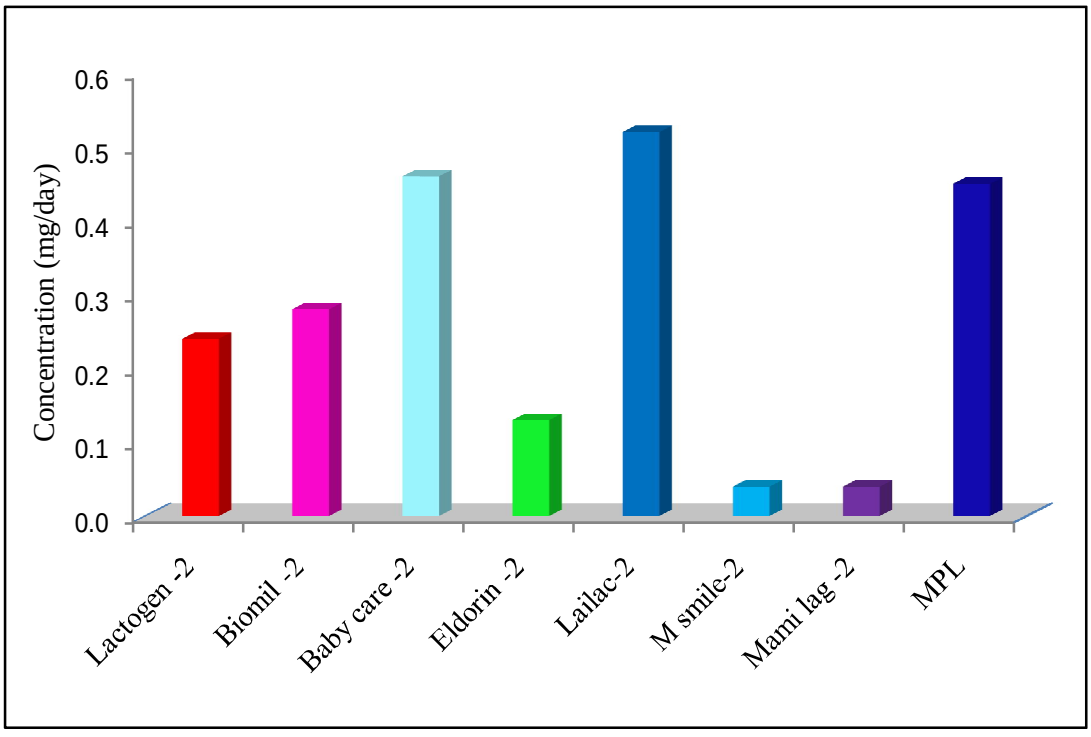

Figure 3.39: The comparison of concentration of nickel in baby (6−24 months) powder milk of different brands with maximum permissible limit.

3.3.7 Estimation of the amount of trace and toxic metals in baby (6−24 months) cereals.

The amount of trace (Co and Zn) and toxic (Pb, Cd, Cr, As, and Ni) metals in baby (6−24 months) cereal samples of selected brands are determined by atomic absorption spectrophotometer and have been arranged in Table 3.15. From our study it is seen that an infant can take the following amount (mg/day) of Co. Zn, Pb, Cd, Cr, As, and Ni metals in baby (6−24 months) cereal samples [Table 3.16.]

Table 3.15: Amount (mg/kg) of trace (Co and Zn) and toxic (Pb, Cd, Cr, As and Ni) metals in baby cereal of selected brands.

Sample	Co	Zn	Pb	Cd	Cr	As	Ni
Biomil-I (Wheat+3 Fruits+Milk)	BDL	330	BDL	BDL	0.38	1.59	32.7
Nestle-I (3 Fruits+Wheat+Milk)	BDL	28.2	BDL	0.59	0.18	BDL	2.9
Nestle - II (Rice+milk)	1.2	180	0.5	0.05	0.2	0.2	BDL
Nestle-III(Rice+Mixed vegetable)	BDL	164	BDL	0.04	0.31	1.68	BDL
Nestle -IV (Wheat +Dal+Palank)	BDL	200	0.3	0.09	0.93	BDL	1.35
Nestle-V (Wheat +Milk)	8.2	419	14.7	0.11	0.15	BDL	BDL
Nestle-VI (Wheat+apple+cornflakes)	3.2	659	BDL	BDL	0.53	1.6	BDL
M smile-I (Rice)	3.2	97.8	BDL	0.04	0.45	0.62	0.2
M smile-II (Relax Fruits+Wheat)	1.2	421	BDL	0.29	BDL	0.58	2.08
M smile-III (Honey +Wheat)	BDL	592	20.4	0.11	0.46	1.43	BDL
Mean	3.4	310	8.98	0.165	0.4	1.1	7.92

BDL: Below Detection Limit, M smile = Mother's smile

From the Table 3.15, it is observed that the highest amount of Co present in Nestle-V (8.2 mg/kg) and the lowest amount in Mother's smile-II and Nestle-II (1.2 mg /kg). It was found below detection limit in Biomil-1, Nestle-I, Nestle-III, Nestle-IV and Mother's smile-III. The permissible limit of Co for infant is 4×10^{-4} mg/day. The five baby cereals have some toxic effect and are not safe for infants.

The amount of zinc in cereal samples varies from 28.2 to 659 mg/kg. Nestle-VI contains the highest amount of Zn (659 mg/kg) and Nestle-I contains the lowest amount (28.2 mg/kg). Recommended level of zinc is 3−5 mg/day. From the Table

3.16 it is seen that each of the baby cereal contains the higher amount of Zn than the recommended value. Zinc is an essential trace element for human but excessive intake of Zn may occur nausea**,** vomiting, pain, cramps and diarrhoea. So due to this excessive intake some adverse effects may occur.

The mean concentration of Pb in baby cereal of selected brands is 8.98 mg/kg [Table 3.15]. The highest concentration of Pb is found in Mother's smile-III (20.4 mg/kg) and the lowest in Nestle-IV (0.3 mg/kg). Recommended level of lead for infants is from 12.5×10^{-3} to 17.5×10^{-3} mg/day. From the Table 3.16, it is seen that four of these cereals contain the higher amount of Pb (mg/day) than the recommended value. Long term accumulation of Pb in human body may causes damage to the kidneys. Hence these brands should be avoided for children.

Table 3.16: Amount of trace (Co and Zn) and toxic (Pb, Cd, Cr, As and Ni) metals in baby cereal of selected brands (mg/day).

Sample	Co	Zn	Pb	Cd	Cr	As	Ni
Biomil-I (Wheat+3 Fruits+Milk)	BDL	19.8	BDL	BDL	0.023	0.095	1.96
Nestle-I (3 Fruits+Wheat+Milk)	BDL	1.69	BDL	0.04	0.011	BDL	0.174
Nestle - II (Rice+milk)	0.07	10.8	0.03	0.003	0.001	0.012	BDL
Nestle-III(Rice+Mixed vegetable)	BDL	9.8	BDL	0.002	0.02	0.10	BDL
Nestle -IV (Wheat +Dal+Palank)	BDL	12	0.02	0.005	0.06	BDL	0.081
Nestle-V (Wheat +Milk)	0.49	25.1	0.88	0.007	0.01	BDL	BDL
Nestle-VI (Wheat+apple+cornflakes)	0.19	39.5	BDL	BDL	0.032	0.096	BDL
M smile-I (Rice)	0.19	5.9	BDL	0.002	0.027	0.62	0.012
M smile-II (Relax Fruits+Wheat)	0.07	25.2	BDL	0.017	BDL	0.037	0.17
M smile-III (Honey +Wheat)	BDL	35.5	1.22	0.007	0.028	0.086	BDL
Mean	0.20	18.6	0.54	0.01	0.024	0.066	0.48

The concentration of cadmium in baby (6−24 moths) cereals is in the range of 0.04−0.59 mg/kg. Recommended level of Cd for infants is $3\times10^{-3} - 4\times10^{-3}$ mg/day. Nestle-I and M smile-II contain the higher concentration [Table 3.16]. Excess intake of Cd is associated with lung damage.

The concentration of chromium in baby (6−24 months) cereal of different brands available in the market is on the average of 0.40 mg/kg. Recommended value of chromium for infants is $(10-120)\times10^{-3}$ mg/day. Most of the cereals contain the higher amount (mg/day) of Cr [Table 3.16] than the recommended value. That is why these samples are not safe for our infants and these should be avoided.

The concentration of As in baby cereals varies between 0.20 and 1.68 mg/kg. Arsenic in Nestle-I, Nestle-IV and Nestle-V [Table 3.15] is below detection limit. The permissible limit of As is 0.61 mg/day for infants. Most of the baby cereals contain lower amount of As than the recommended value except Mother's smile-I [Table 3.16]. Amount of As in Mother's smile-I (0.62 mg/day) is close to the recommended value. Hence these cereals are not harmful.

Recommended value of Ni is 0.45 mg/day. Only Biomil-I contains the higher amount of Ni than the recommended value [Table 3.16]. So, except Biomil-I other baby cereals are safe for our infants.

The variation of concentration (mg/kg) of metals and their comparison with maximum permissible limit (mg/day) in baby (6−24 months) cereal of different brands are shown in the Figure from 3.40 to 3.53.

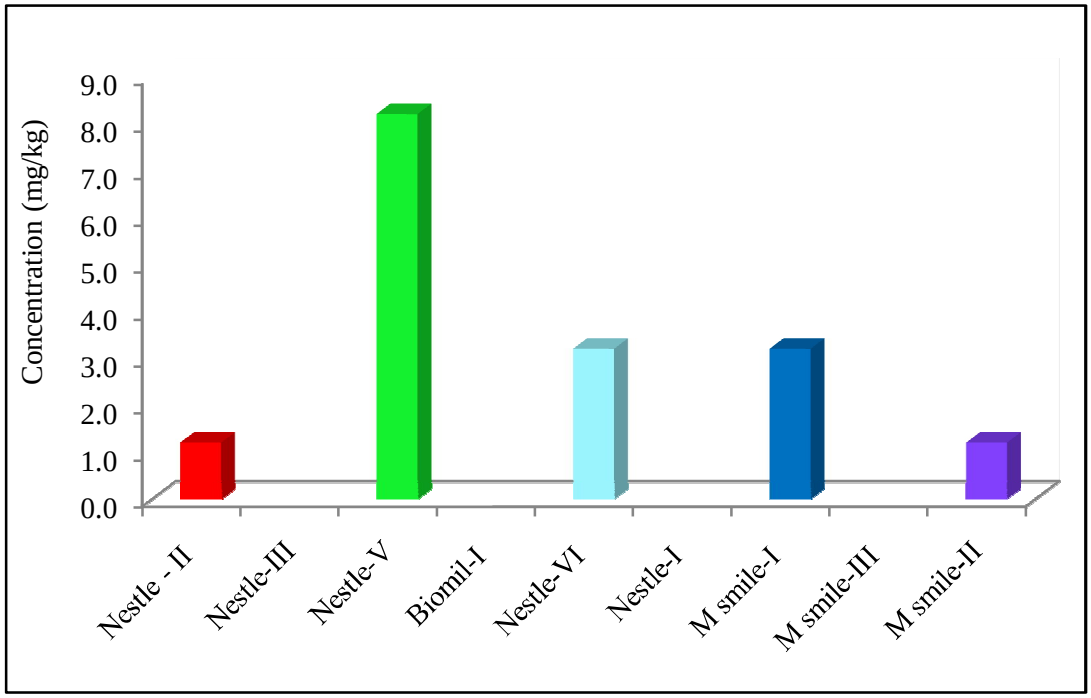

Figure 3.40: The variation of concentration of cobalt in baby (6−24 months) cereal of different brands.

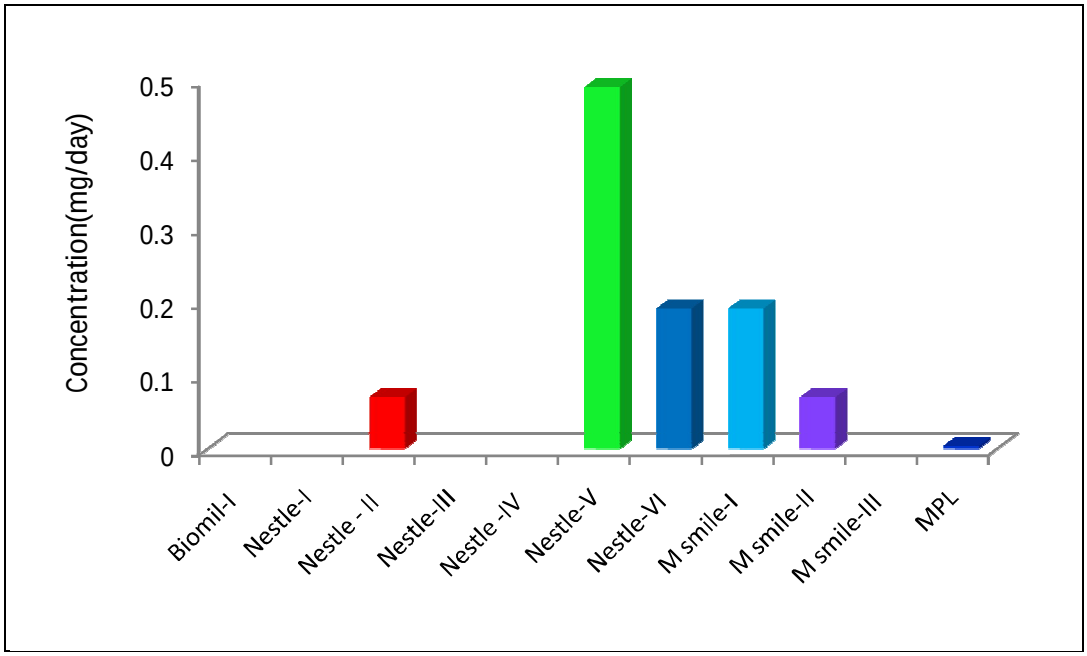

Figure 3.41: The comparison of concentration of cobalt in baby (6−24 months) cereal of different brands with maximum permissible limit.

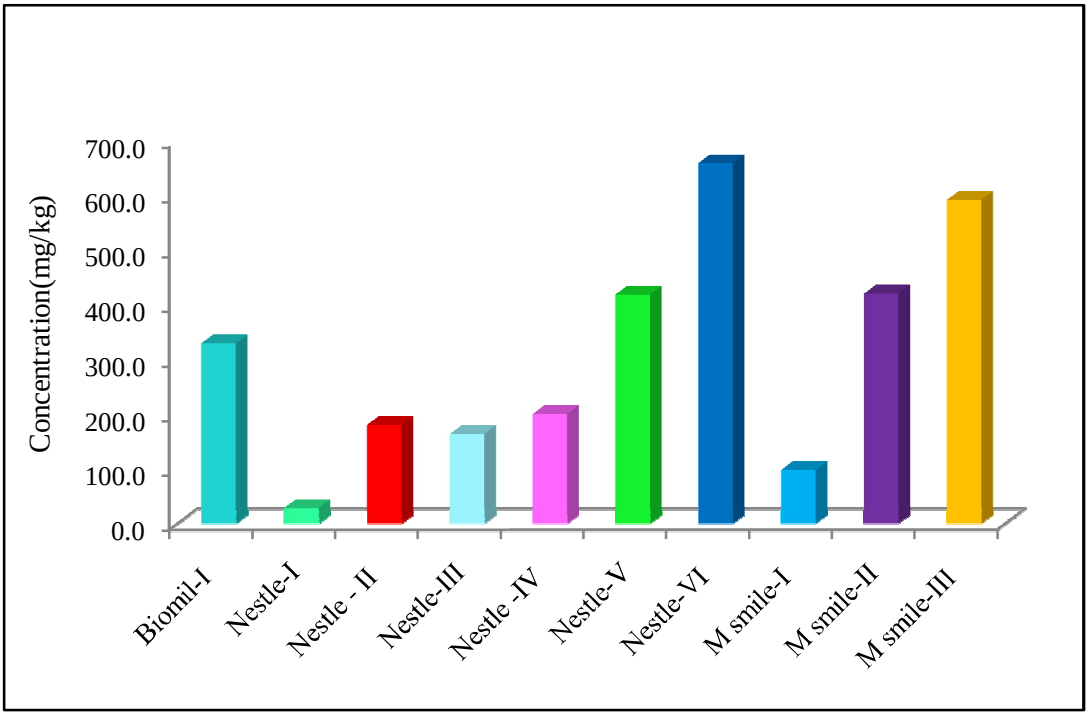

Figure 3.42: The variation of concentration of zinc in baby (6−24 months) cereal of different brands.

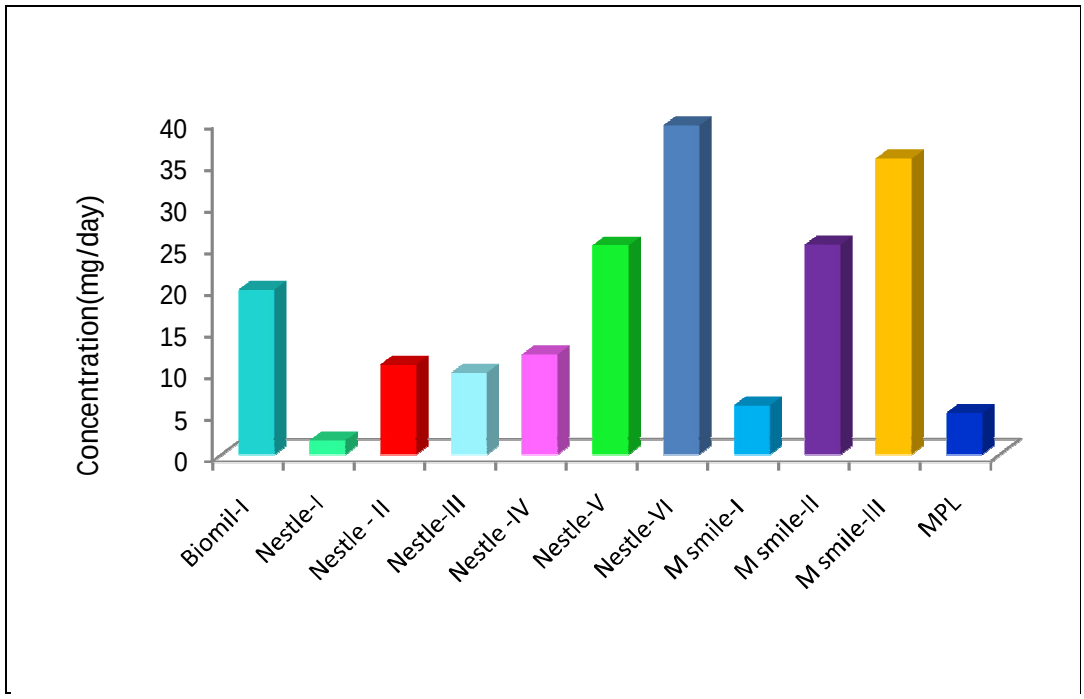

Figure 3.43: The comparison of concentration of zinc in baby (6−24 months) cereal of different brands with maximum permissible limit.

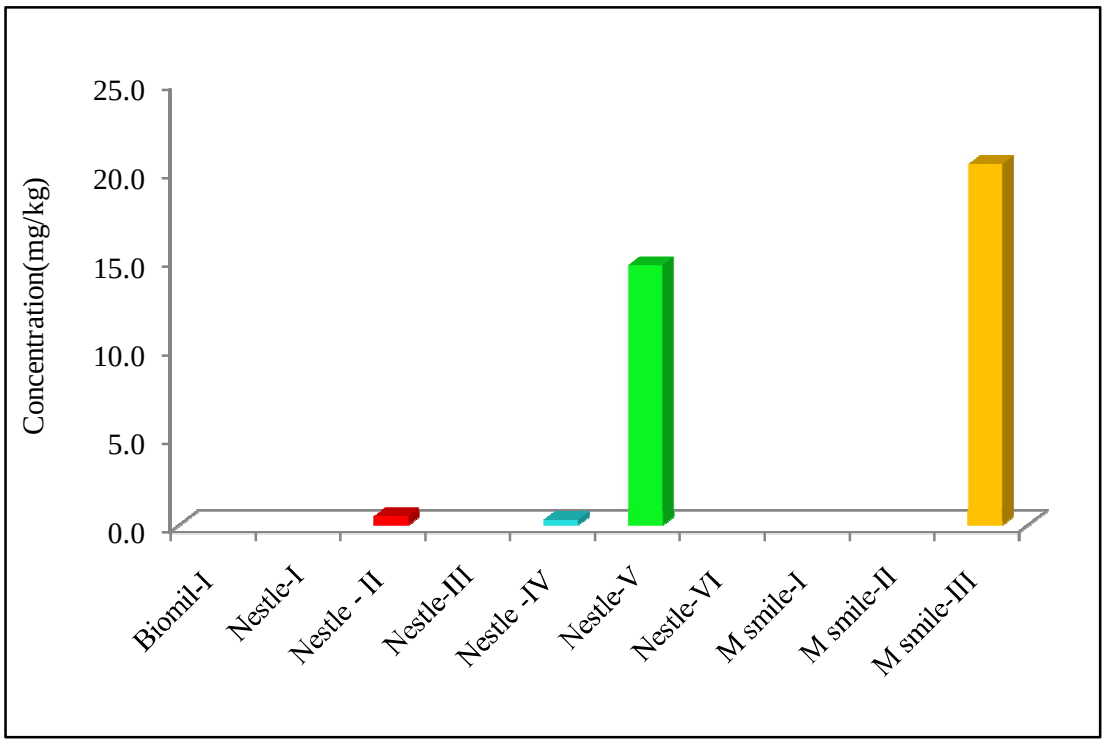

Figure 3.44: The variation of concentration of lead in baby (6−24 months) cereal of different brands.

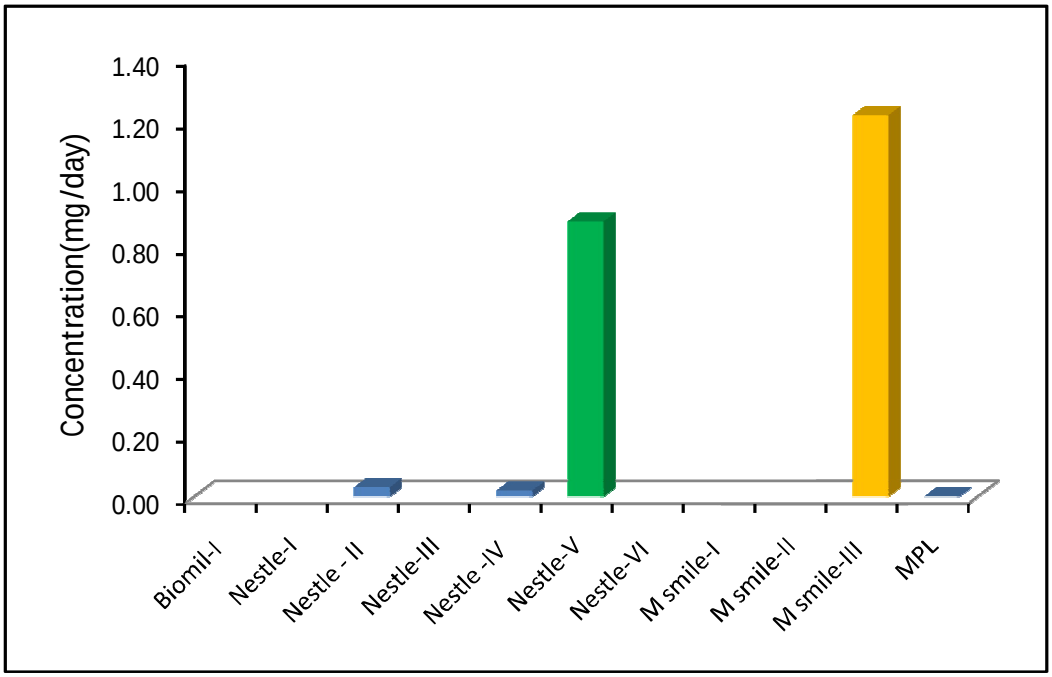

Figure 3.45: The comparison of concentration of lead in baby (6−24 months) cereals of different brands with maximum permissible limit.

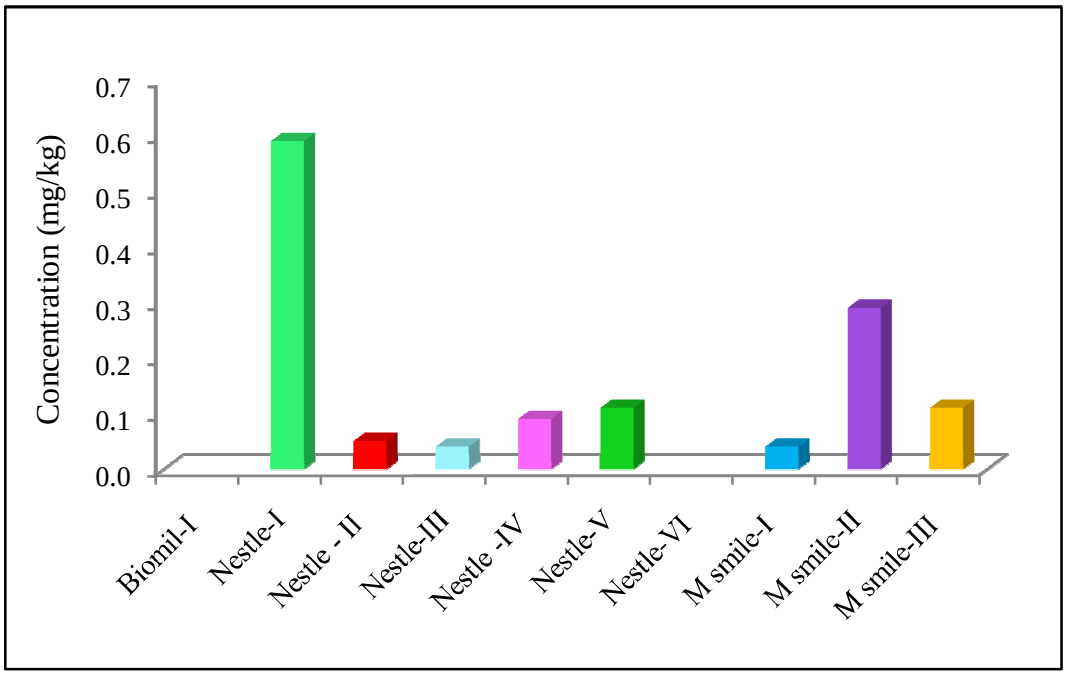

Figure 3.46: The variation of concentration of cadmium in baby (6−24 months) cereals of different brands.

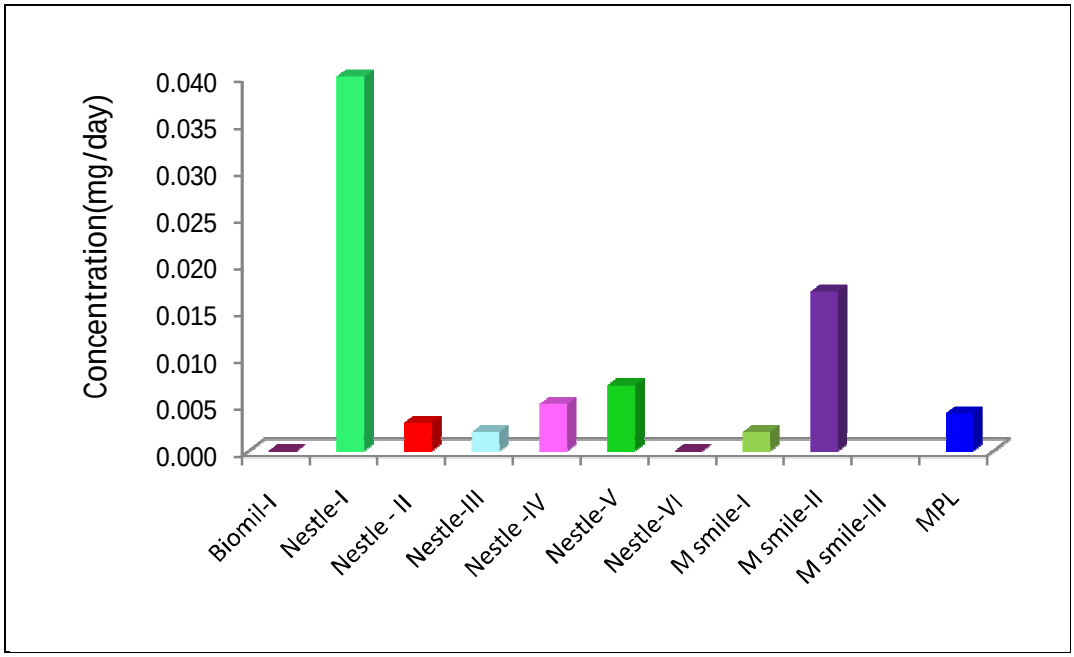

Figure 3.47: The comparison of concentration of cadmium in baby (6−24 months) cereals of different brands with maximum permissible limit.

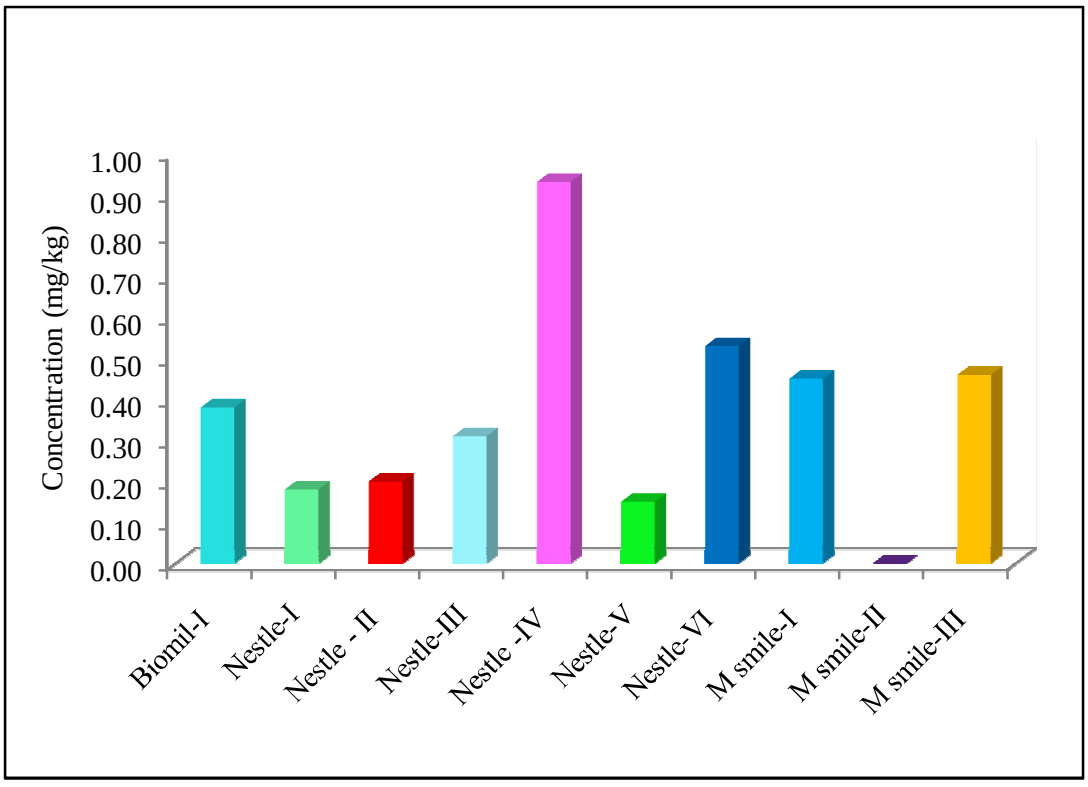

Figure 3.48: The variation of concentration of chromium in baby (6−24 months) cereals of different brands.

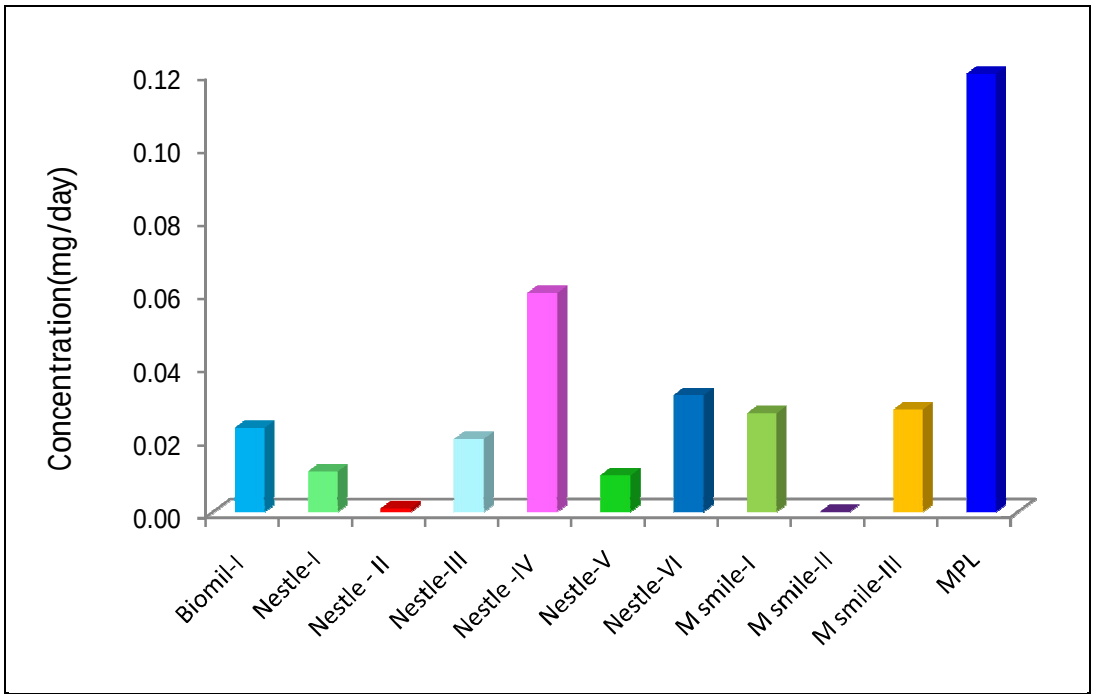

Figure 3.49: The comparison of concentration of chromium in baby (6−24 months) cereals of different brands with maximum permissible limit.

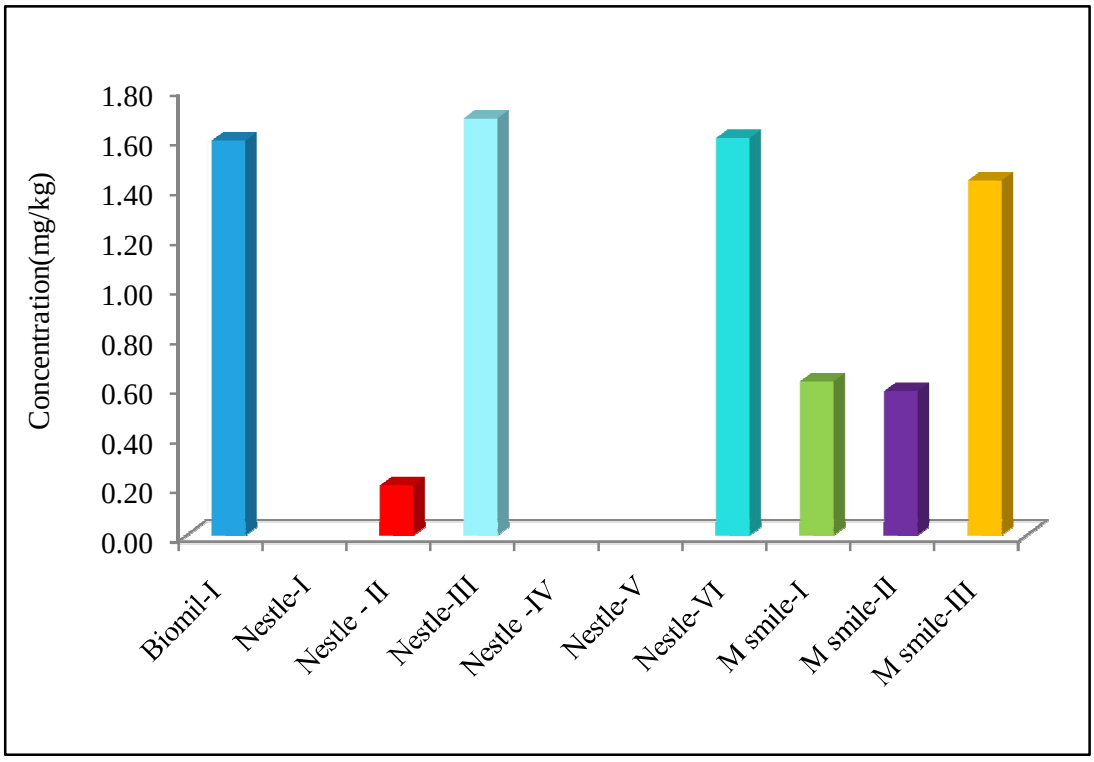

Figure 3.50: The variation of concentration of arsenic in baby (6−24 months) cereals of different brands.

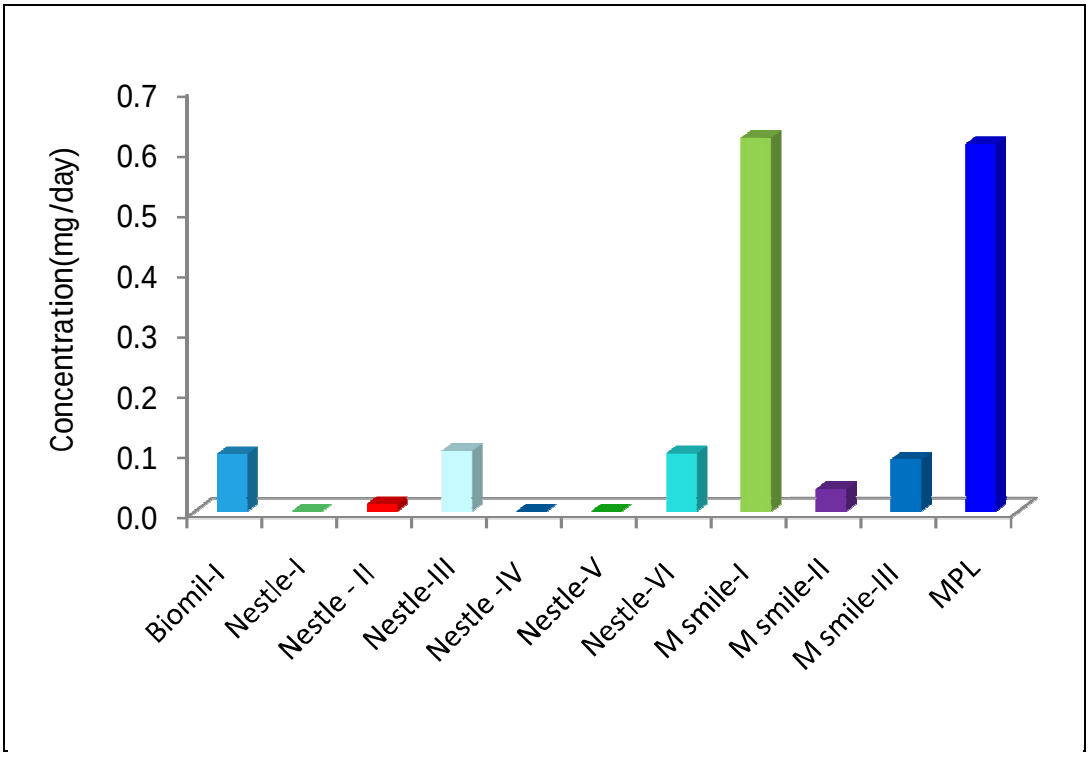

Figure 3.51: The comparison of concentration of arsenic in baby (6−24 months) cereals of different brands with maximum permissible limit**.**

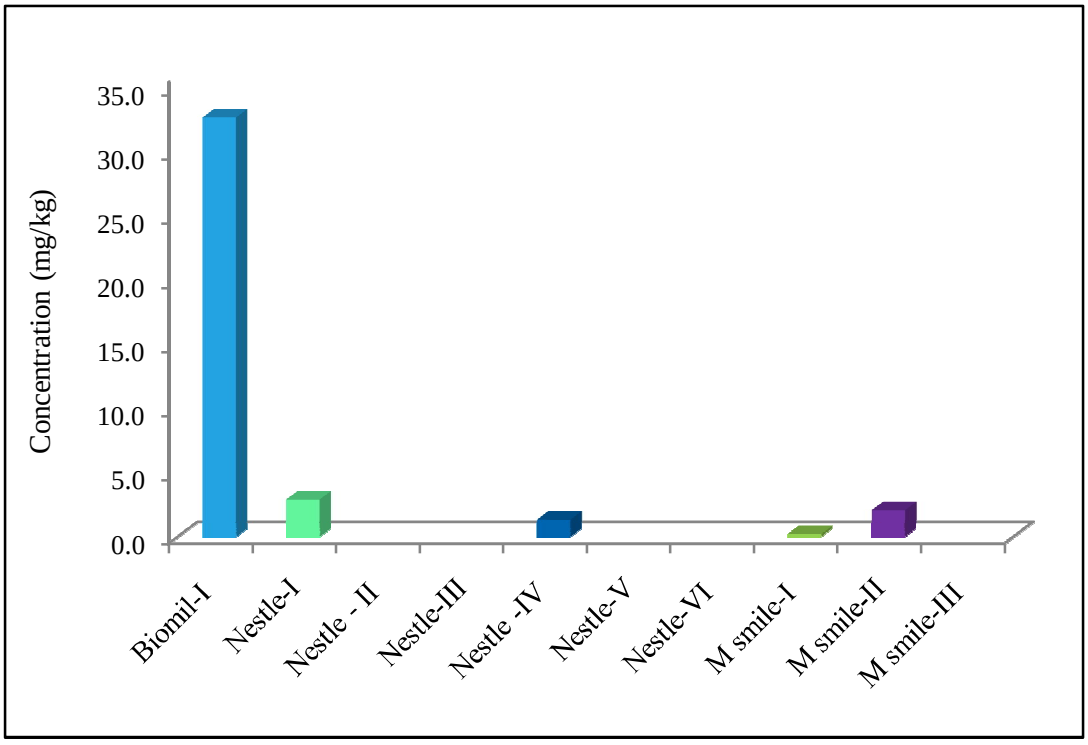

Figure 3.52: The variation of concentration of nickel in baby (6−24 months) cereals of different brands.

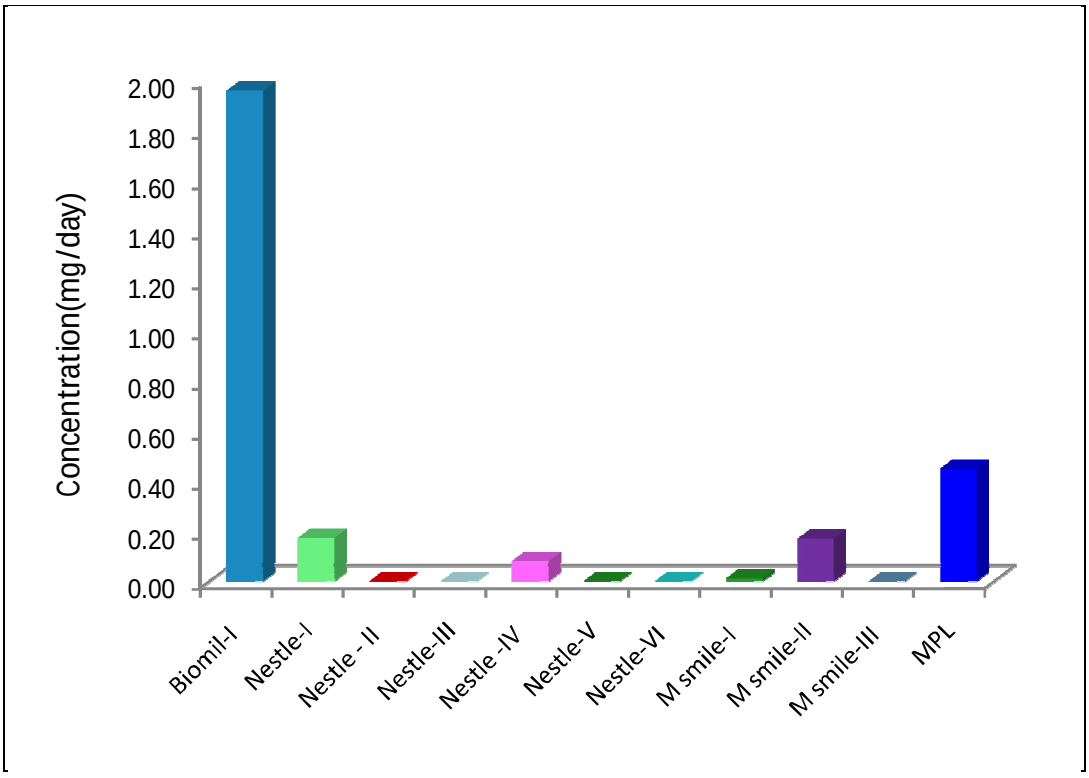

Figure 3.53: The comparison of concentration of nickel in baby (6−24 months) cereals of different brands with maximum permissible limit.

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CHAPTER FOUR

Estimation of Moisture, Ash, Protein, Total Solid, Sugar and the Amount of Trace and Toxic Metals in Jams, Jellies and Juices

CHAPTER FOUR

Estimation of Moisture, Ash, Protein, Total Solid, Sugar and the Amount of Trace and Toxic Metals in Jams, Jellies and Juices

4.1 Introduction

Jams, jellies and juices are made from fruits. Fruit is one of the oldest form of foods known to us. The interest in nutrition increases and people are getting more and more conscious about what they eat. For this reason the interest also grows in fruit and its contents. But there is a lot to discover the fruit contains. We know it is healthy for us, and that people who are sick can be cured by a diet of raw fruits and vegetables. Even cancer can sometimes be cured by this diet. But the substances in fruit which act against cancer are still unknown. Fruit sugar is a simple sugar or monosaccharide that is found in many kinds of ripened fruits. Fruit sugar is extremely sweet and is often used in the preparation of commercial food products. Considering the sweetness of all the naturally occurring carbohydrates, fruit sugar is favorite with food manufactures for two reasons. Firstly, this natural sweetener is less expensive than refined sugar products, making it more cost effective. At the same time, sugar from fruit is so sweet that small amounts are required in order to achieve the same level of sweetness in a given recipe. This factor also makes it possible for producers of commercial foods to manufacture their products with a lower cost per finished unit**.** While it is true, this natural sugar tends to be sweeter than refined sugars. It is important to note that fruit sugar is somewhat dangerous for diabetic patient. Fructose still causes blood sugar levels to peak. In some cases, the rapid increase in levels is even more pronounced than with refined sugar. For this reason, it is important for people attempting to control diabetes with diet to monitor closely the amount of fruit sugar are ingested during the course of the day. Using an up to date glycemic index will make it easier to monitor the amount of fructose absorbed from different fruits and vegetables. Everything we eat or drink has to be digested to extract the energy from it. Our body can extract the energy from it. Our body can get energy from food in two ways: burning with oxygen, for sugar and fat (fruit); burning without oxygen, for proteins. Fruit contains the energy in the form of sugar (glucose). Our body can easily turn this glucose into energy by using oxygen. When the body burns the sugars with the help of

oxygen the waste products are produced. The waste products of this chemical reaction are water and carbon dioxide. Our body can use the water and disposes of the carbon dioxide through the lungs by breathing. It is a very quick, clean and easy way to extract energy. Fruit juice takes only 15 minutes and raw fruit about 30 minutes to digest.

The knowledge of nutrition in different types of fruit products especially jams, jellies and juices are very significant due to their good food values as these are made from fruit, water and sugar. Jam, jelly and juice are produced from fruits, which are produced from flowers and flowers are produced from the ripened ovary and ovaries of a plant together with adjacent tissue [1]. Most fruits are made up of an edible portion combined with some refuse. Fruits as a class are valuable, chiefly for their vitamin, mineral content and for their bulk and indigestible fiber. The main energy constituents present in fruits are carbohydrate. Most fruits have only a trace amount of fat, a small amount of protein and water (85%). The major part of the edible portion of fresh fruits consists of water (75−95%). Fruits are poor sources of protein and oil. The exceptions to these are the olive and also the avocado which may contain as much as 40% oil. Most fruits contain reasonable amount of carbohydrate. The latter may include varying proportions (according to the fruit, maturity, etc.) of dextrose, fructose and sucrose and possibly starch (e.g. banana, apple). The principal acids present in fruits are citric, tartaric and malic acids. The total acidity falls after picking. The P^H of fruits varies from 2.5−4.5. Other constituentents of fruits include cellulose and woody fibers, mineral salts, pectin, gums, tannins, coloring matters and volatile oils. The main feature of the composition from the nutritional point of view is that certain fruits, particularly blackcurrants, most citrus fruits and strawberries are good sources of vitamin C. Certain specified preservatives are found to be present in bananas, grapes and citrus fruits [2].

Jam processing has been known since the eighteenth century. Barconnot was considered to be the first scientist who noticed the formation of jelly in presence of certain concentration of pectin, sugar, acid and water that had happened in French in 1825 [3]. This industry invented an important method for fruit preservation.

Jam is a food product prepared from cooked fruit or vegetable pulps after removal of stones and seeds with addition of sugar, acid and pectin to make slightly cohesive texture to the extent of total soluble solid (not less than 60%) and their natural appearance should not be retained.

During the boiling, the proportion of solid matter in the mixture increases (due to the evaporation of water), a proportion of the sucrose is converted to inverted sugar and a gel is produced on cooling. It has been shown that three separate components, viz. sugar, pectin and acid, play active function in forming the gel.

There are different types of jam which differ from each other in the raw material used, processing method and additives.

According to the texture jam can be classified into solid, semi solid and liquid jam. Jelly is a clear sweet soft fruit-flavoured food that is prepared after the boiling of the fruit with water and then the extract (after filtration) is boiled with the sugar. Artificial coloring matters and preservatives are added to certain varieties of jam and jelly [2].

However every human being requires food for their living as well as for the production of necessary energy like all other animals. Different food contains different proportion of minerals, proteins, carbohydrates and fat. Most of our people are suffering from malnutrition because they are in need of adequate nutritious food. Fruit is occupies larger proportion of daily food item of modern civilized nation certainly due to their great food values. Jams, jellies and juices also have good food value as these are made from fruit, water and sugar. Jams, jellies and juices provide more essential nutrients in significant amounts as well as some heavy metals. Minerals play an important role is the regulation of several essential metabolic processes in the body. The role of minerals in animal and human nutrition has undoubtedly attained increasing significance during the present century. It has been established that thirteen such elements are essential for the normal function of animal and human body processes, each of which has its own specific function. Mineral salts are found in the body and in the food mainly as their ionic forms. Metals form positive ions and non-metals form negative ions. The essential mineral elements are often grouped as macronutrients and micronutrients, depending on the amount of each of them needed in the diet. Sodium, potassium, calcium, magnesium and phosphorus

are considered as macronutrient. Iron, iodine, zinc, copper, chromium, selenium, manganese, molybdenum are often called micronutrients or trace elements. Some elements such as cadmium, lead, mercury, arsenic, lithium and aluminium are not essential for animal body. These elements show toxicity in body if the amount exceeds the tolerance level. Climate conditions are of great importance in the availability of mineral elements. Some environmental conditions such as earth soil, fertilizer used and availability of light also influence the mineral elements in plants. Copper and manganese particularly are not readily available in dry soils [4]. Flood is a natural calamity and Bangladesh suffers from flood frequently. Flood washes away the surface of land and the minerals present in the soil. Particularly zinc and phosphorus are removed by this type of soil erosion.

The importance of minerals as food ingredients is not only due to their nutritional and physiological roles but also contributes to food flavor. This also activates or inhibits enzyme catalyzed and other reactions and they affect the texture of food. Inorganic compounds particularly metallic ions and complexes are essential co-factors in a variety of proteins and enzymes. They conceivably provide essential services, which cannot be or can only poorly be rendered by organic compounds. Essential inorganic elements and compounds carry and transport electron and oxygen, through the oxidation-reduction, acid-base and other reactions.

4.2 Experimental Technique

4.2.1 Estimation of moisture

Discussed in Chapter Two, Section 2.14.1

4.2.2 Estimation of total solids

Discussed In Chapter Two, Section 2.14.2

4.2.3 Estimation of ash

Discussed In Chapter Two, Section 2.14.3

4.2.4 Estimation of protein

Discussed in Chapter Two, Section 2.14.4

4.2.5 Estimation of sugar

Discussed in Chapter Two, Section 2.14.5

4.2.6 Estimation of the amount of trace and toxic metals in jams, jellies and juices

Reagents, chemicals, apparatus and digestion of sample are discussed in Chapter Two, Section 2.15.1−2.15.3. In this chapter, preparation of calibration curves for the estimation of the amount of trace and toxic metals in jams, jellies and juices have been discussed.

4.2.6. a *Preparation of calibration curves for the determination of trace (Co, Zn) and toxic metals (Pb, Cd, Cr, As, Ni) in jams, jellies and juices*

Same techniques have been applied for the preparation of calibration curves of trace (Co, Zn) and toxic metals (Pb, Cd, Cr, As, Ni) in jams, jellies and juices (Please see Chapter Two, Section 3.2.4.a) [Figure 3.1−3.7].

Then the unknown concentrations of these metals are determined with the help of these calibration curves and the obtained results are given in Table 4.26, 4.27 and 4.28.

4.2.7 Estimation of essential metals (Na, K, Ca and Mg) in jams, jellies and juices of different brands

4.2.7. a *Preparation of calibration curves*

0.5, 1.0 and 2.0 ppm solutions of Na in deionized water were prepared from the stock solution (1000 ppm standard Na solution). The absorbances of these solutions have been measured by AAS. From these data the following calibration curve for Na was obtained. The unknown concentration of Na of the investigated samples was determined from the calibration curve and tabulated in Table 4.29, 4.30 and 4.31.

Similarly the calibration curves for K, Ca and Mg were prepared. The unknown concentrations of these metals were determined with the help of these calibration curves and the obtained results are given in Table 4.29, 4.30 and 4.31 respectively.

Table 4.1 Absorbance and concentration of standard solution of sodium

Serial No	Concentration (ppm)	Absorbance
1	0.0	0.00
2	0.5	0.1326
3	1.0	0.2611
4	2.0	0.5121

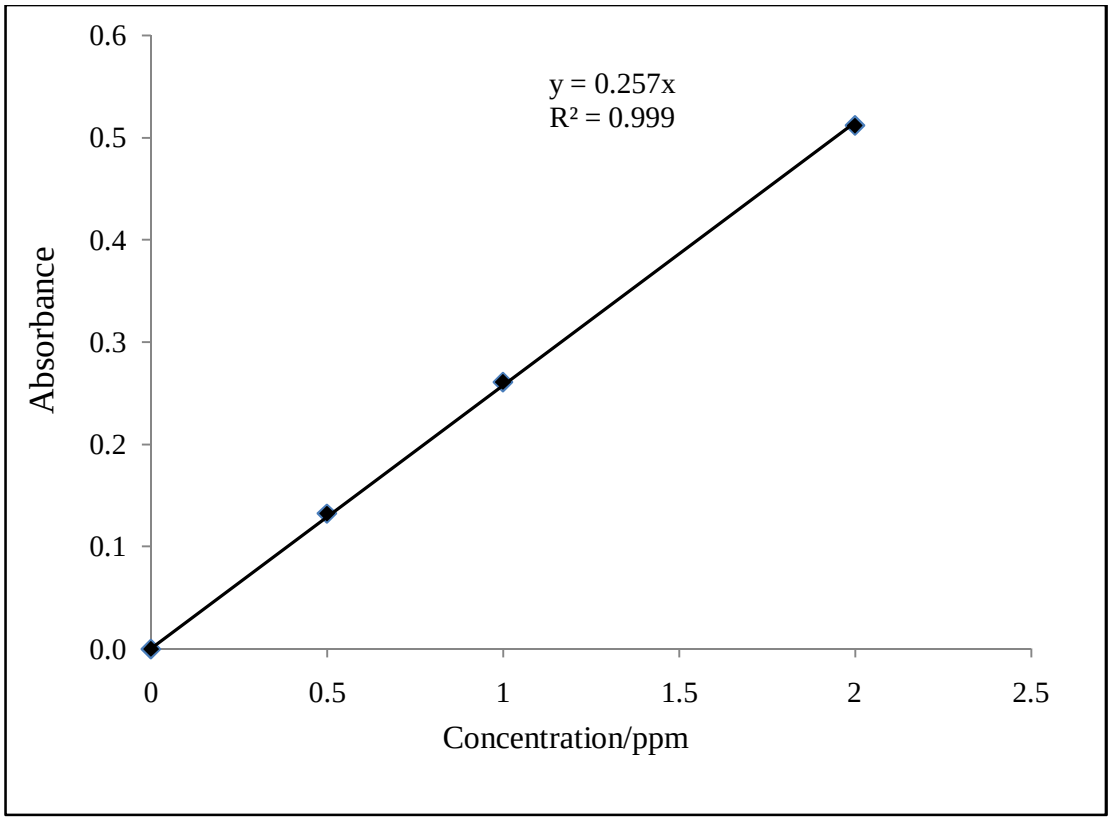

Figure 4.1: Calibration curve for the determination of sodium.

Table 4.2 Absorbance and concentration of standard solution of potassium

Serial No	Concentration (ppm)	Absorbance
1	0.0	0.0000
2	0.5	0.1237
3	1.0	0.2094
4	2.0	0.3808

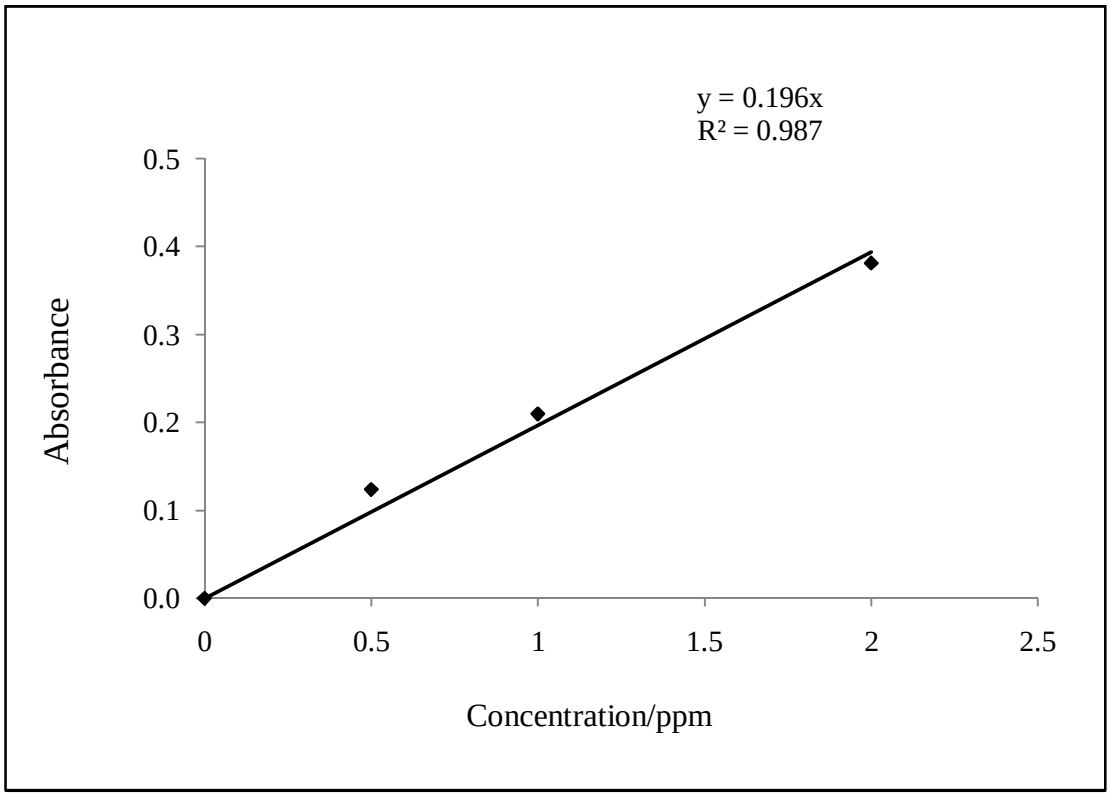

Figure 4.2: Calibration curve for the determination of potassium

Table 4.3 Absorbance and concentration of standard solution of calcium

Serial No	Concentration (ppm)	Absorbance
1	0.0	0.0000
2	0.5	0.1213
3	1.0	0.2265
4	2.0	0.4636

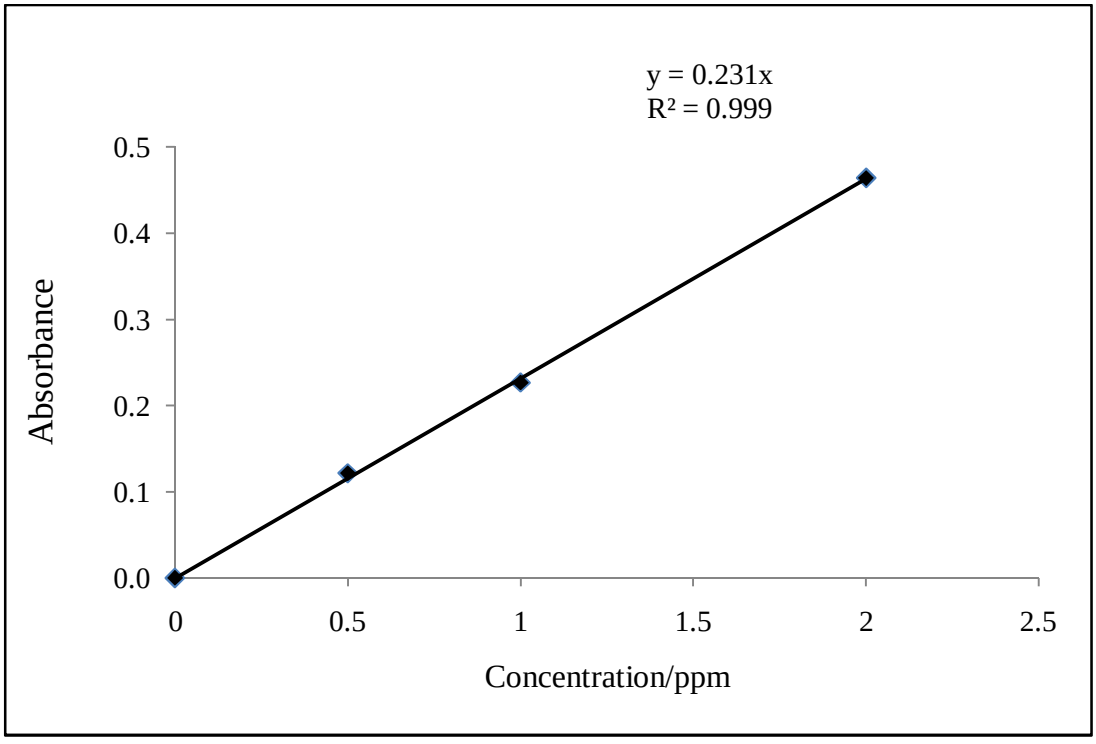

Figure 4.3: Calibration curve for the determination of calcium

Table 4.4 Absorbance and concentration of standard solution of magnesium (Mg)

Serial No	Concentration (ppm)	Absorbance
1	0.0	0.0000
2	0.5	0.1337
3	1.0	0.3107
4	2.0	0.5379

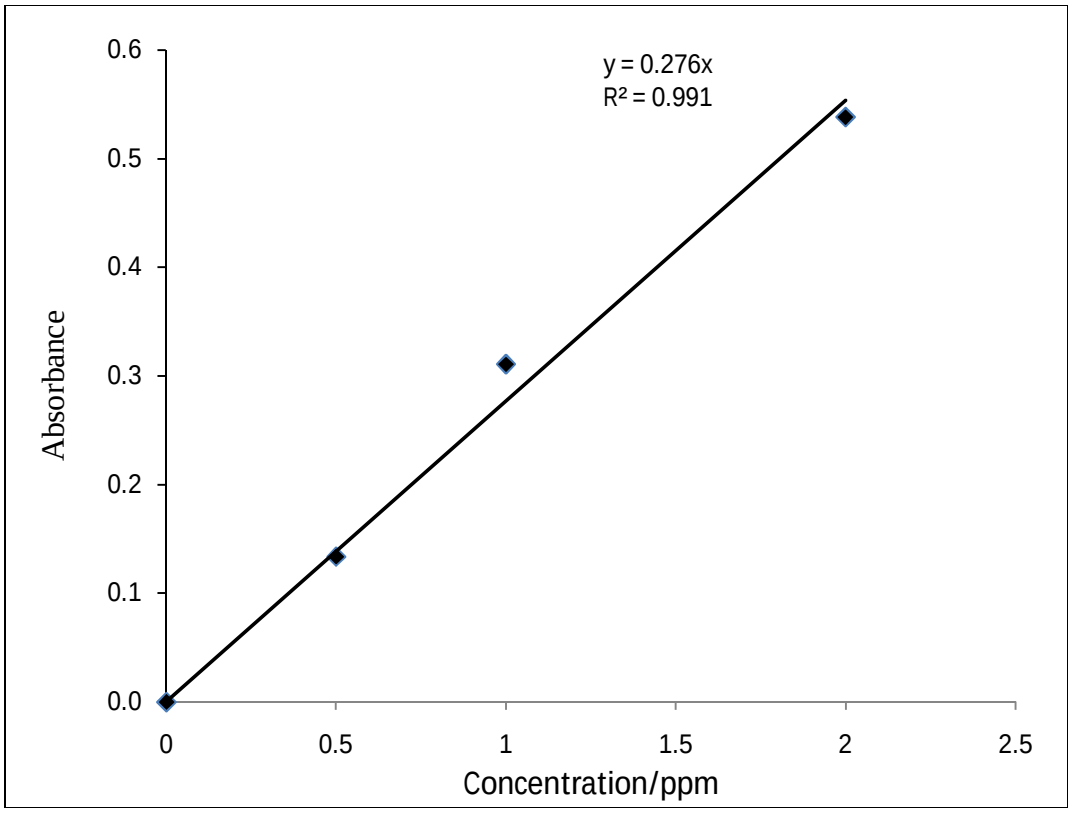

Figure 4.4: Calibration curve for the determination of magnesium

4.3 Results and Discussion

4.3.1 Measurements of moisture in different jams, jellies and juices

Table 4.5: Moisture found (%) in jams of different brands

Name of brands	Moisture (Mean ± SD)
Shezan Mango Jam	23.84 ± 0.36
Pran Mango Jam	21.34 ± 0.30
Freswel Mango Jam	17.89 ± 0.19
Rajshahi Mango Jam	41.77 ± 0.17
Nur Apple Jam	28.73 ± 0.14
Best Food Orange Jam	27.14 ± 0.23
Agrokomerc Pineapple Jam	29.43 ± 0.13
Shezan Mixed Jam	27.08 ± 0.10
Nur Mixed Jam	28.07 ± 0.06

Table 4.5(a): Descriptive statistics of moisture of different jams

Name of brand	Mean	SD	SE	95% CI for mean		Min	Max
				lower bound	upper bound		
Shezan Mango	23.84	0.36	0.21	22.95	24.73	23.55	24.24
Pran Mango	21.34	0.30	0.17	20.59	22.09	21.08	21.67
Freswel Mango	17.89	0.19	0.11	17.42	18.36	17.73	18.10
Rajshahi Mango	41.77	0.17	0.10	41.34	42.20	41.66	41.97
Nur Apple	28.73	0.14	0.08	28.38	29.08	28.62	28.89
B. F. Orange	27.14	0.23	0.13	26.57	27.71	26.94	27.39
Agro. Pineapple	29.43	0.13	0.07	29.11	29.75	29.32	29.57
Shezan Mixed	27.08	0.10	0.06	26.82	27.34	27.01	27.20
Nur Mixed	28.07	0.06	0.04	27.92	28.22	28.03	28.14
Overall	27.25	6.38	1.23	24.73	29.78	17.73	41.97

CI = Confidence Interval, B.F. = Best Food, Agro. = Agrokomerc, SD = Standard Deviation, SE = Standard Error, Min = Minimum, Max = Maximum.

The tests have been carried out for H_0 (Null hypothesis) and H_1 (Alternative hypothesis). Where, H_0 = the mean value of moisture of each jam is same and H_1= the mean value of moisture of each jam is different.

Table 4.5 (b): Analysis of variance for the variation study of moisture of different jams.

	Source of variation	SS	DF	MS	F	P
% of Moisture	Between groups	1057.96	8	132.24	3063.58	0.00
	Within group	0.78	18	0.04	–	–
	Total	1058.73	26	–	–	–

SS = Sum of Square, DF = Degrees of Freedom, MS = Mean of Square, F =Variance comparison test, P =Level of Significant.

Table 4.5(b) contains the statistical information of the experimental results of moisture in selected jams and mentions a significant difference of the results of moisture in these fruit jams. Table 4.5(b) also indicates the level of significant, P = 0.00 < 0.01, so the variance of the results of moisture for each jam is statistically significant at 1% level and we may conclude that the mean variation of the results of moisture for each jam is significantly different.

Table 4.5 (c): Duncan's multiple range test of moisture for mean comparison of different jams.

Duncan[a]

Brand	Subset for alpha = 0.05							
	1	2	3	4	5	6	7	8
Freswel Mango	17.89	–	–	–	–	–	–	–
Pran Mango	–	21.34	–	–	–	–	–	–
Shezan Mango	–	–	23.84	–	–	–	–	–
Shezan Mixed	–	–	–	27.08	–	–	–	–
Best Food Orange	–	–	–	27.14	–	–	–	–
Nur Mixed	–	–	–	–	28.07	–	–	–
Nur Apple	–	–	–	–		28.73	–	–
Agrokomerc Pineapple	–	–	–	–	–	–	29.43	–
Rajshahi Mango	–	–	–	–	–	–	–	41.77
Sig	1.00	1.00	1.00	0.728	1.00	1.00	1.00	1.00

Mean for group in homogeneous subsets are displayed. a. Uses harmonic mean sample size = 3.00.

Table 4.5(c) informs that within the group the mean value of moisture is insignificantly different and between the groups the mean value of moisture is significantly different as well as an interaction effect is found of the results of moisture of selected jams within the group-4.

From the Table 4.5(c), it is seen that the studied jams are divided into eight groups. A significant difference of moisture was observed between the groups and insignificant difference was within the group. The variations of percentage of moisture of different brands of jam are described below and shown in Figure 4.5 constructed from the Table 4.5(a).

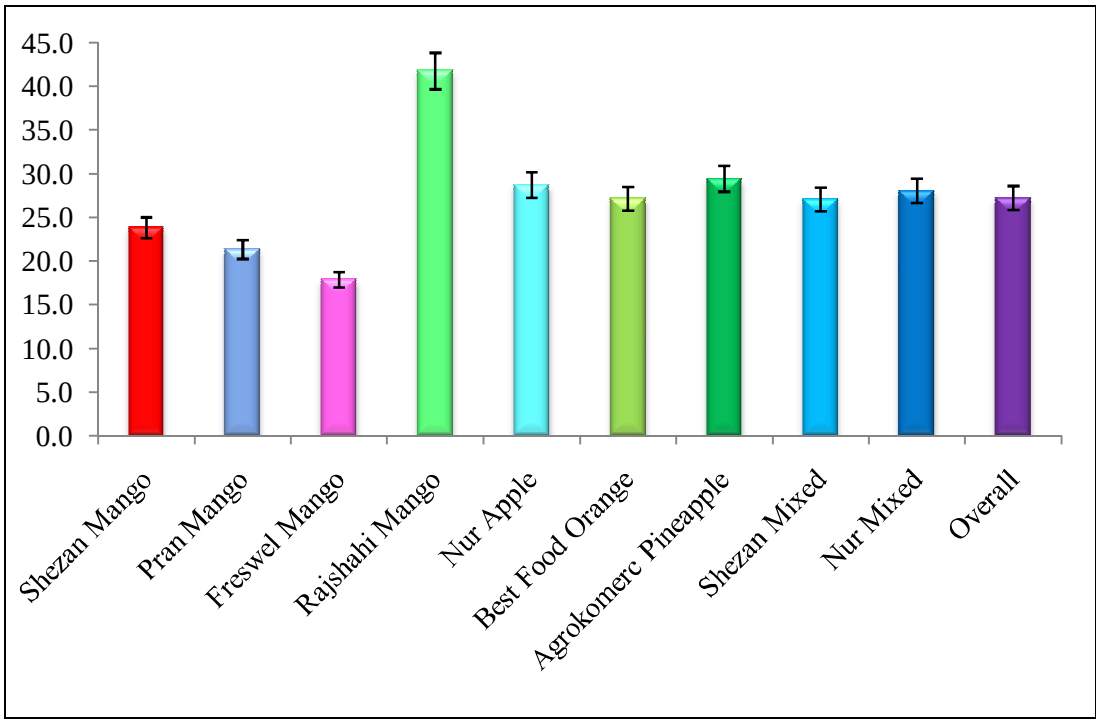

Figure 4.5: Comparison of moisture content in different jams.

Moisture found in jams ranges from 17.89 to 41.77%. The highest moisture content is (41.77%) found in Rajshahi Mango Jam and the lowest (17.89 %) is found in Freswel Mango Jam. The mean value of moisture content in 9 different jams is 27.33% whereas 29.34% is found in Thailand by Winus Puminat [5]. The average values of different fruit jams are shown in the graph [Figure 4.6, different jam vs moisture].

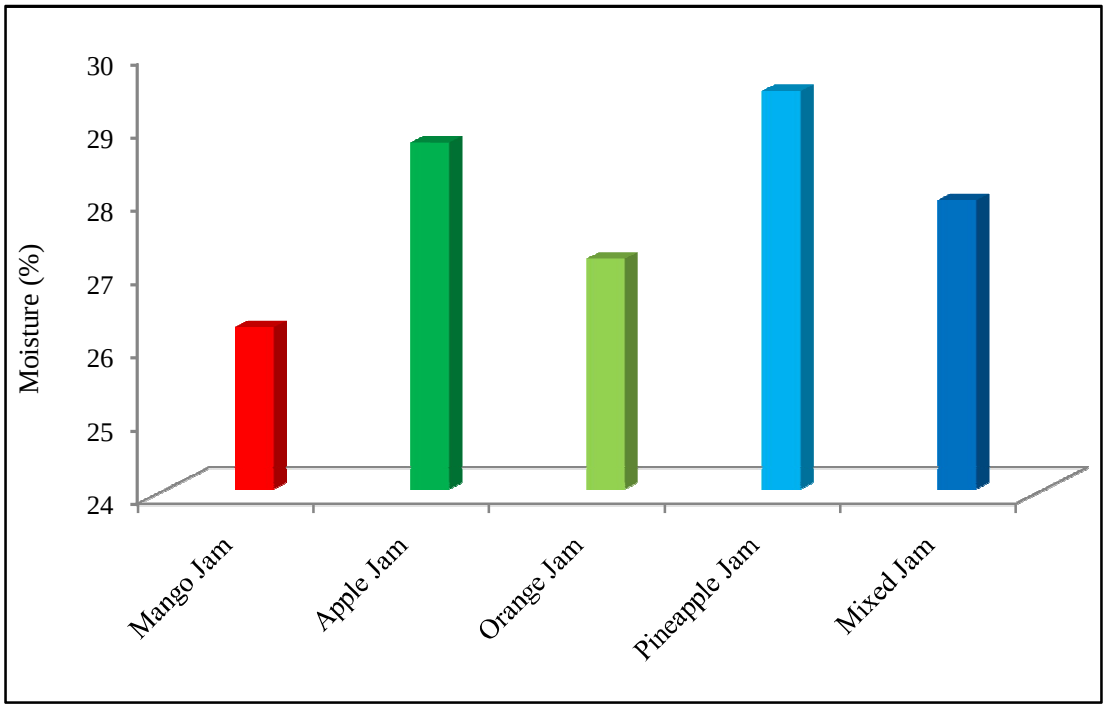

Figure 4.6: Comparison of moisture status in different jams

Table 4.6: Moisture found (%) in jellies of different brands

Name of brands	Moisture (Mean ± SD)
Friends Mango Jelly	43.14 ± 0.43
Agrokomerc Mango Jelly	36.12 ± 0.17
Pran Apple Jelly	25.15 ± 0.27
Friends Orange Jelly	45.23 ± 0.12
BD Orange Jelly	22.52 ± 0.16
Shezan Orange Jelly	25.77 ± 0.12
Pran Orange Jelly	17.13 ± 0.25
Ahmed Guava Jelly	25.97 ± 0.25

Table 4.6(a): Descriptive statistics of moisture of different jellies.

Name of brand	Mean	SD	SE	95% CI for mean		Min	Max
				lower bound	upper bound		
Friends Mango	43.16	0.43	0.25	42.09	44.23	42.77	43.62
Agro.Mango	36.12	0.17	0.10	35.70	36.54	35.93	36.25
Pran Apple	25.15	0.27	0.16	24.47	25.83	24.84	25.35
Friends Orange	45.23	0.12	0.07	44.93	45.53	45.09	45.31
BD Orange	22.52	0.16	0.09	22.13	22.91	22.34	22.64
Shezan Orange	25.77	0.21	0.12	25.24	26.30	25.53	25.93
Pran Orange	17.13	0.12	0.07	16.83	17.43	17.05	17.27
Ahmed Guava	25.97	0.25	0.15	25.34	26.60	25.68	26.14
Overall	30.13	9.70	1.98	26.03	34.23	17.05	45.31

SE = Standard Error, CI = Confidence Interval, Agro. = Agrokomerc

Description of H_0 and H_1 is almost same as earlier.

Table 4.6 (b): Analysis of variance for the variation study of moisture of different jellies.

	Source of variation	SS	DF	MS	F	P
% of Moisture	Between groups	2165.10	7	309.30	5498.66	0.00
	Within group	0.90	16	0.06	–	–
	Total	2166.00	23	–	–	–

SS = Sum of Square, DF = Degrees of Freedom, MS = Mean of Square, F =Variance comparison test, P =Level of Significant.

Statistical explanation of moisture is same as earlier.

Table 4.6(c): Duncan's multiple range test of moisture for mean comparison of different jellies.

Duncan[a]

Brand	Subset for alpha = 0.05						
	1	2	3	4	5	6	7
Pran Orange	17.13	–	–	–	–	–	–
BD Orange	–	22.52	–	–	–	–	–
Pran Apple	–	–	25.15	–	–	–	–
Shezan Orange	–	–	–	25.77	–	–	–
Ahmed Guava	–	–	–	25.97	–	–	–
Agro. Mango	–	–	–	–	36.12	–	–
Friends Mango	–	–	–	–	–	43.16	–
Friends Orange	–	–	–	–	–	–	45.23
Sig	1.00	1.00	1.00	0.32	1.00	1.00	1.00

Mean for group in homogeneous subsets are displayed. a. Uses harmonic mean sample size = 3.00.

From the Table 4.6(c) it is assumed that within the group the mean value of moisture is insignificantly different and between the groups that is significantly different as well as interaction effect is found in the values of moisture of the selected jellies within the group-4.

From the Table 4.6(c), it is seen that the studied jellies are divided into seven groups. A significant difference of moisture is observed between the groups and insignificant difference is within the group. The variation of percentage of moisture of different brands of jellies is described below and shown in Figure 4.7 obtained from the Table 4.6(a).

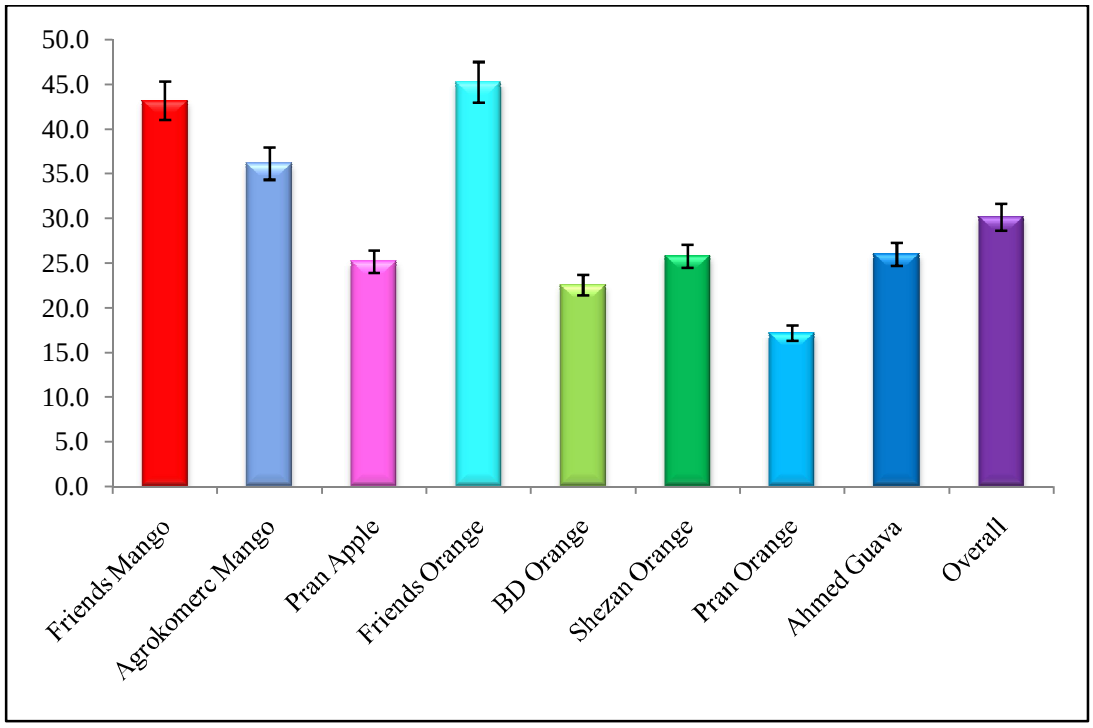

Figure 4.7: Comparison of moisture content in different jellies.

Moisture content in jellies is found in the range of 17.13−45.23%. The highest moisture content (45.23%) found in Friends Orange Jelly and the lowest (17.13%) is found in Pran Orange Jelly. The mean value of moisture content in 8 different jellies is 30.13% whereas 29.34% is found in Thailand by Winus Puminat [5]. The average values of moisture are shown in the Figure 4.8.

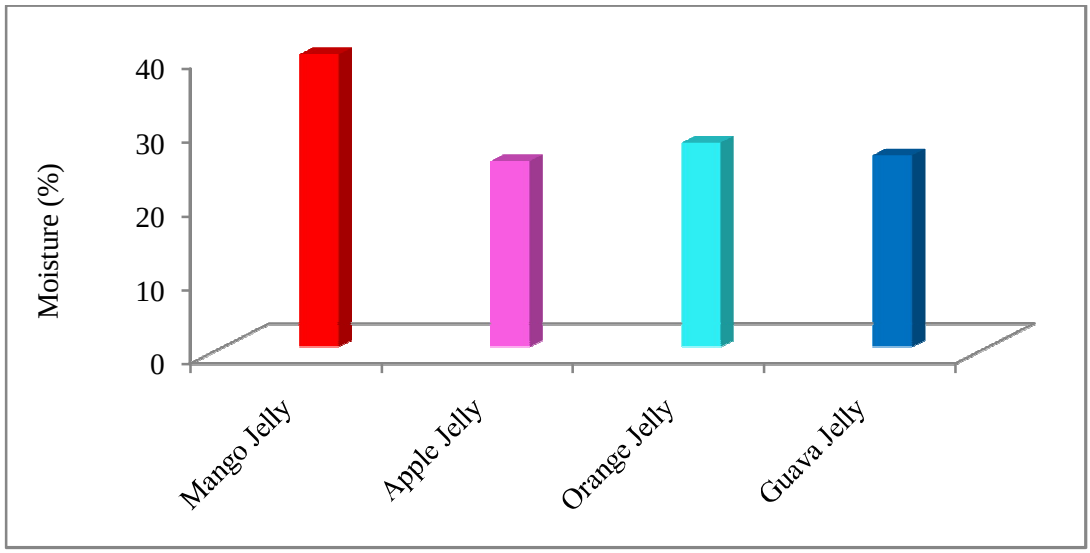

Figure 4.8: Comparison of moisture status in different jellies.

Table 4.7: Moisture found (%) in juices of different brands

Name of brands	Moisture (Mean ± SD)
Danish Mango	83.88 ± 0.36
Shezan Juicepack (Mango	86.12 ± 0.22
Starship (Mango	87.11 ± 0.24
ACME Premium Mango	88.19 ± 0.31
Pran Premium Mango	84.83 ± 0.44
Aarong Orange Flavor	86.74 ± 0.32
Aarong Tamarind	86.15 ± 0.18
Frutika Red Grape	81.93 ± 0.17
Pran Junior (Mango)	86.11 ± 0.30
Pran Frooto Mango	83.11 ± 0.32

Table 4.7 (a): Descriptive statistics of moisture of different juices

Name of brand	Mean	SD	SE	95% CI for mean		Min	Max
				lower bound	upper bound		
Danish Mango	83.88	0.36	0.21	82.99	84.77	83.47	84.14
Shezan Jp (Mango)	86.12	0.22	0.13	85.58	86.66	85.87	86.28
Starship (Mango)	87.11	0.24	0.14	86.52	87.70	86.84	87.29
ACME Pr Mango	88.33	0.31	0.18	87.55	89.11	88.13	88.69
Pran Pr Mango	84.83	0.44	0.25	83.74	85.92	84.33	85.16
Aarong Or Flavor	86.74	0.32	0.19	85.94	87.54	86.51	87.11
Aarong Tamarind	86.15	0.18	0.11	85.69	86.61	85.99	86.35
Frutika Red Grape	81.83	0.17	0.10	81.41	82.25	81.70	82.02
Pran Junior (Mango)	86.11	0.30	0.18	85.35	86.87	85.91	86.46
Pran Frooto Mango	83.80	0.32	0.19	83.00	84.60	83.43	84.03
Total	85.49	1.86	0.34	84.80	86.18	81.70	88.69

Pr = Premium, Jp = Juicepack, Or = Orange

Description of H_0 and H_1 is almost same as earlier.

Table 4.7(b): Analysis of variance for the variation study of moisture of different juices**.**

	Source of variation	SS	DF	MS	F	P
% of Moisture	Between groups	98.25	9	10.92	122.77	0.00
	Within group	1.78	20	0.09	–	–
	Total	100.03	29	–	–	–

Statistical explanation of moisture is same as earlier.

Table 4.7(c): Duncan's multiple range test of moisture for mean comparison of different juices.

Duncan[a]

Brand	Subset for alpha = 0.05					
	1	2	3	4	5	6
Frutika Red Grape	81.83	–	–	–	–	–
Pran Frooto Mango	–	83.80	–	–	–	–
Danish Mango	–	83.88	–	–	–	–
Pran Premium Mango	–	–	84.83	–	–	–
Pran Junior (Mango)	–	–	–	86.11	–	–
Shezan Juicepack (Mango)	–	–	–	86.12	–	–
Aarong Tamarind	–	–	–	86.15	–	–
Aarong Orange Flavor	–	–	–	–	86.74	–
Starship (Mango)	–	–	–	–	87.11	–
ACME Premium Mango	–	–	–	–	–	88.33
Sig	1.00	0.75	1.00	0.88	0.14	1.00

Mean for group in homogeneous subsets are displayed. a. Uses harmonic mean sample size = 3.00.

Table 4.7(c) indicates that within the group the mean value of moisture is insignificantly different whereas between the groups that is significantly different as

well as the interaction effect is found in the results of moisture of the selected juices within the group-2, group-4 and group-5.

From the Table 4.7(c), it is seen that the experimental juices are divided into six groups. A significant difference of moisture of juices is observed between the groups and insignificant difference is observed within the group. The variations of the percentage of moisture of different brands of juices are described below and shown in Figure 4.9 plotted from the Table 4.7(a).

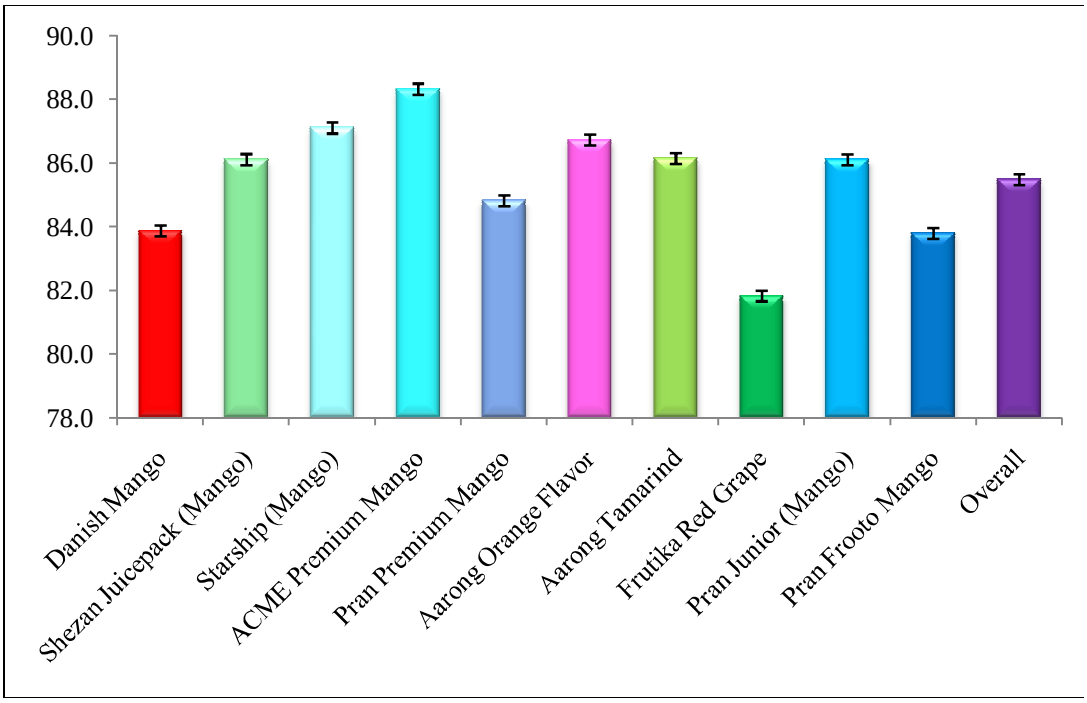

Figure 4.9: Comparison of moisture content in different juices.

Moisture content of juices is found in the range of 81.83−88.83%. The highest moisture content (88.83%) is found in ACME Premium Mango Juice and the lowest (81.837%) is found in Frutika Red Grape Juice. The average values were shown in the graph (Figure 4.10) which indicates the moisture status in various types of juices.

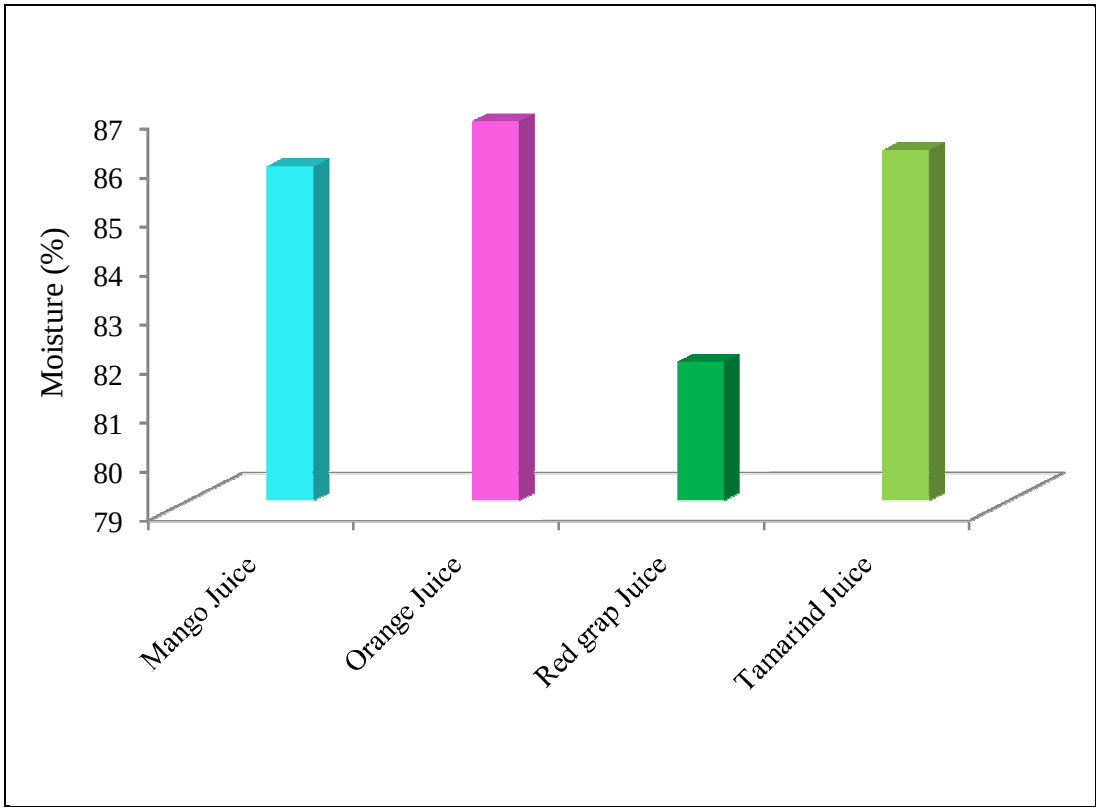

Figure 4.10: Comparison of moisture status in different juices.

4.3.2 Measurements of total solid in different jams, jellies and juices

Table 4.8: Total solid found (%) in jams of different brands

Name of brands	Total solid (Mean ± SD)
Shezan Mango Jam	76.16 ± 0.36
Pran Mango Jam	78.66 ± 0.30
Freswel Mango Jam	82.11 ± 0.19
Rajshahi Mango Jam	58.23 ± 0.17
Nur Apple Jam	71.27 ± 0.14
Best Food Orange Jam	72.86 ± 0.23
Agrokomerc Pineapple Jam	70.57 ± 0.13
Shezan Mixed Jam	72.92 ± 0.10
Nur Mixed Jam	71.93 ± 0.06

Table 4.8(a): Descriptive statistics of total solid of jams**.**

Name of brand	Mean	SD	SE	95% CI for mean		Min	Max
				lower bound	upper bound		
Shezan Mango	76.16	0.36	0.21	75.27	77.05	75.76	76.45
Pran Mango	78.66	0.30	0.17	77.91	79.41	78.33	78.92
Freswel Mango	82.11	0.19	0.11	81.64	82.58	81.90	82.27
Rajshahi Mango	58.23	0.17	0.10	57.80	58.66	58.03	58.34
Nur Apple	71.40	0.34	0.20	70.55	72.25	71.11	71.78
B. F. Orange	72.86	0.23	0.13	72.29	73.43	72.61	73.06
Agro. Pineapple	70.57	0.13	0.07	70.25	70.89	70.43	70.68
Shezan Mixed	72.92	0.10	0.06	72.66	73.18	72.80	72.99
Nur Mixed	71.93	0.06	0.04	71.78	72.08	71.86	71.97
Overall	72.76	6.38	1.23	70.24	75.28	58.03	82.27

B.F. = Best Food

Description of H_0 and H_1 is almost same as earlier.

Table 4.8 (b): Analysis of variance for the variation study of total solid of different jam.

	Source of variation	SS	DF	MS	F	P
% of Total solid	Between groups	1056.82	8	132.10	2447.19	0.00
	Within group	0.97	18	0.05		
	Total	1057.80	26			

Statistical explanation of total solid is same as earlier.

Table 4.8(c): Duncan's multiple range test of total solid for mean comparison of different jam.

Duncan[a]

Brand	Subset for alpha = 0.05							
	1	**2**	**3**	**4**	**5**	**6**	**7**	**8**
Rajshahi Mango	58.23	–	–	–	–	–	–	–
Agro Pineapple	–	70.57	–	–	–	–	–	–
Nur Apple	–	–	71.40	–	–	–	–	–
Nur Mixed	–	–	–	71.93	–	–	–	–
Best Food Orange	–	–	–	–	72.86	–	–	–
Shezan Mixed	–	–	–	–	72.92	–	–	–
Shezan Mango	–	–	–	–	–	76.16	–	–
Pran Mango	–	–	–	–	–	–	78.66	–
Freswel Mango	–	–	–	–	–	–	–	82.11
Sig	1.00	1.00	1.00	1.00	0.76	1.00	1.00	1.00

Mean for groups in homogeneous subsets are displayed. a. Uses harmonic mean sample size = 3.00

Table 4.8(c) shows that within the group the mean value of total solid is insignificantly different and between the groups it is significantly different as well as interaction effect is found in the value of total solid of the selected jams within the group-5. The studied jams are divided into eight groups which are presented in the same table. The same result was observed within the group and between the groups as earlier. The variations of the percentage of total solid of different brands are described below and shown in Figure 4.11 plotted from the Table 4.8(a).

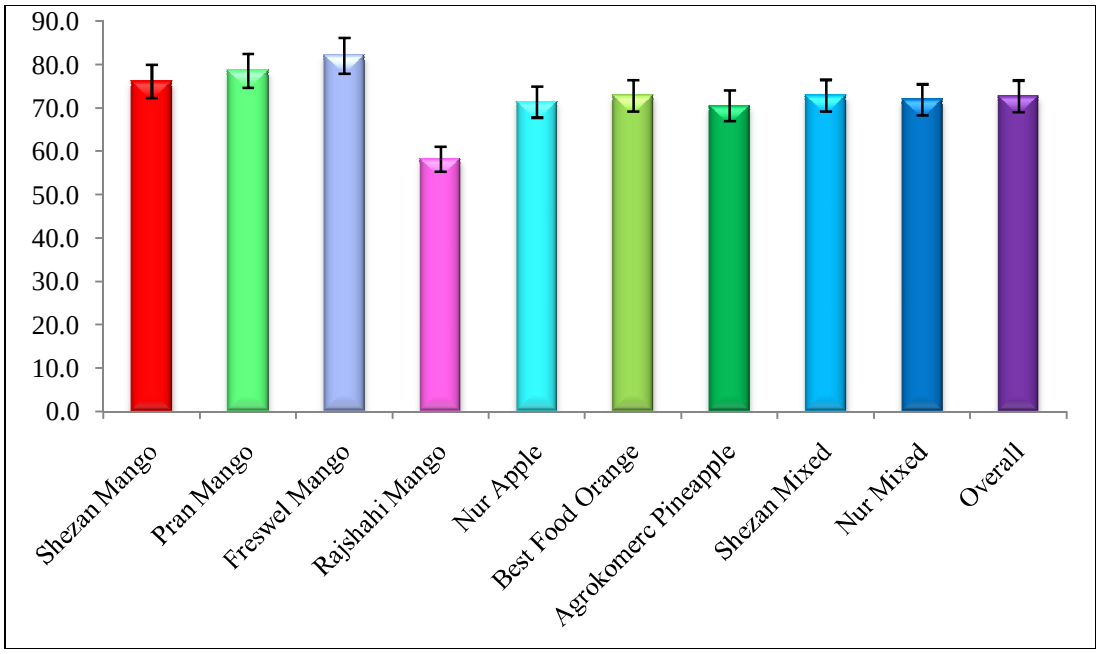

Figure 4.11: Comparison of total solid status in different jams.

In Jams the total solid content ranges from 58.23 to 82.11%. The highest (82.11%) and the lowest (58.23%) amount of total solid content of jams are present in Freswel Mango Jam and Rajshahi Mango Jam respectively. A suitable range of total solid of jams is 65.3−66.6% [5]. If the percentage of total solid of jam is higher than a suitable range, surface of jam will be dry and harsh. Alternatively we can say if the percentage of total solid of jam is less than suitable interval, the wetness and softness of jams will be higher [5].

Table 4.9: Total solid found (%) in jellies of different brands

Name of brands	Total solid (Mean ± SD)
Friends Mango Jelly	56.84 ± 0.43
Agrokomerc Mango Jelly	83.88 ± 0.17
Pran Apple Jelly	74.85 ± 0.27
Friends Orange Jelly	54.77 ± 0.12
BD Orange Jelly	77.48 ± 0.16
Shezan Orange Jelly	74.23 ± 0.21
Pran Orange Jelly	82.87 ± 0.12
Ahmed Guava Jelly	74.03 ± 0.25

Table 4.9(a): Descriptive statistics of total solid of different jellies

Name of brand	Mean	SD	SE	95% CI for mean		Min	Max
				lower bound	upper bound		
Friends Mango	56.84	0.43	0.25	55.77	57.91	56.38	57.23
Agro. Mango	63.88	0.17	0.10	63.46	64.30	63.75	64.07
Pran Apple	74.85	0.27	0.16	74.17	75.53	74.65	75.16
Friends Orange	54.77	0.12	0.07	54.47	55.07	54.69	54.91
BD Orange	77.48	0.16	0.09	77.09	77.87	77.36	77.66
Shezan Orange	74.23	0.21	0.12	73.70	74.76	74.07	74.47
Pran Orange	82.87	0.12	0.07	82.57	83.17	82.73	82.95
Ahmed Guava	74.03	0.25	0.15	73.40	74.66	73.86	74.32
Overall	69.87	9.70	1.98	65.77	73.97	54.69	82.95

Agro. = Agrokomerc

Description of H_0 and H_1 is almost same as earlier.

Table 4.9(b): Analysis of variance for the variation study of total solid of different jellies.

	Source of variation	SS	DF	MF	F	P
% of Total solid	Between groups	2165.10	7	309.30	5498.66	0.00
	Within group	0.90	16	0.06	–	–
	Total	2166.00	23	–	–	–

Statistical explanation of total solid is same as earlier.

Table 4.9 (c): Duncan's multiple range test of total solid for mean comparison of different jellies.

Duncan[a]

Brand	Subset for alpha = 0.05						
	1	**2**	**3**	**4**	**5**	**6**	**7**
Friends Orange	54.77	–	–	–	–	–	–
Friends Mango	–	56.84	–	–	–	–	–
Agrokomerc Mango	–	–	63.88	–	–	–	–
Ahmed Guava	–	–	–	74.03	–	–	–
Shezan Orange	–	–	–	74.23	–	–	–
Pran Apple	–	–	–	–	74.85	–	–
BD Orange	–	–	–	–	–	77.48	–
Pran Orange	–	–	–	–	–	–	82.87
Sig	1.00	1.00	1.00	0.32	1.00	1.00	1.00

Mean for group in homogeneous subsets are displayed. a. Uses harmonic mean sample size = 3.00.

Table 4.9(c) refers that within the group the mean value of total solid is insignificantly different and between the groups it is significantly different as well as interaction effect is found in the value of total solid of the selected jellies within the group-4.

In the same Table the studied jellies are divided into seven groups. Here the same result is observed within the group and between the groups as earlier. The comparisons of total solid status in different jellies are shown in Figure 4.12 which is constructed from the Table 4.9 (a).

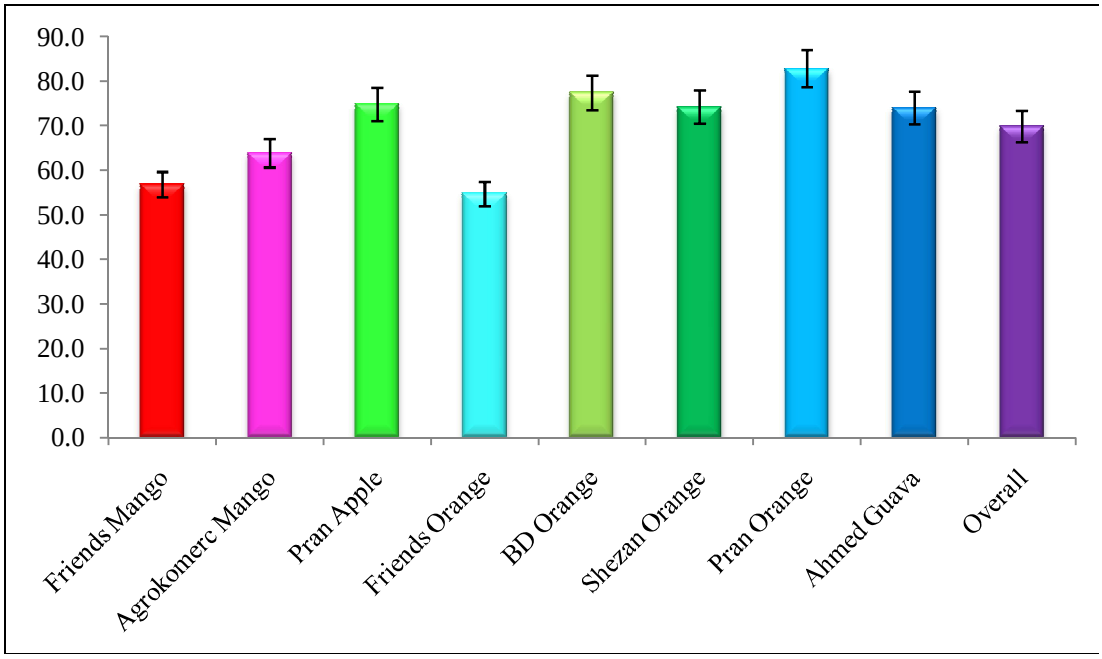

Figure 4.12: Comparison of total solid status in different jellies

In jellies the total solid content ranges from 54.77 to 82.87%. The highest (82.87%) and the lowest (54.77%) amount of total solid content of jellies are present in Pran Orange Jelly and Friends Orange Jelly respectively. A suitable range of total solid of jellies is 65.3−66.6% [5].

Table 4.10: Total solid found (%) in juices of different brands

Name of brands	Total solid (Mean ± SD)
Danish Mango	16.12 ± 0.36
Shezan Juicepack (Mango)	13.88 ± 0.21
Starship (Mango)	12.89 ± 0.24
ACME Premium Mango	11.67 ± 0.31
Pran Premium Mango	15.17 ± 0.44
Aarong Orange Flavor	13.26 ± 0.32
Aarong Tamarind	13.85 ± 0.18
Frutika Red Grape	18.17 ± 0.17
Pran Junior (Mango)	13.89 ± 0.30
Pran Frooto Mango	16.20 ± 0.32

Table 4.10(a): Descriptive statistics of total solid of different juices

Name of brand	Mean	SD	SE	95% CI for mean		Min	Max
				lower bound	upper bound		
Danish Mango	16.12	0.36	0.21	15.23	17.01	15.86	16.53
Shezan Juicepack (Ma)	13.94	0.21	0.12	13.43	14.45	13.72	14.13
Starship (Mango)	12.89	0.24	0.14	12.30	13.48	12.71	13.16
ACME Pr Mango	11.67	0.31	0.18	10.89	12.45	11.31	11.87
Pran Premium Mango	15.17	0.44	0.25	14.08	16.26	14.84	15.67
Aarong Orange Flavor	13.26	0.32	0.19	12.46	14.06	12.89	13.49
Aarong Tamarind	13.85	0.18	0.11	13.39	14.31	13.65	14.01
Frutika Red Grape	18.17	0.17	0.10	17.75	18.59	17.98	18.30
Pran Junior (Mango)	13.89	0.30	0.18	13.13	14.65	13.54	14.09
Pran Frooto Mango	16.20	0.32	0.19	15.40	17.00	15.97	16.57
Total	14.52	1.86	0.34	13.82	15.21	11.31	18.30

Pr = Premium, (Ma) = Mango

Description of H_0 and H_1 is almost same as earlier.

Table 4.10 (b): Analysis of variance for the variation study of total solid of different juices

	Source of variation	SS	DF	MS	F	P
% of Total solid	Between groups	98.03	9	10.89	123.24	0.00
	Within group	1.77	20	0.09	–	–
	Total	99.80	29	–	–	–

Statistical explanation of total solid is same as earlier.

Table 4.10(c): Duncan's multiple range test of total solid for mean comparison of different juices

Duncan[a]

Brand	Subset for alpha = 0.05					
	1	2	3	4	5	6
ACME Premium Mango	11.67	–	–	–	–	–
Starship (Mango)	–	12.89	–	–	–	–
Aarong Orange Flavor	–	13.26	–	–	–	–
Aarong Tamarind	–	–	13.85	–	–	–
Pran Junior (Mango)	–	–	13.89	–	–	–
Shezan Juicepack (Mango)	–	–	13.94	–	–	–
Pran Premium Mango	–	–	–	15.17	–	–
Danish Mango	–	–	–	–	16.12	–
Pran Frooto Mango	–	–	–	–	16.20	–
Frutika Red Grape	–	–	–	–	–	18.17
Sig	1.00	0.14	0.73	1.00	0.75	1.00

Mean for group in homogeneous subsets are displayed. a. Uses harmonic mean sample size = 3.00.

From the Table 4.10(c), we get the same results as well as interaction effect is found in the results of total solid of the selected juice within the group-2, group-3 and group-5.

In the same Table the studied juices are divided into six groups. In this case the same results are obtained between and within the groups. The variation of the percentage of total solid of different brands is described below and shown in Figure 4.13 plotted from the Table 4.10 (a).

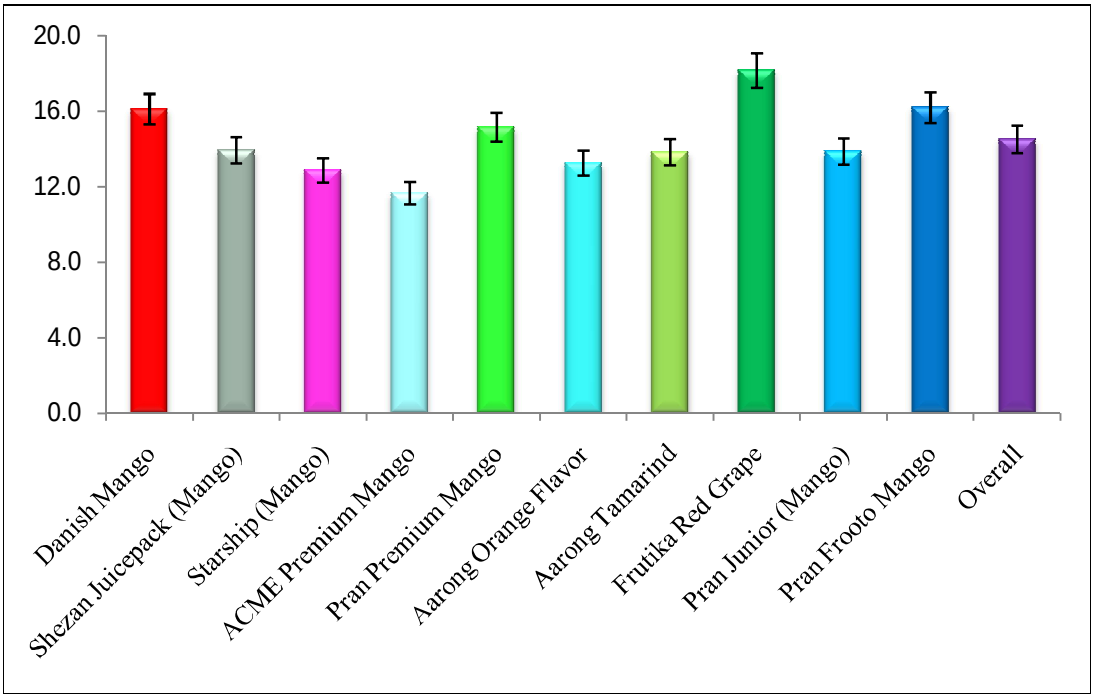

Figure 4.13: Comparison of total solid content in different juices.

The total solid found in different juices is in the range of 11.67−18.17%. The highest solid (18.17%) is found in Frutika Red Grape Juice and the lowest (11.67%) in ACME Premium Mango Juice.

4.3.3 Measurements of ash in different jams, jellies and juices

Ash found in jams ranges from 0.15 to 1.52%. The highest ash is found (1.52%) in Rajshahi Mango Jam and the lowest (0.15%) is found in Best Food Orange Jam and Agrokomerc Pineapple Jam.

Ash found in jellies ranges from 0.11 % to 0.42 %. The highest amount of ash is observed (0.42%) in Friends Mango Jelly and the lowest amount is (0.11%) found in Pran Orange Jelly and Agrokomerc Mango Jelly. The total amount of ash in juices of different brands is ranges from 0.05 to 0. 0.31 %. The highest amount is present in Aarong Tamarind Juice (0.31 %) and the lowest in Shezan Juicepac (mango 0.05%). The experimental values of ash in jams, jellies as well as juices of different brands are given in Tables 4.21, 4.22 and 4.23 respectively.

The statistical model of Descriptive, ANOVA (Analysis of variance) and DMRT (Duncan multiple range test) are not applicable for interpretation of ash because jams, jellies and juices contain insignificant amount of ash of the investigated samples.

Table 4.11: Ash found (%) in jams of different brands

SI. No	Sample	Ash content (%)
1	Shezan Mango Jam	0.27
2	pran Mango Jam	0.16
3	Freswel Mango Jam	0.27
4	Rajshahi Mango Jam	1.52
5	Nur Apple Jam	0.24
6	Best Food Orange Jam	0.15
7	Agrokomerc Pineapple Jam	0.15
8	Shezan mixed Jam	0.26
9	Nur Mixed Jam	0.22

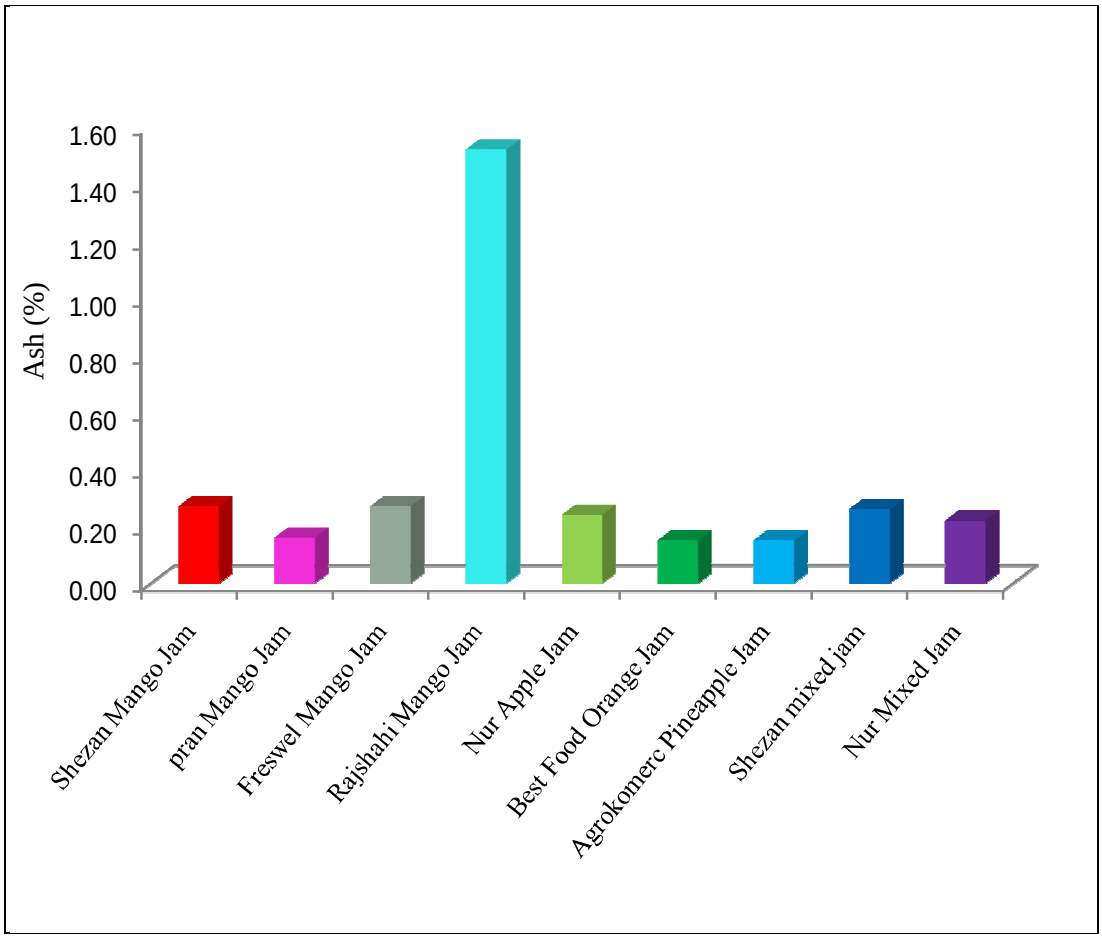

Figure 4.14: Comparison of ash status in different jams

Table 4.12: Ash found (%) in jellies of different brands

Sl. No	Sample	Ash content (%)
1	Friends Mango Jelly	0.42
2	Agrokomerc Mango Jelly	0.11
3	Pran Apple jelly	0.22
4	Friends Ornge Jelly	0.25
5	BD Orange Jelly	0.21
6	Shezan Orange jelly	0.13
7	Pran Orange Jelly	0.11
8	Ahmed Guava Jelly	0.21

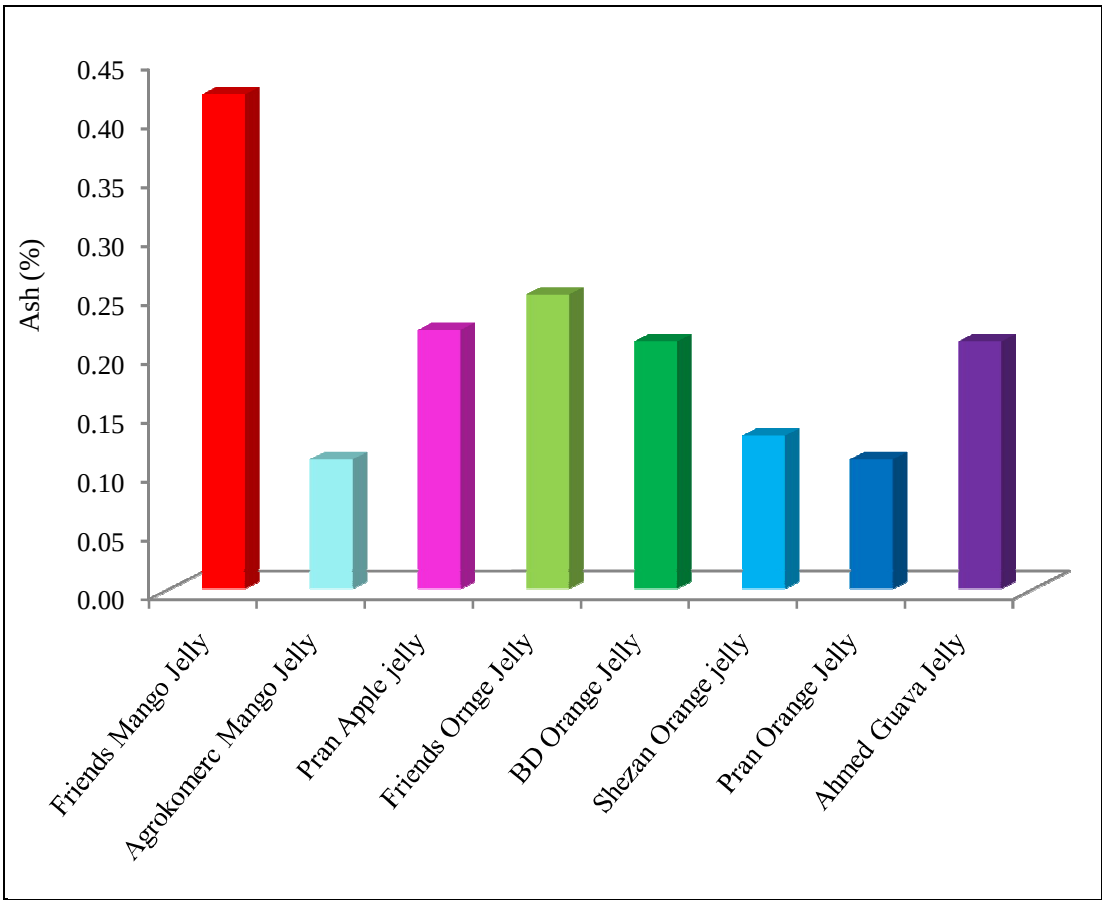

Figure 4.15: Comparison of ash content in different jellies.

Table 4.13: Ash found (%) in juices of different brands

SI.No	Sample	Ash (%)
1	Danish Mango	0.19
2	Shezan Juice (Mango)	0.05
3	Starship (Mango)	0.16
4	ACME pr. mango	0.07
5	Pran pr. mango	0.18
6	Aarong Orange Flavor	0.08
7	Aarong Tamarind	0.31
8	Frutika Red Grape	0.06
9	Pran Junior (Mango)	0.21
10	Pran Frooto Mango	0.23

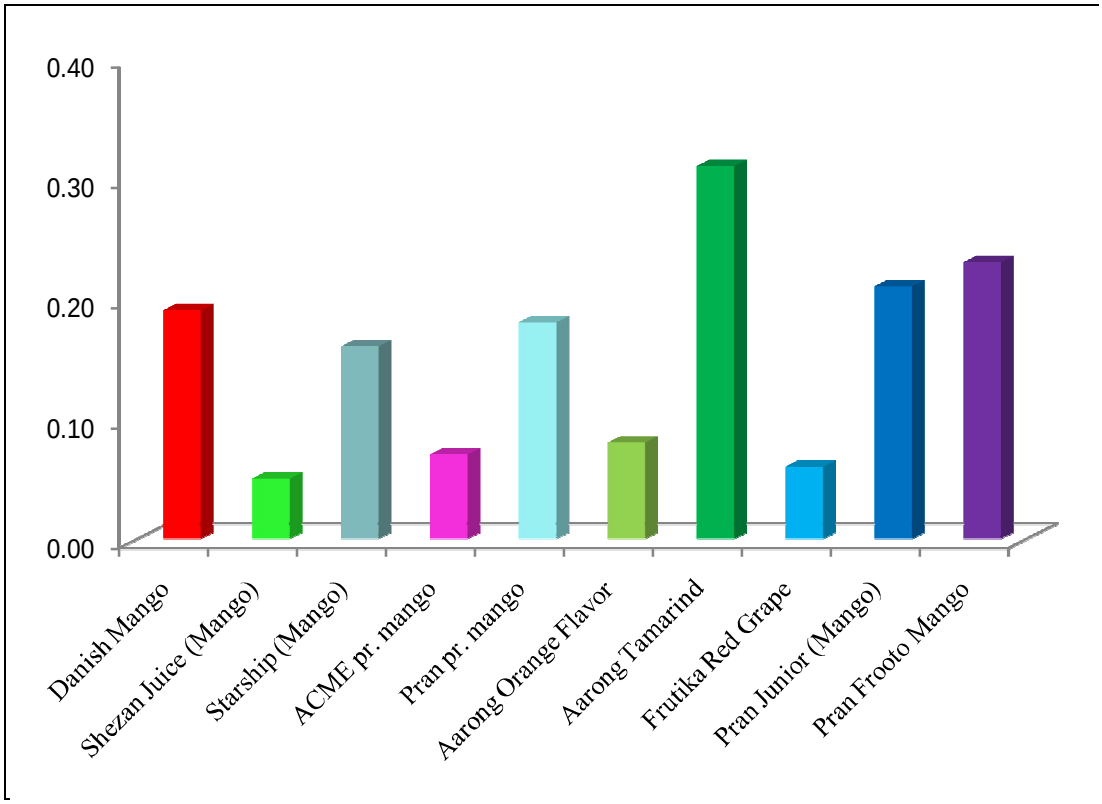

Figure 4.16: Comparison of ash content in different juice.

4.3.4 Measurements of protein in different jams, jellies and juices

Table 4.14: Protein found (%) in jams of different brands

Name of brands	Protein (Mean ± SD)
Shezan Mango Jam	0.35 ± 0.00
Pran Mango Jam	0.45 ± 0.00
Freswel Mango Jam	0.00 ± .00
Rajshahi Mango Jam	0.00 ± 0.00
Nur Apple Jam	0.00 ± 0.00
Best Food Orange Jam	0.79 ± 0.01
Agrokomerc Pineapple Jam	0.35 ±0.00
Shezan Mixed Jam	0.00 ± 0.00
Nur Mixed Jam	0.00 ± 0.00

Table 4.14 (a): Descriptive statistics of protein of different jams**.**

Name of brand	Mean	SD	SE	95% CI for mean		Min	Max
				lower bound	upper bound		
Shezan Mango	0.35	0.00	0.00	0.35	0.35	0.35	0.35
Pran Mango	0.45	0.00	0.00	0.45	0.45	0.45	0.45
Freswel Mango	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Rajshahi Mango	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Nur Apple	0.00	0.00	0.00	0.00	0.00	0.00	0.00
B. F. Orange	0.79	0.01	0.01	0.77	0.81	0.78	0.80
Agro Pineapple	0.35	0.00	0.00	0.35	0.35	0.35	0.35
Shezan Mixed	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Nur Mixed	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Overall	0.22	0.27	0.05	0.11	0.32	0.00	0.80

B.F. = Best Food, Agro = Agrokomerc

Description of H_0 and H_1 is almost same as earlier.

Table 4.14 (b): Analysis of Variance for the variation study of protein of different jams.

	Source of variation	SS	DF	MS	F	P
% of Protein	Between groups	1.96	8	0.25	22053.00	0.00
	Within group	0.00	18	0.00		
	Total	1.96	26			

Statistical explanation of protein in jams is almost same as earlier.

Table 4.14(c): Duncan's Multiple Range Test of protein for mean comparison of different jams.

Duncan[a]

Brand	Subset for alpha = 0.05			
	1	2	3	4
Freswel Mango	0.00	–	–	–
Rajshahi Mango	0.00	–	–	–
Nur Apple	0.00	–	–	–
Shezan Mixed	0.00	–	–	–
Nur Mixed	0.00	–	–	–
Shezan Mango	–	0.35	–	–
Agrokomerc Pineapple	–	0.35	–	–
Pran Mango	–	–	0.45	–
Best Food Orange	–	–	–	0.79
Sig.	1.00	1.00	1.00	1.00

Mean for group in homogeneous subsets are displayed. a. Uses Harmonic Mean Sample Size = 3.00.

The result of mean value of protein is same as earlier and the interaction effect is found in jams within the group-2.

In this case the studied jams are divided into four groups. A significant difference of protein is observed between the groups and insignificant difference is within the group. The variation of the percentage of protein of different brands is narrated below and shown in Figure 4.17 constructed from the Table 4.14 (a).

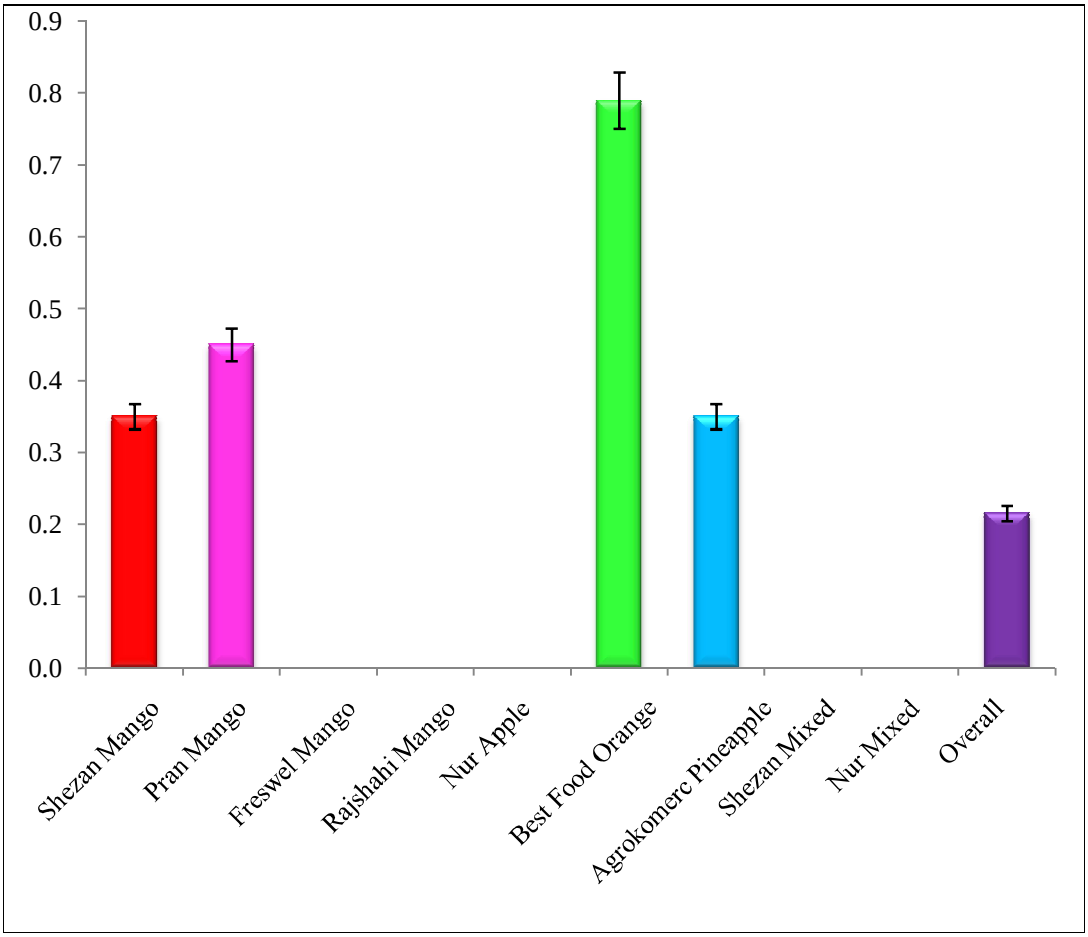

Figure 4.17: Comparison of protein status in different jams.

Among the 9 brands of jams only 4 brands contain small amount of protein and other 5 contain no protein. Presence of protein in jams ranges from 0.35 to 0.79%. The highest amount (0.79%) is found in Best Food Orange Marmalade brand and the lowest amount (0.35%) in Shezan Mango Jam and Agrokomerc Pineapple brand. Pran Mango Jam contains 0.45% protein. The average values are shown in the graph (Figure 4.17) which indicates the protein status in various types of jams.

Table 4.15: Protein found (%) in jellies of different brands

Name of brands	Protein (Mean ± SD)
Friends Mango Jelly	0.50 ± 0.00
Agrokomerc Mango Jelly	0.35 ± 0.00
Pran Apple Jelly	0.00 ± .00
Friends Orange Jelly	0.00 ± 0.00
BD Orange Jelly	0.00 ± 0.00
Shezan Orange Jelly	0.00 ± 0.01
Pran Orange Jelly	0.00 ± 0.00
Ahmed Guava Jelly	0.00 ± 0.00

Table 4.15 (a): Descriptive statistics of protein of different jellies

Name of brand	Mean	SD	SE	95% CI for mean		Min	Max
				lower bound	upper bound		
Friends Mango	0.50	0.00	0.00	0.50	0.50	0.50	0.50
Agro. Mango	0.35	0.00	0.00	0.35	0.35	0.35	0.35
Pran Apple	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Friends Orange	0.00	0.00	0.00	0.00	0.00	0.00	0.00
BD Orange	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Shezan Orange	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Pran Orange	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Ahmed Guava	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Overall	0.11	0.19	0.04	0.03	0.19	0.00	0.50

Description of H_0 and H_1 is almost same as earlier.

Table 4.15 (b): Analysis of variance for the variation study of protein of different jellies

	Source of variation	SS	DF	MS	F	P
% of Protein	Between groups	0.85	7	0.12	–	–
	Within group	0.00	16	0.00	–	–
	Total	0.85	23	–	–	–

Table 4.15(b) gives us the statistical information of the experimental results of protein in jellies and informs a significant difference of the protein values of these fruit jellies.

From the same Table it is seen that the level of significant, P is absent. Mean Square value of protein in the selected jellies are within group is 0.00. So F value is not generated. That is why the level of significant and Duncan's multiple range test are not found in this case.

The variation of the percentage of protein of different brands of jellies is narrated below and shown in Figure 4.18 plotted from the Table 4.15 (a).

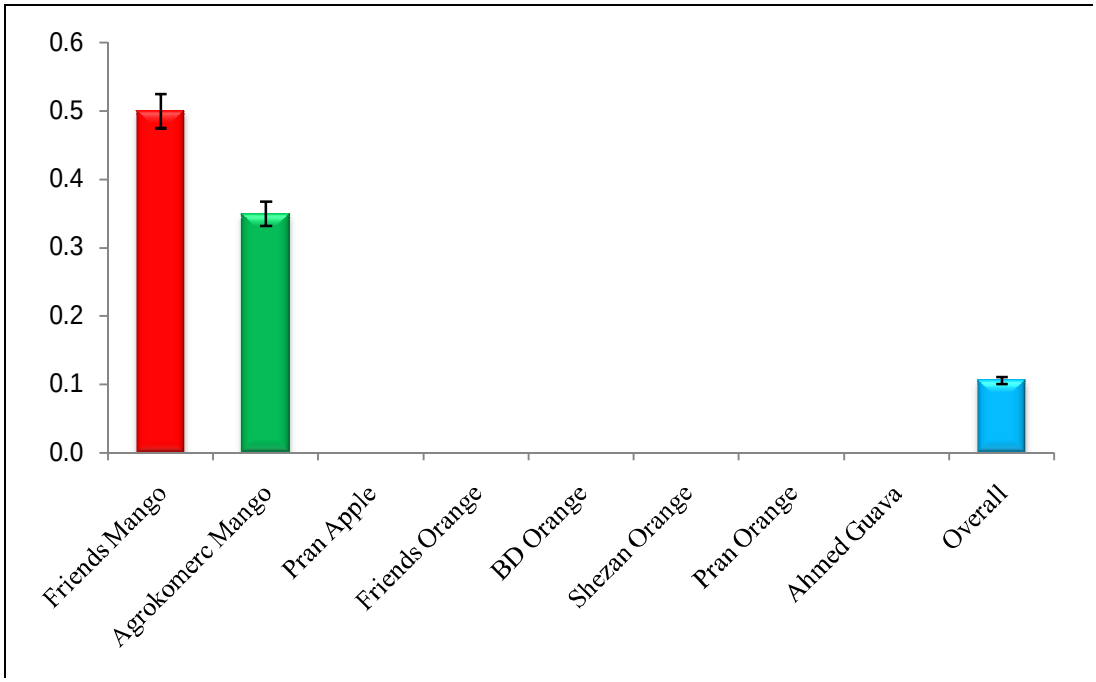

Figure 4.18: Comparison of protein status in different jellies.

Among 8 brands of jellies only 2 brands contain small amount of protein. Protein found in jellies ranges from 0.035 to 0.50%. The highest amount of protein (0.50%) is found in Friends Mango Jelly and the lowest (0.35%) amount in Agrokomerc Mango Jelly. Protein is absent in other 6 brands also.

Table 4.16: Protein found (%) in juices of different brands

Name of brands	Protein content (Mean ± SD)
Danish Mango	0.00 ± 0.00
Shezan Juicepack (Mango)	0.35 ± 0.00
Starship (Mango)	0.00 ± .00
ACME Premium Mango	0.00 ± 0.00
Pran Premium Mango	0.35 ± 0.00
Aarong Orange Flavor	0.00 ± 0.00
Aarong Tamarind	0.00 ± 0.00
Frutika Red Grape	0.00 ± 0.00
Pran Junior (Mango)	0.00 ± 0.00
Pran Frooto Mango	0.00 ± 0.00

Table 4.16(a): Descriptive statistics of protein of different juices

Name of brand	Mean	SD	SE	95% CI for mean		Min	Max
				lower bound	upper bound		
Danish Mango	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Shezan Jpk (Ma)	0.35	0.00	0.00	0.35	0.35	0.35	0.35
Starship (Ma)	0.00	0.00	0.00	0.00	0.00	0.00	0.00
ACME Pr Ma	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Pran Premium Ma	0.35	0.00	0.00	0.35	0.35	0.35	0.35
Aarong Or Flavor	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Aarong Tamarind	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Frutika Red Grape	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Pran Junior (Ma)	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Pran Frooto Ma	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Total	0.07	0.14	0.03	0.02	0.12	0.00	0.35

Ma = Mango, Jpk =Juice pack, Pr = premium, Or = Orange

Description of H_0 and H_1 is almost same as earlier.

Table 4.16 (b): Analysis of Variance for the variation study of protein of different juices

	Source of variation	SS	DF	MS	F	P
% of Protein	Between groups	98.03	9	10.89	123.24	0.00
	Within group	1.77	20	0.09	–	–
	Total	99.80	29	–	–	–

Table 4.16(b) shows the statistical information of the experimental results of protein in juices and informs a significant difference of the protein values of these fruit juices. This Table also informs us that the level of significant, P is absent. Mean square value of protein in juices within group is 0.00; so F value is not generated. That is why the level of significant and Duncan's multiple range test are absent in protein in the experimental juices.

The variation of the percentage of protein of different juices is described in the following figure constructed from the Table 4.16 (a).

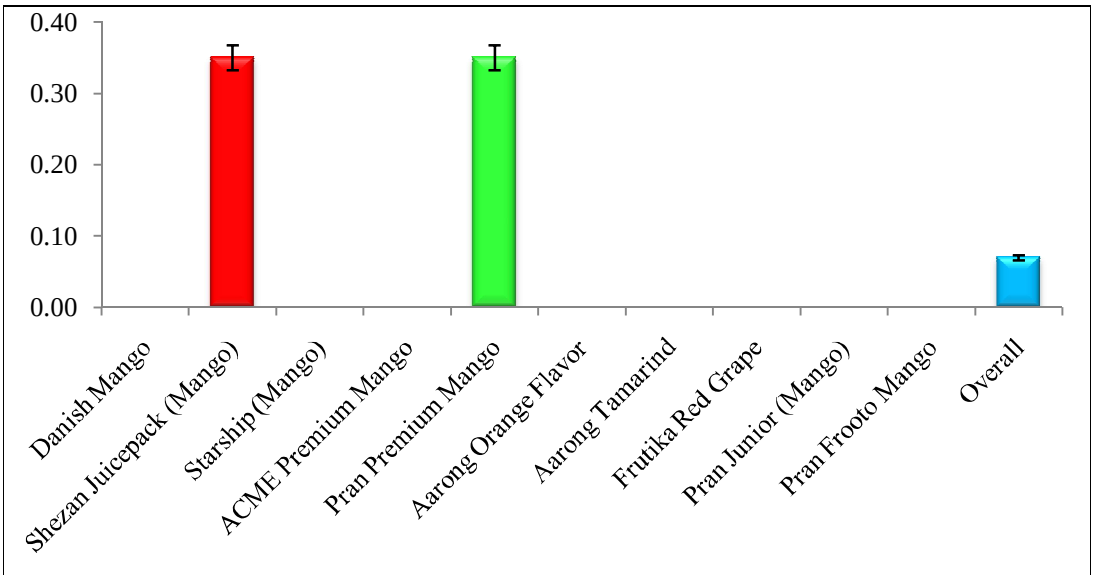

Figure 4.19: Comparison of protein status in different juices.

Protein found in juices ranges from 0.00 to 0.35%. Pran Premium Mango Juice and Shezan Juice (Mango) both contain protein 0.35%, which is absent in other 8 brands.

Protein is indispensable part of the food for animal as it is the chief constituent of the protoplasm which forms the vital part of every living cell. It repairs body tissue by continuous catabolism in the body and synthesis of new proteins from the amino acids. Plasma protein regulates water balance. Protein is involved in the regulation of acid-base balance.

4.3.5 Measurements of reducing sugar, non-reducing sugar and total sugar in different jams

Reducing sugar in four different mango jams ranges from 46.10 to 62.15%. Khalid found reducing sugar of mango jams that ranges from 33.63 to 21.94% [6]. The range is very close to the present investigation.

In this study four varieties of mango jams are analyzed. The lowest total sugar is found (46.10%) in Rajshahi Mango Jam and the highest (62.15%) in Freswel Mango Jam. Best Food Orange Marmalade Jam contains 57.27% total sugar. Pearson reported that the percentage of total sugar present in jam was 53.00−68.00 % [7].

Table 4.17: Reducing sugar found (%) in jams of different brands

Name of brands	Reducing sugar (Mean ± SD)
Shezan Mango Jam	48.17 ± 0.25
Pran Mango Jam	35.26 ± 0.12
Freswel Mango Jam	40.16 ± 0.17
Rajshahi Mango Jam	38.00 ± 0.20
Nur Apple Jam	57.20 ± 0.40
Best Food Orange Jam	32.10 ± 0.22
Agrokomerc Pineapple Jam	41.00 ±0.70
Shezan Mixed Jam	28.00 ± 0.00
Nur Mixed Jam	60.30 ± 0.42

Table 4.17(a): Descriptive statistics of reducing sugar of different jams**.**

Name of brand	Mean	SD	SE	95% CI for Mean		Min	Max
				lower bound	upper bound		
Shezan Mango	48.17	0.25	0.14	47.55	48.79	47.89	48.37
Pran Mango	35.26	0.12	0.07	34.97	35.55	35.16	35.39
Freshwel Mango	40.16	0.17	0.10	39.74	40.58	39.97	40.29
Rajshahi Mango	38.00	0.20	0.12	37.50	38.50	37.87	38.23
Nur Apple	57.20	0.40	0.23	56.20	58.20	56.88	57.65
B. F. Orange	32.10	0.22	0.13	31.56	32.64	31.86	32.29
Agro. Pineapple	41.00	0.70	0.40	39.26	42.74	40.50	41.80
Shezan Mixed	28.00	0.00	0.00	28.00	28.00	28.00	28.00
Nur Mixed	60.30	0.42	0.24	59.25	61.35	59.95	60.77
Overall	42.24	10.55	2.03	38.07	46.42	28.00	60.77

Description of H_0 and H_1 is almost same as earlier.

Table 4.17 (b): Analysis of variance for the variation study of reducing sugar of different jam.

	Source of variation	SS	DF	MS	F	P
% of Reducing sugar	Between groups	2889.87	8	361.23	3179.87	0.00
	Within group	2.05	18	0.11	–	–
	Total	2891.91	26	–	–	–

Table 4.17(b) represents the statistical information of the experimental results of reducing sugar of fruit jams and indicates a significant difference of these fruit jams according to the variation results of reducing sugar.

The level of significant, P = 0.00 < 0.01, so the variance of the result of reducing sugar of each jam is statistically significant at 1% level and we may conclude that the mean variation of the result of reducing sugar of each jam is significantly different.

Table 4.17(c): Duncan's multiple range test of reducing sugar for mean comparison of different jam.

Duncan[a]

Brand		Subset for alpha = 0.05							
	1	2	3	4	5	6	7	8	9
Shezan Mixed	28.00	–	–	–	–	–	–	–	–
Best Food Orange	–	32.10	–	–	–	–	–	–	–
Pran Mango	–	–	35.26	–	–	–	–	–	–
Rajshahi Mango	–	–	–	38.00		–	–	–	–
Freshwel Mango	–	–	–	–	40.16	–	–	–	–
Agrokomerc Pineapple	–	–	–	–	–	41.00	–	–	–
Shezan Mango	–	–	–	–	–	–	48.17	–	–
Nur Apple	–	–	–	–	–	–	–	57.20	–
Nur Mixed	–	–	–	–	–	–	–	–	60.30
Sig	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00

Mean for group in homogeneous subsets are displayed. a. Uses Harmonic Mean Sample Size = 3.00.

Table 4.17(c) shows that within the group the mean value of reducing sugar is insignificantly different and between the groups it is significantly different. The interaction effect is not found in the values of reducing sugar of the selected jams within the same group.

The studied jams are divided into nine groups [Table 4.17(c)]. A significant difference of reducing sugar is observed between the groups and insignificant difference is within the group. The variation of the percentage of reducing sugar of different brands is described below and shown in Figure 4.20 plotted from the Table 4.17(a).

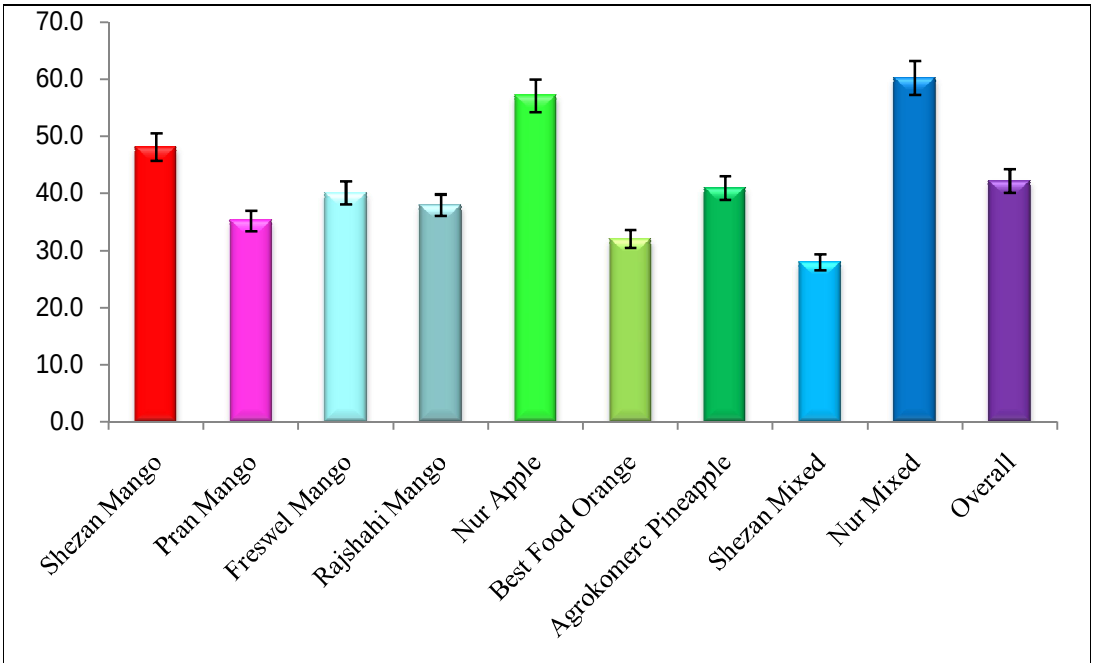

Figure 4.20: Comparison of reducing sugar status in different jams.

The reducing sugar present in jams ranges from 28.00 to 60.30 %. The highest amount was (60.30%) is found in Nur Mixed Jam and the lowest (28.00%) in Agrokomerc Pineapple Jam. The literature value is in the range of 22.0−40.0% [7]. It is lower than the experimental results. Hence it is suitable for diabetic patient.

Table 4.18: Non-reducing sugar found (%) in jams of different brands

Name of brands	Non-reducing sugar (Mean ± SD)
Shezan Mango Jam	11.08 ± 0. 20
Pran Mango Jam	16.92 ± 0.03
Freswel Mango Jam	21.99 ± 0.04
Rajshahi Mango Jam	08.10 ± 0.42
Nur Apple Jam	05.33 ± 0.49
Best Food Orange Jam	25.17 ± 0.23
Agrokomerc Pineapple Jam	10.86 ± 0.60
Shezan Mixed Jam	09.90 ± 0.45
Nur Mixed Jam	01.30 ± 0.94

Table 4.18 (a): Descriptive statistics of non reducing sugar of different jams**.**

Name of brand	Mean	SD	SE	95% CI for mean		Min	Max
				lower bound	upper bound		
Shezan Mango	48.17	0.25	0.14	47.55	48.79	47.89	48.37
Pran Mango	35.26	0.12	0.07	34.97	35.55	35.16	35.39
Freswel Mango	40.16	0.17	0.10	39.74	40.58	39.97	40.29
Rajshahi Mango	38.00	0.20	0.12	37.50	38.50	37.87	38.23
Nur Apple	57.20	0.40	0.23	56.20	58.20	56.88	57.65
B. F. Orange	32.10	0.22	0.13	31.56	32.64	31.86	32.29
Agro.Pineapple	41.00	0.70	0.40	39.26	42.74	40.50	41.80
Shezan Mixed	28.00	0.00	0.00	28.00	28.00	28.00	28.00
Nur Mixed	60.30	0.42	0.24	59.25	61.35	59.95	60.77
Overall	42.24	10.55	2.03	38.07	46.42	28.00	60.77

Description of H_0 and H_1 is almost same as earlier.

Table 4.18 (b): Analysis of variance for the variation study of non reducing sugar of different jams.

	Source of variation	SS	DF	MS	F	P
% of Non-reducing sugar	Between groups	1432.26	8	179.03	818.37	0.00
	Within group	3.94	18	0.22	–	–
	Total	1436.20	26	–	–	–

Statistical explanation for non reducing sugar in jams is almost same as earlier.

Table 4.18 (c): Duncan's multiple range test of non reducing sugar for mean comparison of different jams.

Duncan[a]

Brand	Subset for alpha = 0.05							
	1	**2**	**3**	**4**	**5**	**6**	**7**	**8**
Nur Mixed	1.30	–	–	–	–	–	–	–
Nur Apple	–	5.33	–	–	–	–	–	–
Rajshahi Mango	–	–	8.10	–	–	–	–	–
Shezan Mixed	–	–	–	9.90	–	–	–	–
Agro. Pineapple	–	–	–	–	10.86	–	–	–
Shezan Mango	–	–	–	–	11.08	–	–	–
Pran Mango	–	–	–	–	–	16.92	–	–
Freshwel Mango	–	–	–	–	–	–	21.99	–
Best Food Orange	–	–	–	–	–	–	–	25.17
Sig	1.00	1.00	1.00	1.00	0.57	1.00	1.00	1.00

Mean for group in homogeneous subsets are displayed. a. Uses harmonic mean sample size = 3.00

The results of non-reducing sugar in both groups (between and within) are similar as earlier and the interaction effect is found within the group-5.

The studied jams are divided into eight groups [Table 4.18(c)]. In this case similar result is observed. The variation of the percentage of non-reducing sugar of different brands is described below and shown in Figure 4.21 prepared from the Table 4.18(a).

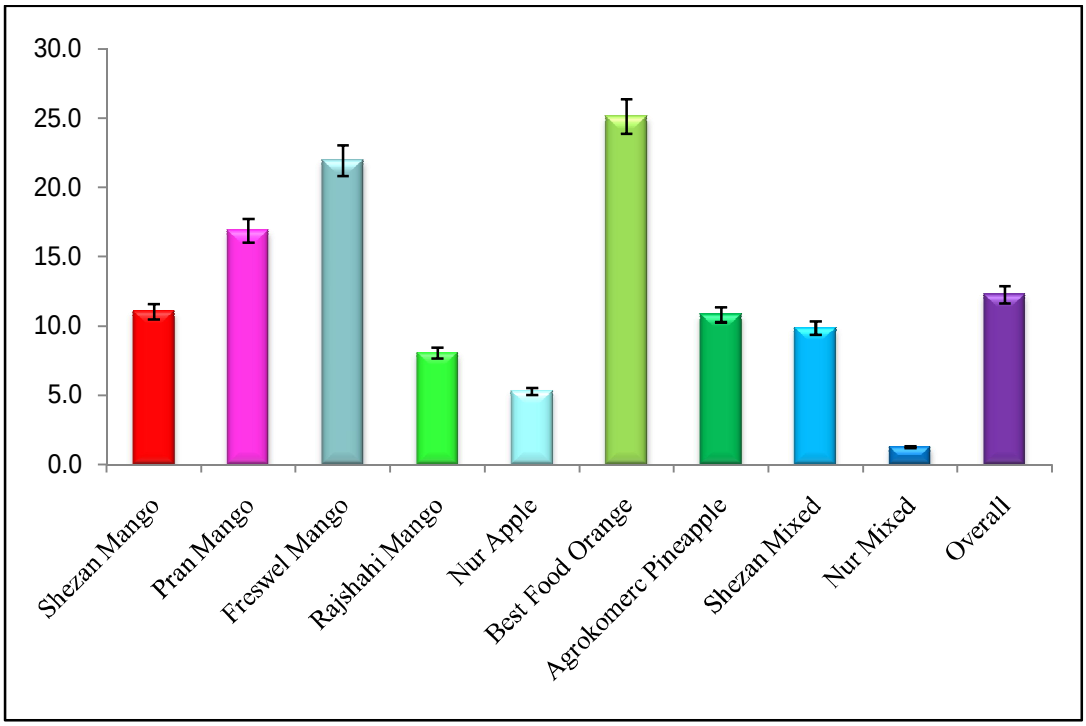

Figure 4.21: Comparison of non reducing sugar status in different jams

Non-reducing sugar found in jams ranges from 5.33 to 25.17%. The highest amount of non-reducing sugar (25.17%) is found in Best Food Orange Marmalade Jam and the lowest (5.33 %) in Nur Apple Jam.

Table 4.19: Total sugar found (%) in jams of different brands

Name of brands	Total sugar (Mean ± SD)
Shezan Mango Jam	59.25 ± 0.33
Pran Mango Jam	52.18 ± 0.15
Freswel Mango Jam	62.15 ± 0.20
Rajshahi Mango Jam	46.10 ± 0.30
Nur Apple Jam	62.53 ± 0.35
Best Food Orange Jam	57.27 ± 0.40
Agrokomerc Pineapple Jam	38.86 ± 0.60
Shezan Mixed Jam	50.90 ± 0.25
Nur Mixed Jam	61.60 ± 0.52

Table 4.19(a): Descriptive statistics of total sugar of different jams**.**

Name of brand	Mean	SD	SE	95% CI for mean		Min	Max
				lower bound	upper bound		
Shezan Mango	59.25	0.33	0.19	58.42	60.08	59.01	59.63
Pran Mango	52.18	0.15	0.09	51.80	52.56	52.06	52.35
Freshwel Mango	62.15	0.20	0.12	61.65	62.65	61.92	62.29
Rajshahi Mango	46.10	0.30	0.17	45.36	46.84	45.88	46.44
Nur Apple	62.53	0.35	0.20	61.66	63.40	62.13	62.78
Best Food Orange	57.27	0.40	0.23	56.27	58.27	56.95	57.72
Agro. Pineapple	38.86	0.60	0.35	37.37	40.35	38.41	39.54
Shezan Mixed	50.90	0.25	0.15	50.27	51.53	50.73	51.19
Nur Mixed	61.60	0.52	0.30	60.31	62.89	61.03	62.05
Overall	54.54	7.88	1.52	51.42	57.65	38.41	62.78

Description of H_0 and H_1 is almost same as earlier.

Table 4.19 (b): Analysis of variance for the variation study of total sugar of different jams.

	Source of variation	SS	DF	MS	F	P
% of Total sugar	Between groups	1611.44	8	201.43	1461.88	0.00
	Within group	2.48	18	0.14	–	–
	Total	1613.92	26	–	–	–

Statistical explanation for analysis of variance for the variation study of total sugar of different jam is almost same as earlier.

Table 4.19 (c): Duncan's Multiple Range Test of total sugar for mean comparison of different jams.

Duncan[a]

Brand	Subset for alpha = 0.05							
	1	**2**	**3**	**4**	**5**	**6**	**7**	**8**
Agro. Pineapple	38.86	–	–	–	–	–	–	–
Rajshahi Mango	–	46.10	–	–	–	–	–	–
Shezan Mixed	–	–	50.90	–	–	–	–	–
Pran Mango	–	–	–	52.18	–	–	–	–
Best Food Orange	–	–	–	–	57.27	–	–	–
Shezan Mango	–	–	–	–	–	59.25	–	–
Nur Mixed	–	–	–	–	–	–	61.60	–
Freswel Mango	–	–	–	–	–	–	62.15	62.15
Nur Apple	–	–	–	–	–	–	–	62.53
Sig	1.00	1.00	1.00	1.00	1.00	1.00	0.09	0.23

Mean for group in homogeneous subsets are displayed. a. Uses harmonic mean sample size = 3.00.

Table 4.19(c) shows that within the group the mean value of total sugar is insignificantly different and between the groups it is significantly different. The interaction effect is found in the values of total sugar of the selected jams within the group-7.

The jams are divided into eight groups [Table 4.19(c)]. In this case significant difference of total sugar is observed between the groups and insignificant difference is within the group. The variations of the percentage of total sugar of different brands are given below which are shown in Figure 4.22 drawn from the Table 4.19(a).

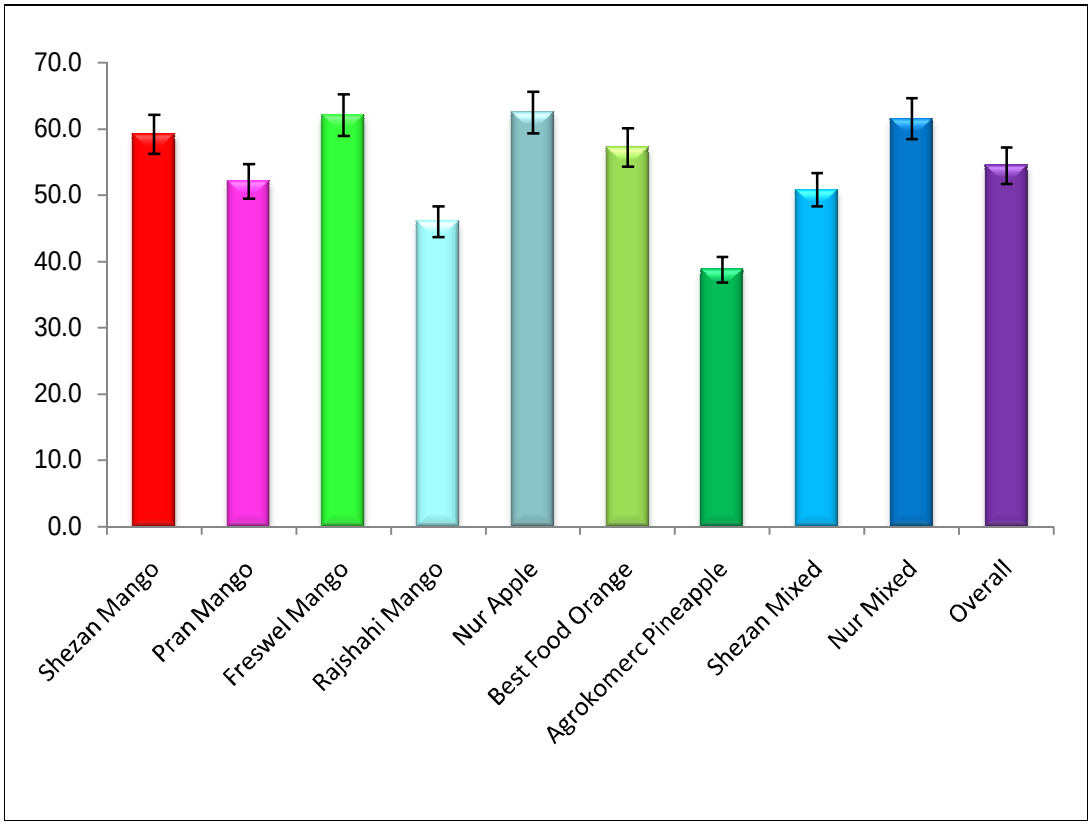

Figure 4.22: Comparison of total sugar status in different jams.

Total sugar present in jams ranges from 38.86 to 62.53%. The highest amount of total sugar (62.53%) is found in Nur Apple Jam and the lowest amount (38.86%) in Agrokomerc Pineapple Jam.

4.3.6 Measurements of reducing sugar, non-reducing sugar and total sugar in different jellies.

Reducing sugar was found in two mango jellies ranges from 31.12 to 23.21%. Khalid found reducing sugar in mango jellies ranges from 33.63 to 21.94% [6]. The range is very close to our present investigation.

Total sugar was found in different kinds of jellies ranges from 59.97 to 28.44%. The highest amount of total sugar was found in BD Orange Jelly and the lowest in Friends Orange Jelly. Total sugar in jellies was reported by Pearson (53.00−68.00%) [7]. The results obtained in this study are within this range except Friends Orange Jelly and Agrokomerc Mango jelly. In our study, 28.44% total sugar was found in Friends Orange Jelly and 42.37% was in Agrokomerc Mango Jelly.

Table 4.20: Reducing sugar found (%) in jellies of different brands

Name of brand	Reducing sugar (Mean ± SD)
Friends Mango Jelly	23.21± 0.03
Agrokomerc Mango Jelly	31.12 ± 0.03
Pran Apple Jelly	29.25 ±0.01
Friends Orange Jelly	16.32 ± 0.00
BD Orange Jelly	29.87 ± 0.01
Shezan Orange Jelly	49.66 ± 0.01
Pran Orange Jelly	44.55 ± 0.01
Ahmed Guava Jelly	31.15 ± 0.01

Table 4.20 (a): Descriptive statistics of reducing sugar of different jellies

Name of brand	Mean	SD	SE	95% CI for mean		Min	Max
				lower bound	upper bound		
Friends Mango	23.21	0.03	0.02	23.14	23.28	23.18	23.24
Agrokomerc Mango	31.12	0.03	0.02	31.05	31.19	31.09	31.15
Pran Apple	29.25	0.01	0.01	29.23	29.27	29.24	29.26
Friends Orange	16.32	0.00	0.00	16.32	16.32	16.32	16.32
BD Orange	29.87	0.01	0.01	29.85	29.89	29.86	29.88
Shezan Orange	49.66	0.02	0.01	49.61	49.71	49.64	49.68
Pran Orange	44.55	0.02	0.01	44.50	44.60	44.53	44.57
Ahmed Guava	31.15	0.01	0.01	31.13	31.17	31.14	31.16
Overall	31.89	10.25	2.09	27.56	36.22	16.32	49.68

Description of H_0 and H_1 is almost same as earlier.

Table 4.20 (b): Analysis of variance for the variation study of reducing sugar of different jellies.

	Source of variation	SS	DF	MS	F	P
% of Reducing sugar	Between groups	2418.02	7	345.43	952913.84	0.00
	Within group	0.01	16	0.00	–	–
	Total	2418.03	23	–	–	–

Table 4.20 (b) indicates the statistical information of the experimental results of reducing sugar in fruit jellies and informs a significant difference of these fruit jellies according to the variation results of reducing sugar. The level of significant, P was found to be 0.00 < 0.01. So the variance of the result of reducing sugar is statistically significant at 1% level and we may conclude that the mean variation of the result is significantly different.

Table 4.20 (c): Duncan's multiple range test of reducing sugar for mean comparison of different jellies

Duncan[a]

Brand	Subset for alpha = 0.05						
	1	2	3	4	5	6	7
Friends Orange	16.32	–	–	–	–	–	–
Friends Mango	–	23.21	–	–	–	–	–
Pran Apple	–	–	29.25	–	–	–	–
BD Orange	–	–	–	29.87	–	–	–
Agrokomerc Mango	–	–	–	–	31.12	–	–
Ahmed Guava	–	–	–	–	31.15	–	–
Pran Orange	–	–	–	–	–	44.55	–
Shezan Orange	–	–	–	–	–	–	49.66
Sig	1.00	1.00	1.00	1.00	0.07	1.00	1.00

Mean for group in homogeneous subsets are displayed. a. Uses harmonic mean sample size=3.00.

Table 4.20 (c) shows that the group mean value is as earlier. The interaction effect is found within the group-5.

The studied jellies are divided into seven groups [Table 4.20(c)]. A significant difference of reducing sugar is observed between the groups and insignificant difference is within the group. The variations of the percentage of reducing sugar of different brands of jellies are described below.

The comparisons of reducing sugar status in different jellies are shown in Figure 4.23 plotted from the Table 4.20 (a).

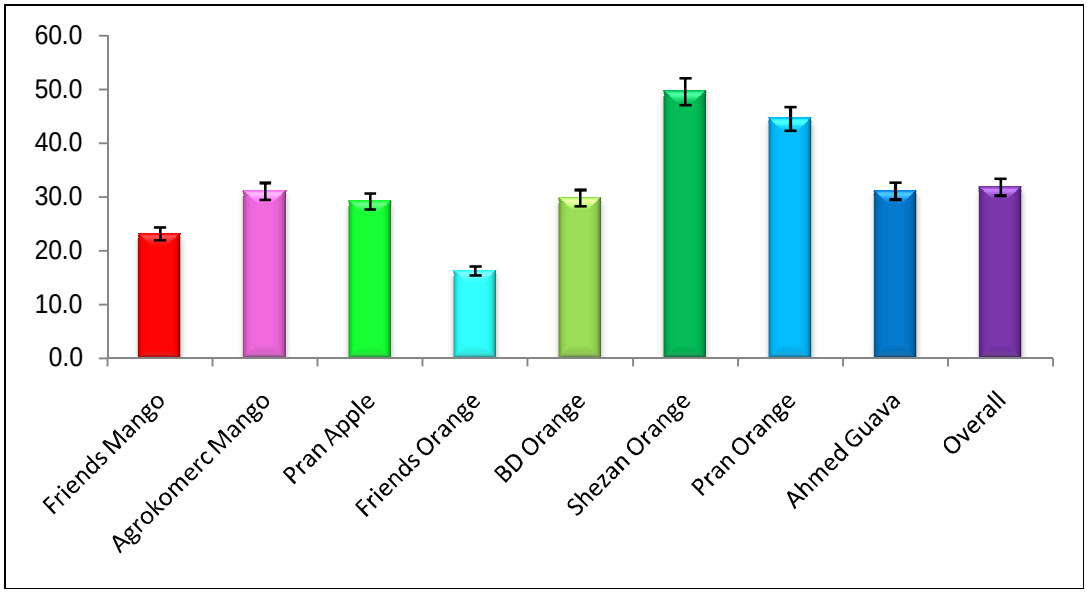

Figure 4.23: Comparison of reducing sugar status in different jellies.

The reducing sugar present in jellies ranges from 16.32 to 49.66**%.** The highest amount of reducing sugar **(**49.66%**)** is found in Shezan Orange Jelly and the lowest (16.32 %) in Friends Orange Jelly.

Table 4.21: Non-reducing sugar found (%) in jellies of different brands

Name of brands	Non-reducing sugar (Mean ± SD)
Friends Mango Jelly	29.28 ± 0.02
Agrokomerc Mango Jelly	11.25 ± 0.04
Pran Apple Jelly	28.93 ± 0.01
Friends Orange Jelly	12.12 ± 0.00
BD Orange Jelly	30.10 ± 0.02
Shezan Orange Jelly	06.43 ± 0.03
Pran Orange Jelly	10.33 ±0.00
Ahmed Guava Jelly	22.17 ± 0.02

Table 4.21(a): Descriptive statistics of non reducing sugar of different jellies.

Name of brand	Mean	SD	SE	95% CI for mean		Min	Max
				lower bound	upper bound		
Friends Mango	29.28	0.02	0.01	29.23	29.33	29.26	29.30
Agro. Mango	11.25	0.04	0.02	11.16	11.34	11.22	11.29
Pran Apple	28.86	0.12	0.07	28.55	29.17	28.72	28.94
Friends Orange	12.12	0.00	0.00	12.12	12.12	12.12	12.12
BD Orange	30.10	0.02	0.01	30.06	30.14	30.08	30.11
Shezan Orange	6.43	0.03	0.02	6.36	6.50	6.40	6.46
Pran Orange	10.33	0.00	0.00	10.33	10.33	10.33	10.33
Ahmed Guava	22.17	0.02	0.01	22.13	22.21	22.15	22.18
Overall	18.82	9.39	1.92	14.85	22.78	6.40	30.11

Description of H_0 and H_1 is almost same as earlier.

Table 4.21 (b): Analysis of variance for the variation study of non reducing sugar of different jellies

	Source of variation	SS	DF	MS	F	P
% of Non-reducing sugar	Between groups	2029.58	7	289.94	124482.56	0.00
	Within group	0.04	16	0.00	–	–
	Total	2029.62	23		–	–

The above Table refers the statistical information of the experimental results of non-reducing sugar in fruit jellies and mentions a significant difference of these fruit jellies according to the variation results of non-reducing sugar.

From the Table 4.21 (b), it has been observed that the level of significant, $P = 0.00 < 0.01$, so the variance of the result of non reducing sugar of each jelly is statistically significant at 1% level and we may conclude that the mean variation of the result of non-reducing sugar of each jelly is significantly different.

Table 4.21 (c): Duncan's multiple range test of non reducing sugar for mean comparison of different jellies

Duncan[a]

Brand	Subset for alpha = 0.05							
	1	**2**	**3**	**4**	**5**	**6**	**7**	**8**
Shezan Orange	6.43	–	–	–	–	–	–	–
Pran Orange	–	10.33	–	–	–	–	–	–
Agro. Mango	–	–	11.25	–	–	–	–	–
Friends Orange	–	–	–	12.12	–	–	–	–
Ahmed Guava	–	–	–	–	22.17	–	–	–
Pran Apple	–	–	–	–	–	28.86	–	–
Friends Mango	–	–	–	–	–	–	29.28	–
BD Orange	–	–	–	–	–	–	–	30.10
Sig	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00

Mean for group in homogeneous subsets are displayed. a. Uses Harmonic Mean Sample Size = 3.00.

The mean value within the group of non-reducing sugar is insignificantly different and between the groups it is significantly different as well as no interaction effect is found in the values of non-reducing sugar within the group.

Jellies are divided into eight groups [Table4.21(c)]. A significant difference of non-reducing sugar is observed between the groups and within the group it has insignificant difference. The variation of the percentage of non-reducing sugar is described below in Figure 4.24 obtained from the Table 4.21 (a).

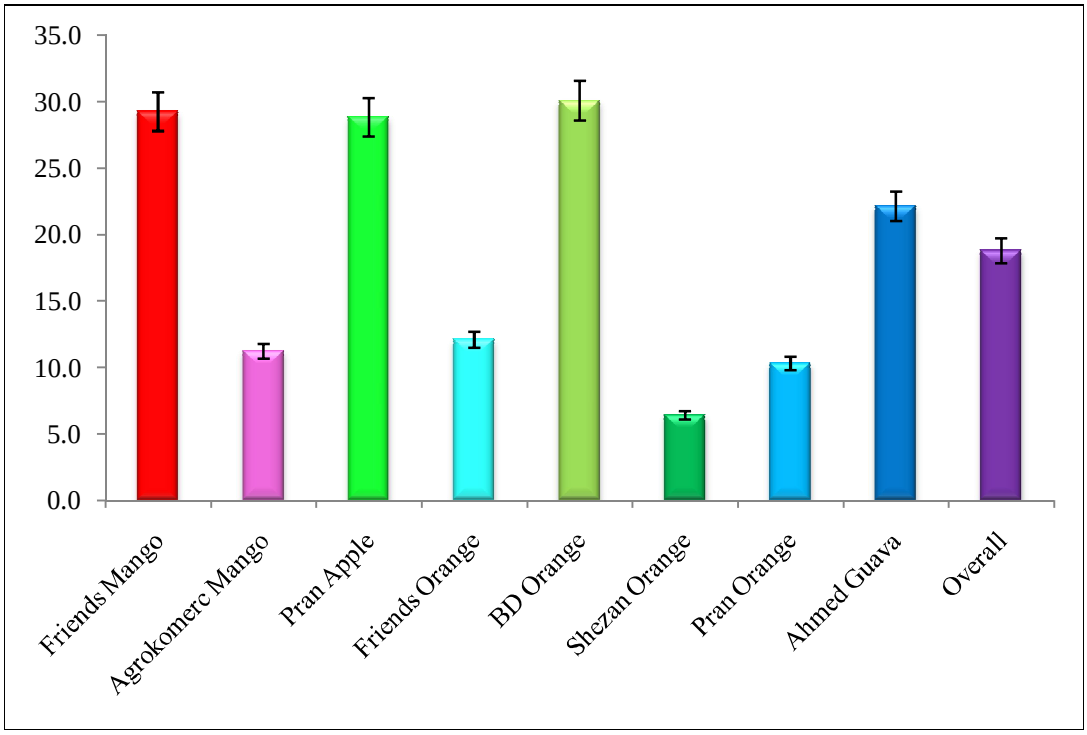

Figure 4.24: Comparison of non reducing sugar status in different jellies.

Non-reducing sugar found in jellies ranges from 6.43 to 30.10%**.** The highest amount **(**30.10%**)** was found in BD Orange Jelly and the lowest **(**6.43**%)** in Shezan Orange Jelly.

Table 4.22: Total sugar detected (%) in jellies of different brands

Name of brands	**Total sugar (Mean ± SD)**
Friends Mango Jelly	52.49 ± 0.01
Agrokomerc Mango Jelly	42.37 ± 0.01
Pran Apple Jelly	58.18 ± 0.02
Friends Orange Jelly	28.44 ± 0.00
BD Orange Jelly	59.97 ± 0.01
Shezan Orange Jelly	56.09 ± 0.01
Pran Orange Jelly	54.88 ±0.02
Ahmed Guava Jelly	53.32 ± 0.02

Table 4.22(a): Descriptive statistics of total sugar of different jellies

Name of brand	Mean	SD	SE	95% CI for mean		Min	Max
				lower bound	upper bound		
Friends Mango	52.49	0.01	0.01	52.47	52.51	52.48	52.50
Agro. Mango	42.37	0.01	0.01	42.35	42.39	42.36	42.38
Pran Apple	58.18	0.02	0.01	58.13	58.23	58.16	58.20
Friends Orange	28.44	0.00	0.00	28.44	28.44	28.44	28.44
BD Orange	59.97	0.01	0.01	59.95	59.99	59.96	59.98
Shezan Orange	56.09	0.01	0.01	56.07	56.11	56.08	56.10
Pran Orange	54.88	0.02	0.01	54.83	54.93	54.86	54.90
Ahmed Guava	53.32	0.02	0.01	53.27	53.37	53.30	53.34
Overall	50.72	9.98	2.04	46.50	54.93	28.44	59.98

Description of H_0 and H_1 is almost same as earlier.

Table 4.22(b): Analysis of variance for the variation study of total sugar of different jellies

	Source of variation	SS	DF	MS	F	P
% of Total sugar	Between groups	2290.11	7	327.16	1635793.61	0.00
	Within group	0.00	16	0.00	–	–
	Total	2290.11	23	–	–	–

Table 4.22((b) provides statistical information of the experimental results of total sugar of fruit jellies and indicates a significant difference of these fruit jellies according to the variation results of total sugar. The level of significant, $P = 0.00 < 0.01$, so the variance of the result of total sugar of each jelly is statistically significant at 1% level and we may conclude that the mean variation of the result of total sugar of each jelly is significantly different.

Table 4.22 (c): Duncan's multiple range test of total sugar for mean comparison of different jellies

Duncan[a]

Brand	Subset for alpha = 0.05							
	1	**2**	**3**	**4**	**5**	**6**	**7**	**8**
Friends Orange	28.44	–	–	–	–	–	–	–
Agrokomerc Mango	–	42.37	–	–	–	–	–	–
Friends Mango	–	–	52.49	–	–	–	–	–
Ahmed Guava	–	–	–	53.32	–	–	–	–
Pran Orange	–	–	–	–	54.88	–	–	–
Shezan Orange	–	–	–	–	–	56.09	–	–
Pran Apple	–	–	–	–	–	–	58.18	–
BD Orange	–	–	–	–	–	–	–	59.97
Sig	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00

Mean for group in homogeneous subsets are displayed. a. Uses harmonic mean sample size = 3.00.

From the Table 4.22 (c) it is evident that within the group mean value of total sugar is insignificantly different and between the groups it is significantly different and no interaction effect is found of total sugar within the group.

The studied jellies are divided into eight groups [Table 4.22 (c)]. In this case between and within the groups same result of total sugar was observed. The variation of the percentage of total sugar of different brands is given below and shown in Figure 4.25 prepared from the Table 4.22 (a).

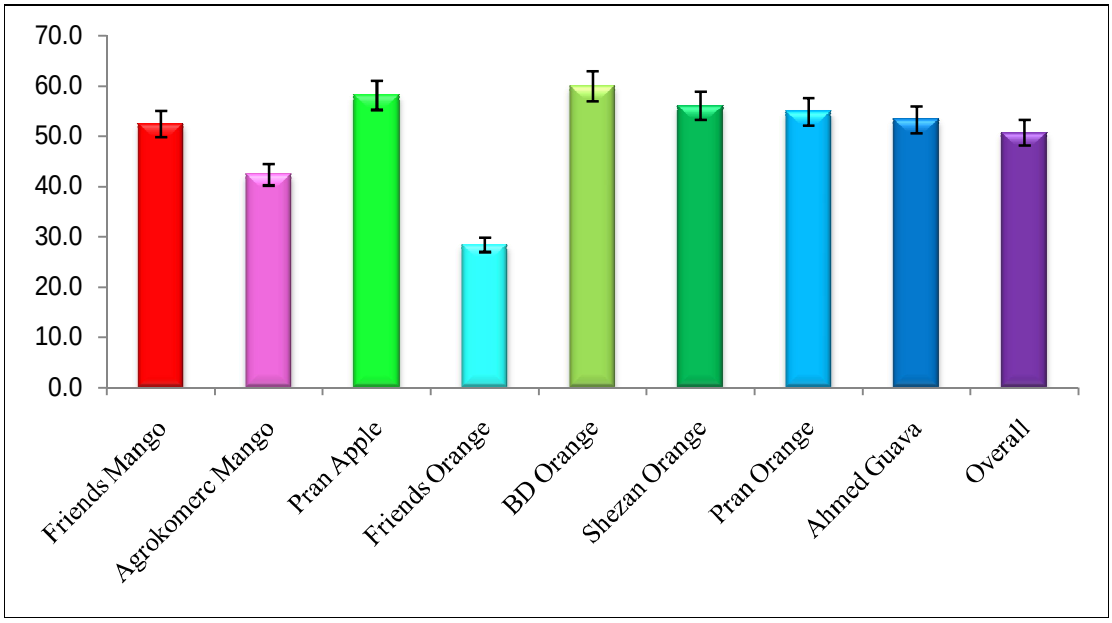

Figure 4.25: Comparison of total sugar status in different jellies.

Total sugar found in jellies is in the range of 28.44–59.97 %. The highest amount of total sugar **(**59.97%**)** is found in BD Orange Jelly and the lowest **(**28.44**%)** in Friends Orange Jelly.

4.3.7 Measurements of reducing sugar, non-reducing sugar and total sugar in different juices.

Table 4.23: Reducing sugar detected (%) in juices of different brands

Name of brands	Reducing sugar (Mean ± SD)
Danish Mango	2.65 ± 0.04
Shezan Juicepack (Mango)	4.45 ± 0.04
Starship (Mango)	4.11 ± 0.03
ACME Premium Mango	3.22 ± 0.5
Pran Premium Mango	6.24 ± 0.03
Aarong Orange Flavor	3.65 ± 0.12
Aarong Tamarind	11.60 ± 0.06
Frutika Red Grape	11.25 ± 0.05
Pran Junior (Mango)	8.43 ± 0.02
Pran Frooto Mango	4.12 ± 0.02

Table 4.23 (a): Descriptive statistics of reducing sugar of different juices.

Name of brand	Mean	SD	SE	95% CI for mean		Min	Max
				lower bound	upper bound		
Danish Mango	2.65	0.04	0.02	2.56	2.74	2.61	2.68
Shezan Jpk. (Ma)	4.45	0.04	0.03	4.34	4.56	4.40	4.48
Starship (Ma)	4.11	0.03	0.02	4.02	4.20	4.09	4.15
ACME Pr. Ma	3.22	0.05	0.03	3.09	3.35	3.16	3.26
Pran Premium Ma	6.24	0.03	0.02	6.17	6.31	6.22	6.27
Aarong Or. Flavor	3.65	0.12	0.07	3.34	3.96	3.55	3.79
Aarong Tamarind	11.60	0.06	0.04	11.44	11.76	11.53	11.65
Frutika Red Grape	11.25	0.05	0.03	11.12	11.38	11.19	11.28
Pran Junior (Ma)	8.43	0.02	0.01	8.39	8.47	8.41	8.44
Pran Frooto Ma	4.12	0.02	0.01	4.07	4.17	4.10	4.14
Overall	5.97	3.20	0.58	4.78	7.17	2.61	11.65

Description of H_0 and H_1 is almost same as earlier.

Table 4.23 (b): Analysis of variance for the variation study of reducing sugar of different juices.

	Source of variation	SS	DF	MS	F	P
% of Reducing sugar	Between groups	296.58	9	32.95	10699.09	0.00
	Within group	0.06	20	0.00	–	–
	Total	296.64	29	–	–	–

This Table provides the statistical information as earlier.

Table 4.23(c): Duncan's multiple range test of reducing sugar for mean comparison of different juices.

Duncan[a]

Brand	Subset for alpha = 0.05								
	1	2	3	4	5	6	7	8	9
Danish Mango	2.65	–	–	–	–	–	–	–	–
ACME Premium Mango	–	3.22	–	–	–	–	–	–	–
Aarong Orange Flavor	–	–	3.65	–	–	–	–	–	–
Starship (Mango)	–	–	–	4.11	–	–	–	–	–
Pran Frooto Mango	–	–	–	4.12	–	–	–	–	–
Shezan Juicepack (Mango)	–	–	–	–	4.45	–	–	–	–
Pran Premium Mango	–	–	–	–	–	6.24	–	–	–
Pran Junior (Mango)	–	–	–	–	–	–	8.43	–	–
Frutika Red Grape	–	–	–	–	–	–	–	11.25	–
Aarong Tamarind	–	–	–	–	–	–	–	–	11.60
Sig	1.00	1.00	1.00	0.83	1.00	1.00	1.00	1.00	1.00

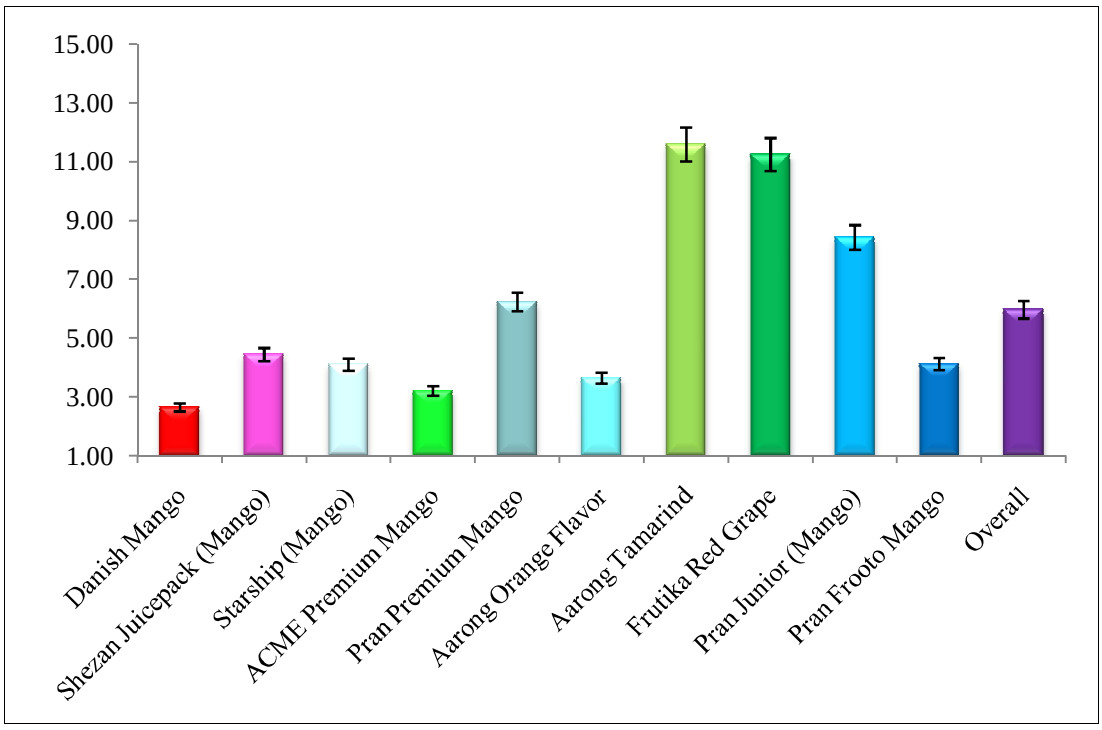

Figure 4.26: Comparison of reducing sugar status in different juices.

Reducing sugar found in juices is in the range of 11.60−2.65%. The highest amount (11.60%) is present in Aarong Tamarind Juice and the lowest amount (2.65%) present

in Danish Mango Juice. The amount of total sugar in juices is found from 14.41% to 10.06%. The highest amount (14.41%) is found in Frutika Red Grape Juice and the lowest amount (10.06 %) in ACME Premium Mango Juice.

Table 4.24: Non-reducing sugar present (%) in juices of different brands

Name of brands	**Non-reducing sugar (Mean ± SD)**
Danish Mango	11.32 ± 0.02
Shezan Juicepack (Mango)	7.13 ± 0.08
Starship (Mango)	6.66 ± 0.05
ACME Premium Mango	6.84 ± 0.02
Pran Premium Mango	7.18 ± 0.07
Aarong Orange Flavor	7.45 ± 0.12
Aarong Tamarind	2.53 ± 0.02
Frutika Red Grape	3.16 ± 0.03
Pran Junior (Mango)	4.37 ± 0.07
Pran Frooto Mango	7.25 ± 0.10

Table 4.24 (a): Descriptive statistics of non reducing sugar of different juices.

Name of brand	**Mean**	**SD**	**SE**	**95% CI for mean**		**Min**	**Max**
				lower bound	**upper bound**		
Danish Mango	11.32	0.02	0.01	11.27	11.37	11.30	11.34
Shezan Juicepack (Ma)	7.13	0.09	0.05	6.91	7.35	7.07	7.23
Starship (Ma)	6.66	0.05	0.03	6.53	6.79	6.60	6.69
ACME Premium Ma	6.84	0.02	0.01	6.79	6.89	6.82	6.86
Pran Premium Ma	7.18	0.07	0.04	7.01	7.35	7.10	7.23
Aarong Orange Flavor	7.45	0.12	0.07	7.14	7.76	7.31	7.55
Aarong Tamarind	2.53	0.02	0.01	2.48	2.58	2.51	2.55
Frutika Red Grape	3.16	0.03	0.02	3.09	3.23	3.14	3.19
Pran Junior (Ma)	4.37	0.07	0.04	4.20	4.54	4.30	4.44
Pran Frooto Ma	7.25	0.10	0.06	7.01	7.49	7.16	7.35
Overall	6.39	2.43	0.44	5.48	7.30	2.51	11.34

Description of H_0 and H_1 is almost same as earlier.

Table 4.24 (b): Analysis of variance for the variation study of non-reducing sugar of different juices.

	Source of variation	SS	DF	MS	F	P
% of Non-reducing sugar	Between groups	296.58	9	32.95	10699.09	0.00
	Within group	0.06	20	0.00	–	–
	Total	296.64	29	–	–	–

The statistical analyses are same as earlier of Table 4.24 (b).

Table 4.24 (c): Duncan's multiple range test of non reducing sugar for mean comparison of different juices

Duncan[a]

Brand	Subset for alpha = 0.05							
	1	2	3	4	5	6	7	8
Aarong Tamarind	2.53	–	–	–	–	–	–	–
Frutika Red Grape	–	3.16	–	–	–	–	–	–
Pran Junior (Mango)	–	–	4.37	–	–	–	–	–
Starship (Mango)	–	–	–	6.66	–	–	–	–
ACME Premium Mango	–	–	–	–	6.84	–	–	–
Shezan Juicepack (Mango)	–	–	–	–	–	7.13	–	–
Pran Premium Mango	–	–	–	–	–	7.18	–	–
Pran Frooto Mango	–	–	–	–	–	7.25	–	–
Aarong Orange Flavor	–	–	–	–	–	–	7.45	–
Danish Mango	–	–	–	–	–	–	–	11.32
Sig	1.00	1.00	1.00	1.00	1.00	0.05	1.00	1.00

Mean for group in homogeneous subsets are displayed. a. Uses harmonic mean sample size = 3.00

Table 4.24 (c) informs that within the group mean value of non-reducing sugar is insignificantly different and between the groups is significantly different and the interaction effect is found in the values of non-reducing sugar of the selected juices within the group-6.

From the Table 4.24 (c), it is seen that the studied juices are divided into eight groups. Here the significant difference of non-reducing sugar is observed between the groups and insignificant difference is within the group. The variation of the percentage of

non- reducing sugar of different brands of juices is described below and shown in Figure 4.27.

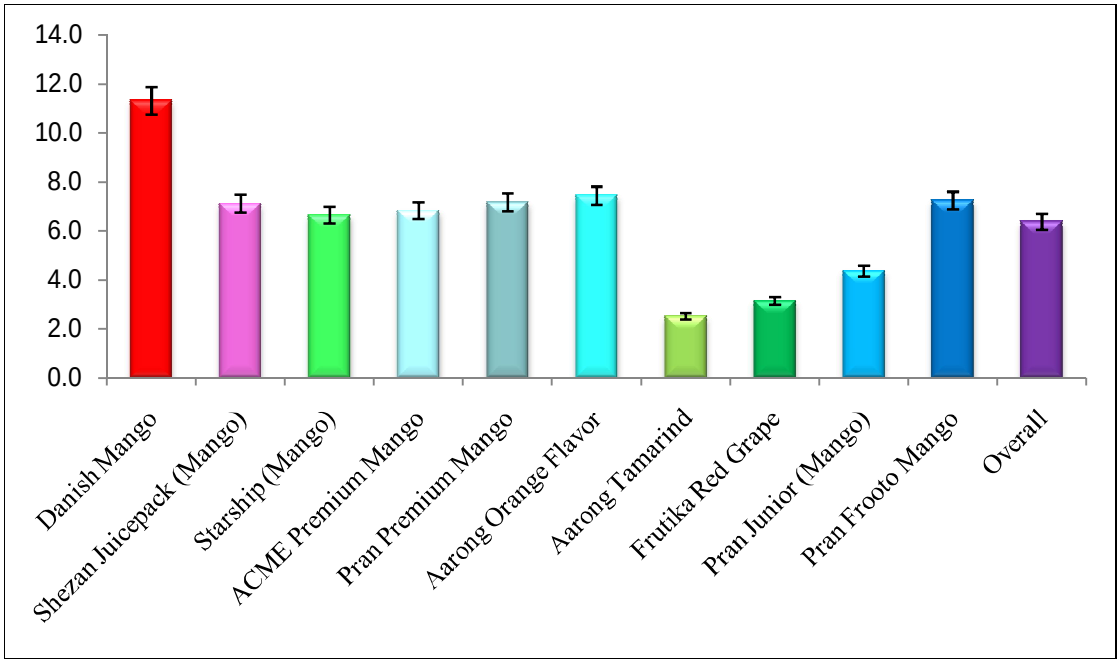

Figure 4.27: Comparison of non-reducing sugar status in different juices.

The amount of non-reducing sugar is found in the range of 2.53−11.32%. The highest amount (11.23%) is found in Danish Mango Juice and the lowest amount (2.53%) in Aarong Tamarind Juice.

Table 4.25: Total sugar detected (%) in juices of different brands

Name of brands	Total sugar (Mean ± SD)
Danish Mango	13.97 ± 0.05
Shezan Juicepack (Mango)	11.58 ± 0.04
Starship (Mango)	10.77 ± 0.02
ACME Premium Mango	10.06 ± 0.05
Pran Premium Mango	13.42 ± 0.09
Aarong Orange Flavor	11.10 ± 0.00
Aarong Tamarind	14.13 ± 0.06
Frutika Red Grape	14.41 ± 0.07
Pran Junior (Mango)	12.80 ± 0.07
Pran Frooto Mango	11.37 ± 0.09

Table 4.25(a): Descriptive statistics of total sugar of different juices.

Name of brand	Mean	SD	SE	95% CI for mean		Min	Max
				lower bound	upper bound		
Danish Mango	13.97	0.05	0.03	13.86	14.08	13.93	14.02
Shezan Juicepack (Ma)	11.58	0.04	0.03	11.47	11.69	11.55	11.63
Starship (Mango)	10.77	0.02	0.01	10.73	10.81	10.75	10.78
ACME Premium Ma	10.06	0.05	0.03	9.93	10.19	10.00	10.10
Pran Premium Mango	13.42	0.09	0.05	13.19	13.65	13.32	13.50
Aarong Orange Flavor	11.10	0.00	0.00	11.10	11.10	11.10	11.10
Aarong Tamarind	14.13	0.06	0.04	13.98	14.28	14.06	14.17
Frutika Red Grape	14.41	0.07	0.04	14.23	14.59	14.33	14.47
Pran Junior (Mango)	12.80	0.07	0.04	12.62	12.98	12.74	12.88
Pran Frooto Mango	11.37	0.09	0.05	11.16	11.58	11.28	11.45
Overall	12.36	1.52	0.28	11.79	12.93	10.00	14.47

Description of H_0 and H_1 is almost same as earlier.

Table 4.25 (b): Analysis of variance for the variation study of total sugar of different juices.

	Source of variation	SS	DF	MS	F	P
% of Total sugar	Between groups	66.72	9	7.41	2008.93	0.00
	Within group	0.07	20	0.00	–	–
	Total	66.79	29	–	–	–

Table 4.25 (b) indicates statistical information of the experimental results of total sugar of fruit juices and shows a significant difference of these fruit juices according to the variation results of total sugar and also describes that the level of significant, $P = 0.00 < 0.01$. So the variance of the result of total sugar of each juice is statistically significant at 1% level and we may conclude that the mean variation of the result of total sugar of each juice is significantly different.

Table 4.25 (c): Duncan's multiple range test of total sugar for mean comparison of different juices.

Duncan[a]

Brand	Subset for alpha = 0.05									
	1	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**
ACME Pr Ma	10.06	–	–	–	–	–	–	–	–	–
Starship (Ma)	–	10.77	–	–	–	–	–	–	–	–
Aarong Or Fla	–	–	11.10	–	–	–	–	–	–	–
Pran Fro Ma	–	–	–	11.37	–	–	–	–	–	–
Shezan Jpk(Ma)	–	–	–	–	11.58	–	–	–	–	–
Pran Jr (Ma)	–	–	–	–	–	12.80	–	–	–	–
Pran Pr Mango	–	–	–	–	–	–	13.42	–	–	–
Danish Mango	–	–	–	–	–	–	–	13.97	–	–
Aarong Tamar	–	–	–	–	–	–	–	–	14.13	–
Frutika Red Gr	–	–	–	–	–	–	–	–	–	14.41
Sig	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00

Mean for group in homogeneous subsets are displayed. a. Uses harmonic mean sample size = 3.00.

Fla = Flavor, Jr = Junior, Tamar = Tamarind, Gr = Grape, Fro = Frooto

The group mean value [Table 4.25 (c)] of total sugar is as earlier and the interaction effect is not found in the values of total sugar of the selected juices within the group.

The studied juices are divided into ten groups [Table 4.25 (c)]. A significant difference of total sugar is observed between the groups and insignificant difference is within the group.

The variation of the percentage of total sugar of different brands of juices is given below and shown in Figure 4.28.

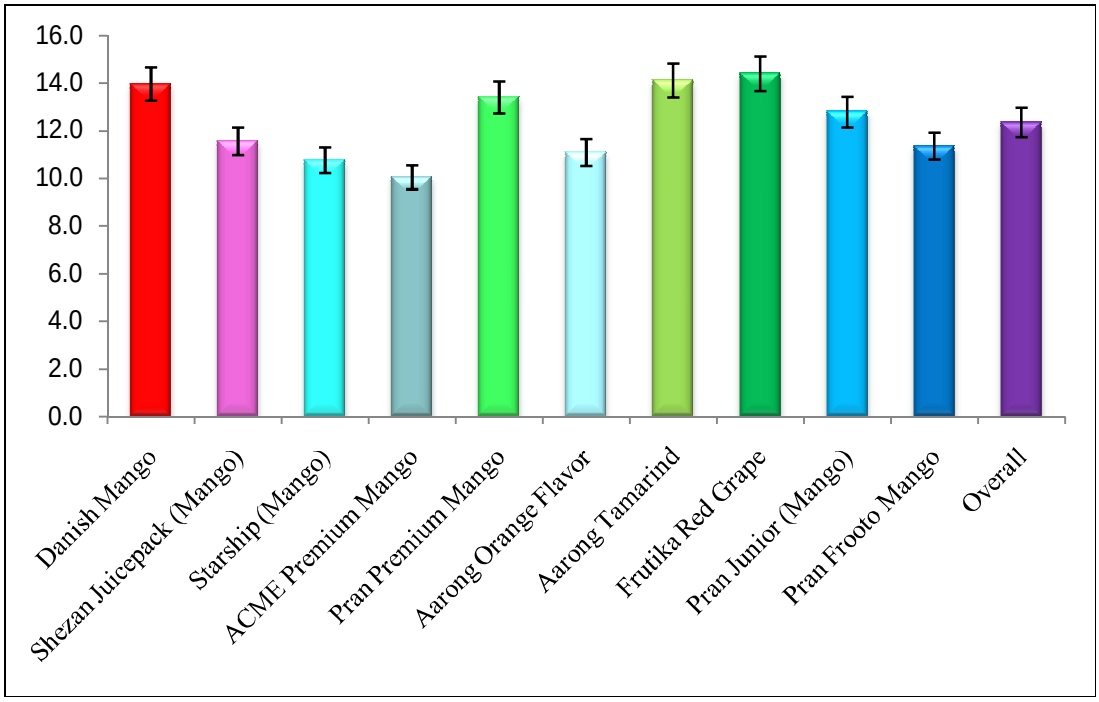

Figure 4.28: Comparison of total sugar status in different juices.

The amount of total sugar found in juices ranges from 14.41 to 10.06%. The highest amount (14.41%) was found in Frutika Red Grape Juice and the lowest amount (10.06 %) in ACME Premium Mango Juice.

The amount of total sugar in mango juices is 13.97 % (Danish Mango Juice), 11.58 % (Shezan Juicepac), 10.77% (Starship), 10.06% (ACME Premium Mango Juice), 13.42% (Pran Premium Mango Juice), 12.80 % (Pran Junior Juice) and 11.37% (Pran Frooto Mango Juice).

The average values are shown in the graphs (Figures 4.29 and 4.30) which indicate the total sugar and reducing sugar status in various types of jams, jellies and juices respectively.

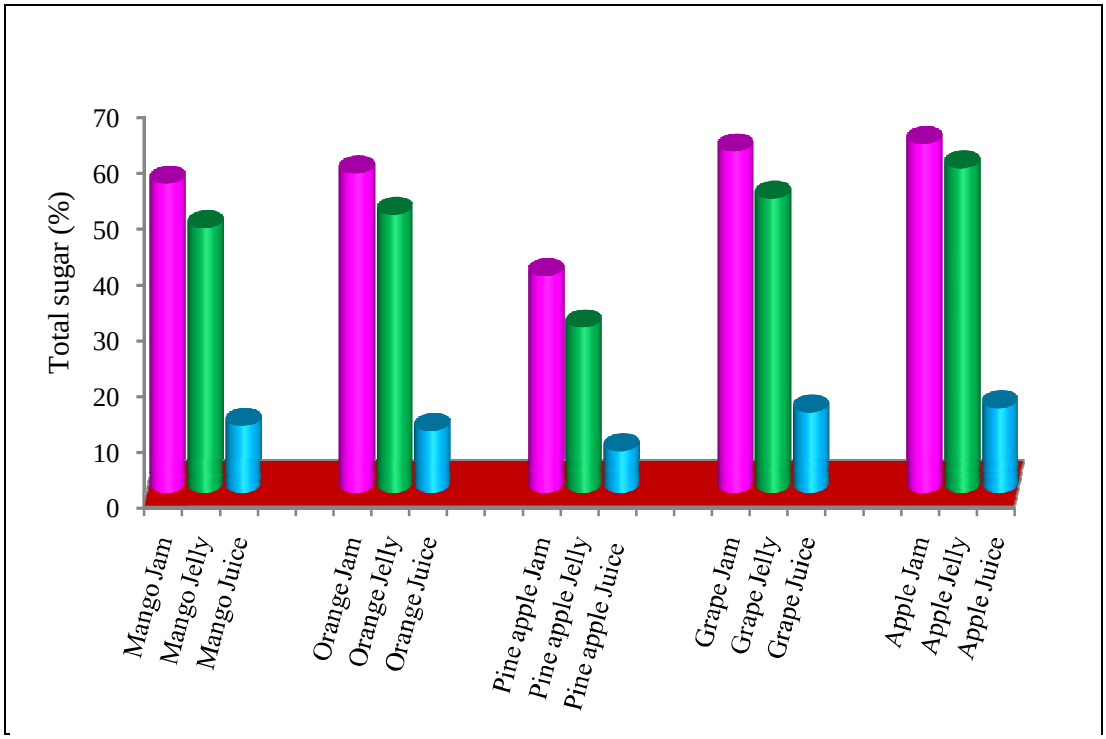

Figure 4.29: Comparison of total sugar status in jams, jellies and juices.

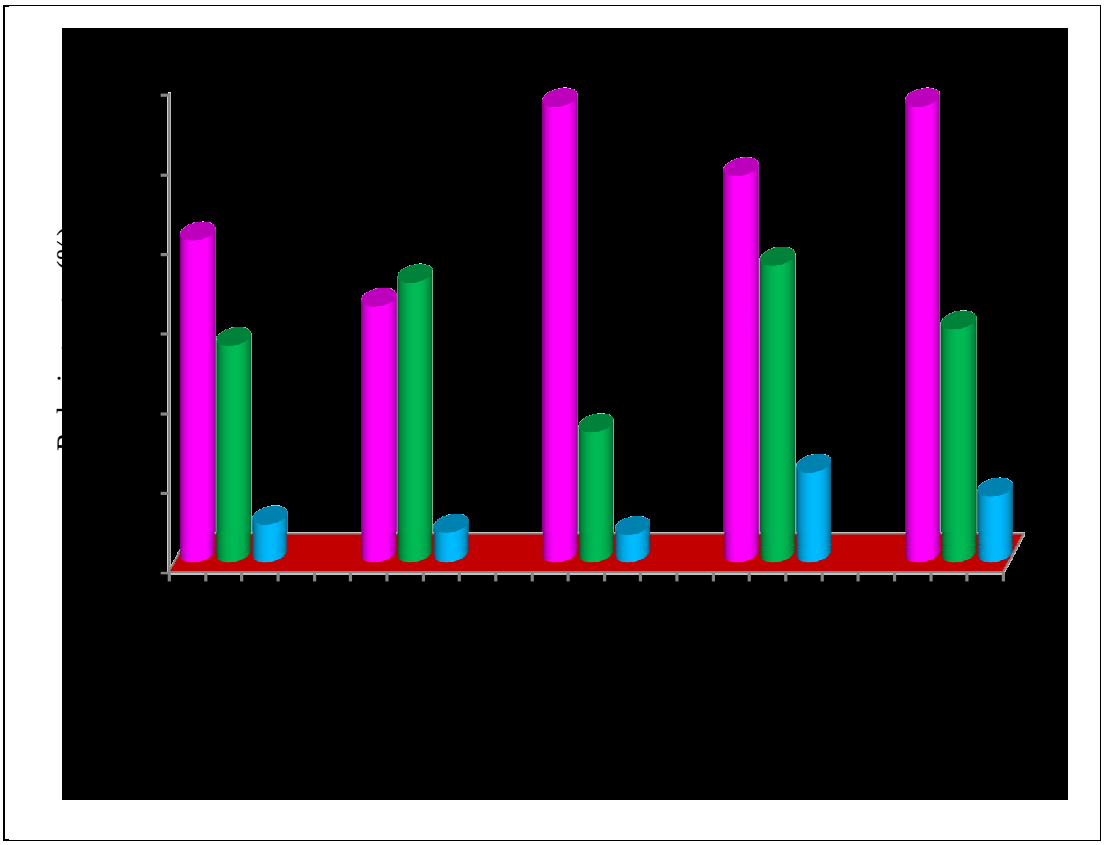

Figure 4.30: Comparison of reducing sugar status in jams, jellies and juices.

4.3.8 Estimation of the amount of trace and toxic metals in jams, jellies and juices.

4.3.8.a Estimation of the amount of trace and toxic metals in jams

The concentration of trace (Co and Zn) and toxic metals (Pb, Cd, Cr, As, and Ni) in jam, jelly and juice samples of selected brands are estimated by atomic absorption spectrophotometer and tabulated in the Table 4.26, 4.27 and 4.28 respectively.

From the Table 4.26, it is seen that the highest concentration of Co is found in Freswel Mango Jam (0.05 mg/kg) and the lowest in Shezan Mixed Jam (0.01 mg/kg). It is also obtained 0.03 mg/kg in Pran Mango Jam, 0.02 mg/kg in Nur Mixed Jam, 0.04 mg/kg in Agrokomerc Pineapple Jam, 0.03 mg/kg in Rajshahi Mango Jam, 0.04 mg/kg in Ahmad Mango Jam and 0.03 mg/kg in Nur Apple Jam. The Recommended value of Co is 29 µg/day or 0.029 mg/day [8]. So the experimental results of the selected jams show that the amount of Co is higher than the recommended value. Hence it is not safe to health. The amount of zinc found in jams ranges from 0.32 to 0.72 mg/kg. Rajshahi Mango Jam contains the highest amount of Zn (0.72mg/kg). Nur Mixed Jam and Ahmad Mango Jam contain the lower amount of Zn (0.32 mg/kg). Recommended value of zinc is 12 mg/day [9]. It shows that the concentration of Zn in jams is lower compared to the recommended value. So it is within the permissible limit. Hence the jam samples are safe but more Zn is required to improve the quality of the jam samples especially for our babies.

Table 4.26: Amount of trace and toxic metals in jam of different brands (mg/kg)

Sl. No	Sample	Co	Zn	Pb	Cd	Cr	As	Ni
1	Pran Mango Jam	0.03	0.43	0.09	0.01	0.13	0.39	BDL
2	Nur mixed Jam	0.02	0.32	0.07	0.01	0.26	0.14	BDL
3	Agrok Pineapple Jam	0.04	0.66	0.08	0.02	0.16	0.45	BDL
4	Rajshahi Mango Jam	0.03	0.72	0.04	0.01	0.22	0.18	BDL
5	Freswel Mango Jam	0.05	0.53	0.05	0.02	0.33	0.88	BDL
6	Ahmad mango Jam	0.04	0.32	0.03	0.01	0.27	0.06	BDL
7	Nur Apple Jam	0.03	0.42	0.12	0.02	0.25	0.36	BDL
8	Shezan mixed Jam	0.01	0.51	0.24	0.01	0.31	0.44	BDL

Agrok=Agrokomerc

BDL: Below Detection Limit

The highest concentration of Pb is found in Shezan Mixed Jam (0.24 mg/kg) and the lowest concentration in Ahmad Mango Jam (0.03 mg/kg) **[**Table 4.26]. Pb also found in Pran Mango Jam (0.09 mg/kg), Nur Mixed Jam (0.07 mg/kg), Agrokomerc Pineapple Jam (0.08 mg/kg), Rajshahi Mango Jam (0.04 mg/kg), Freswel Mango Jam (0.05 mg/kg) and Nur Apple Jam (0.12 mg/kg). Reference Health Standard (mg/kg body wt.) of lead is zero (0.0) [10]. Our findings reveal that the intake level of Pb is higher than the recommended value for consumption of jams as a part of diet of our daily life. Hence the jam samples are not safe for us.

The concentration of cadmium was found in jams ranges from 0.01 to 0.02 mg/kg. It is seen that the lower concentration ~ 0.01 mg/kg is found in Pran Mango Jam, Nur Mixed Jam, Rajshahi Mango Jam, Ahmad Mango Jam and Shezan Mixed Jam The higher concentration ~ 0.02 mg/kg is found in Agrokomerc Pineapple Jam, Freswel Mango Jam and Nur Apple Jam. Reference Health Standard of Cd is 0.025 mg/kg body wt. (Provisional Tolerable Monthly Intake) [10]. From our study it is seen that all the selected brands of jam contain the higher amount of Cd than the recommended value and these are not safe for us.

The concentration of chromium in jams of different brands available in the market ranges from 0.13 to 0.33 mg/kg. Cr found in Nur Mixed Jam, Agrokomerc Pineapple Jam, Rajshahi Mango Jam, Ahmad Mango Jam, Nur Apple Jam and Shezan Mixed Jam is 0.26, 0.16, 0.22, 0.27, 0.25 and 0.31 mg/kg respectively. Recommended value of chromium is 33 µg/day or 0.033 mg/day [11].

From our study it is found that all jam samples contain higher amount of Cr than the recommended value. Hence these jam samples are not safe for us. Excess intake of Cr can causes asthma, bronchitis, pneumonitis and inflammation of the liver.

The concentration of As in jams varies between 0.88 and 0.06 mg/kg. The highest concentration of As is present in Freswel Mango Jam (0.88 mg/kg). The Reference Health Standard (mg/kg body wt.) of As is zero (0.0) [12]. From the above data it is found that all jams contain higher amount of As than the recommended value. So these jam samples are not safe for us. Cancer of skin and lung, peripheral neuropathy, vertigo, gastrointestinal disturbances, muscle spasms etc. are usually caused by excessive arsenic intake.

Recommended level of nickel is 3−7 mg/kg [13]. It is seen that the concentration of nickel in jams is below the detection limit. So there is no toxic effect of Ni in our samples. The variations of concentration (mg/kg) of metals in jams of different brands are shown in Figures 4.31, 4.32, 4.33, 4.34, 4.35 and 4.36.

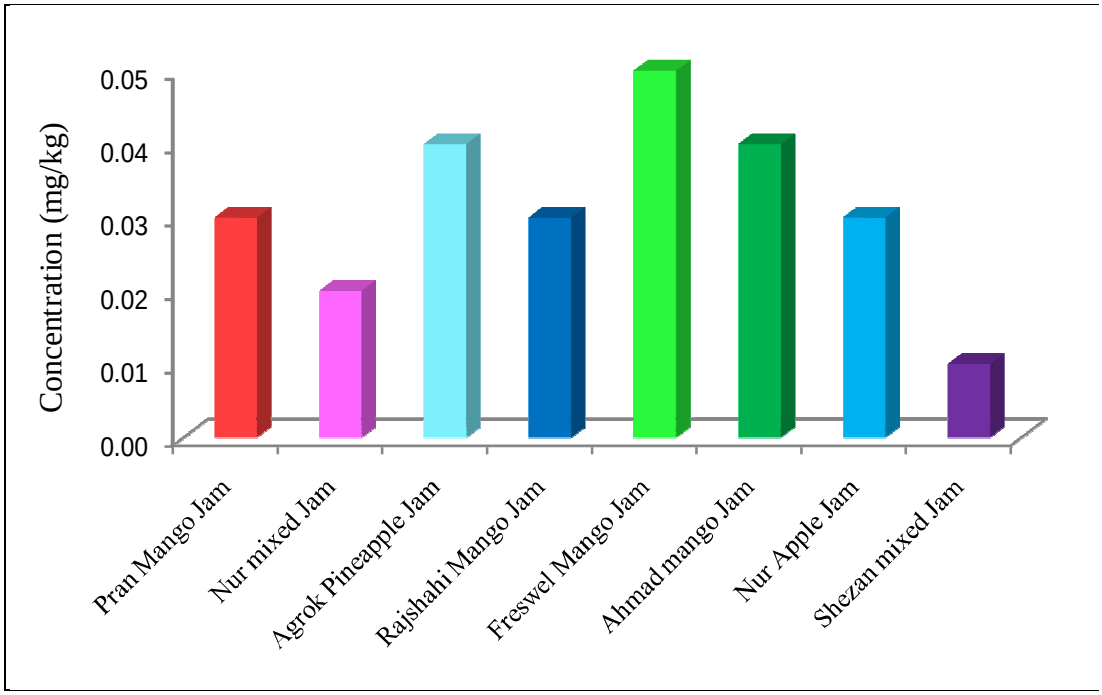

Figure 4.31: The variation of concentration of cobalt in jams of different brands.

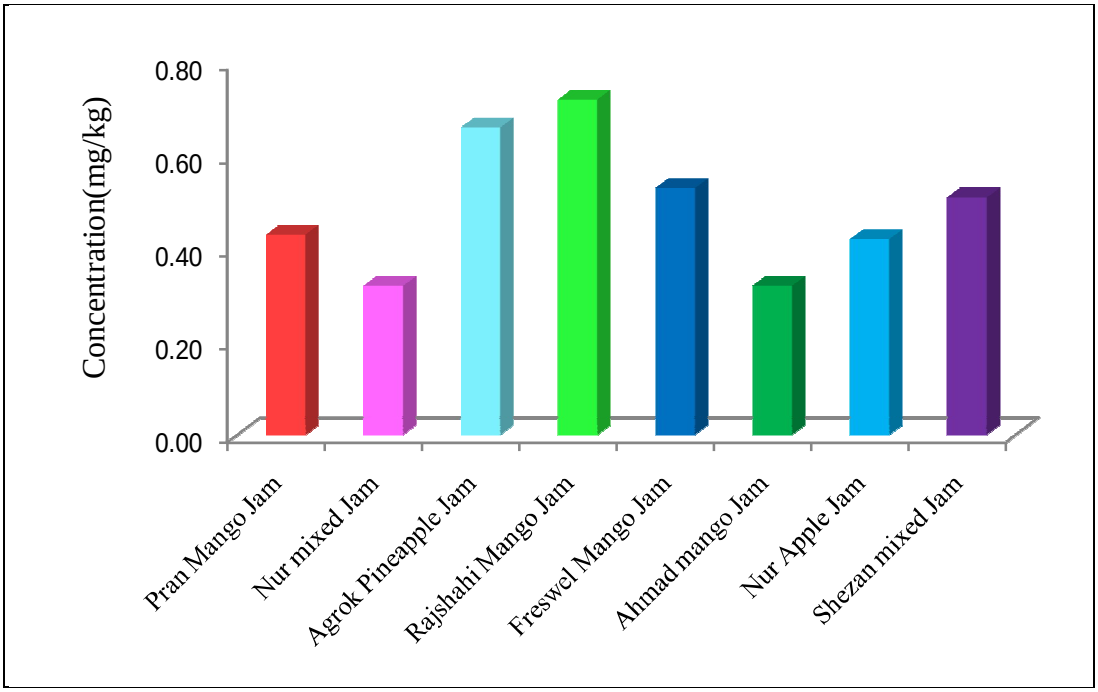

Figure 4.32: The variation of concentration of zinc in jams of different brands

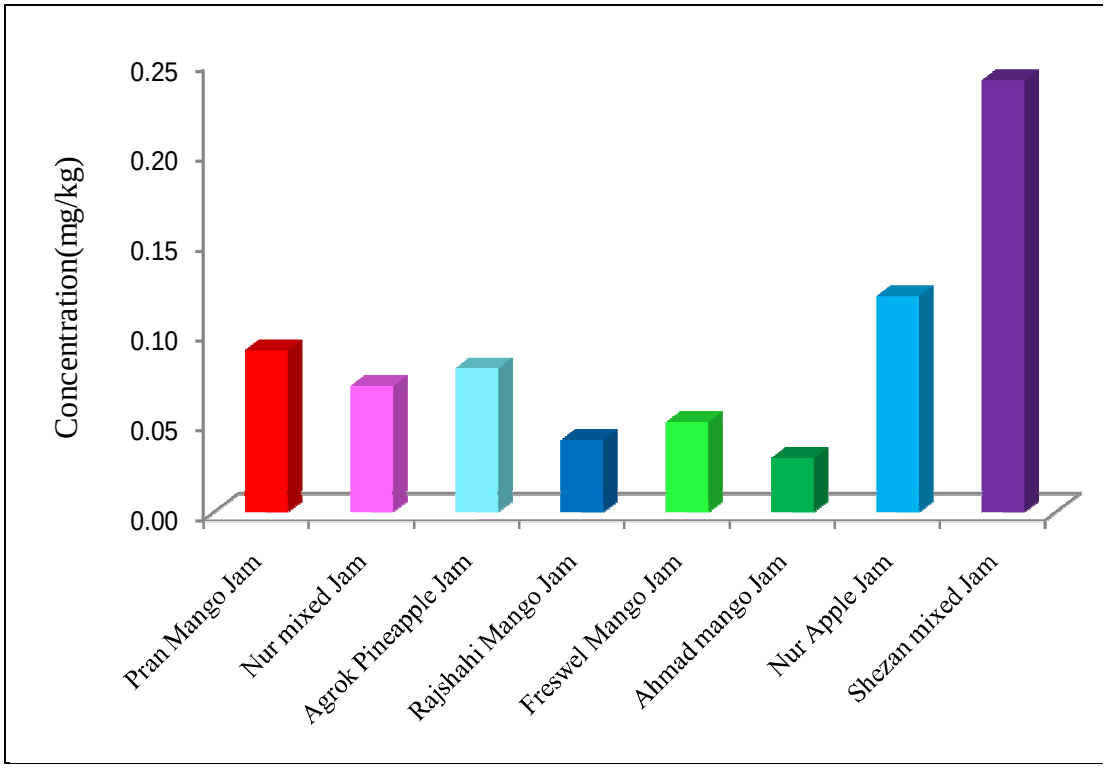

Figure 4.33: The variation of concentration of lead in jams of different brands.

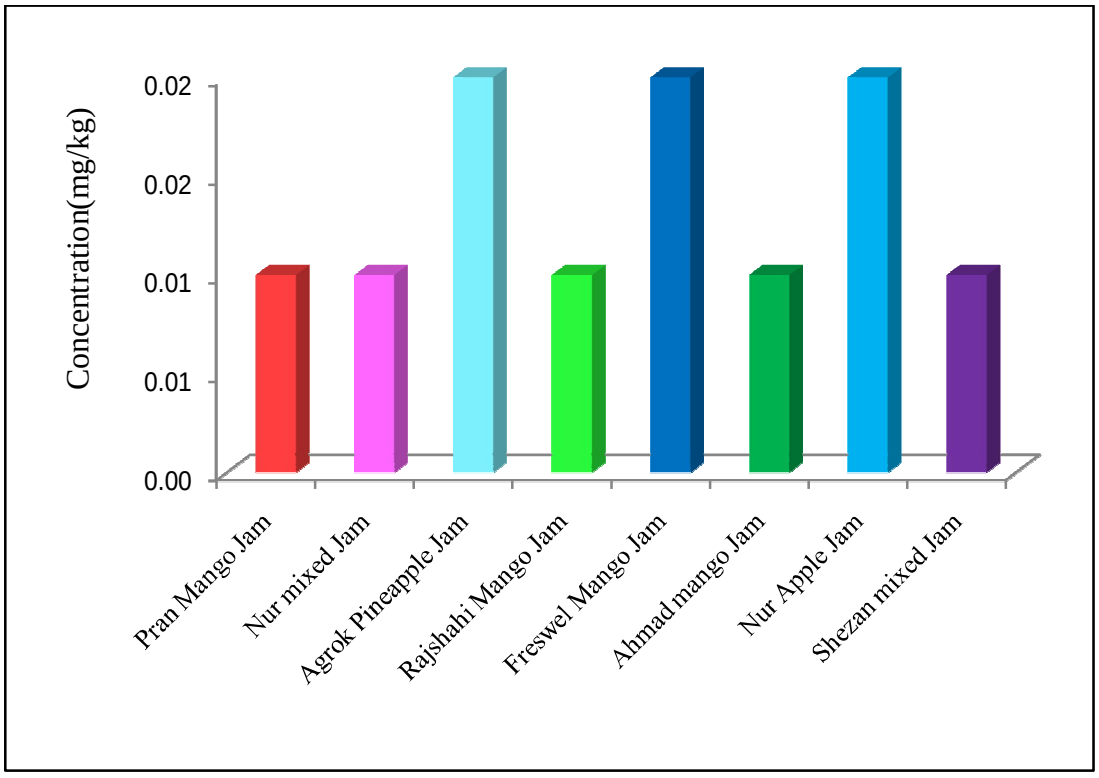

Figure 4.34: The variation of concentration of cadmium in jams of different brands.

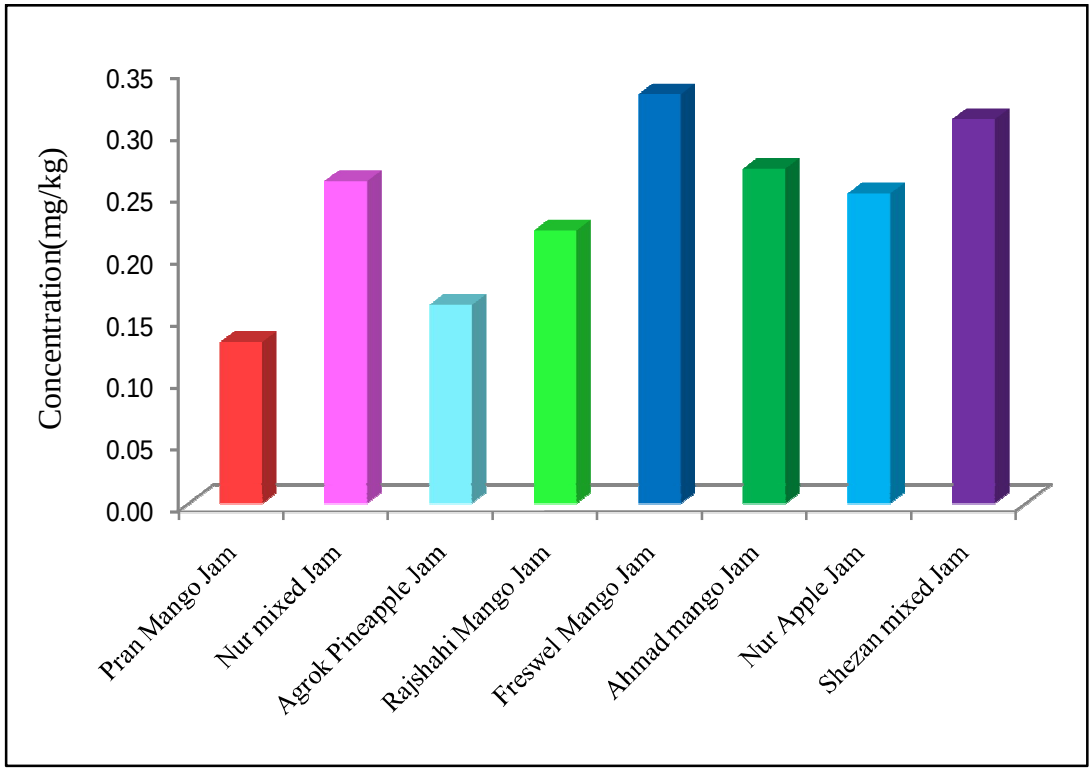

Figure 4.35: The variation of concentration of chromium in jams of different brands.

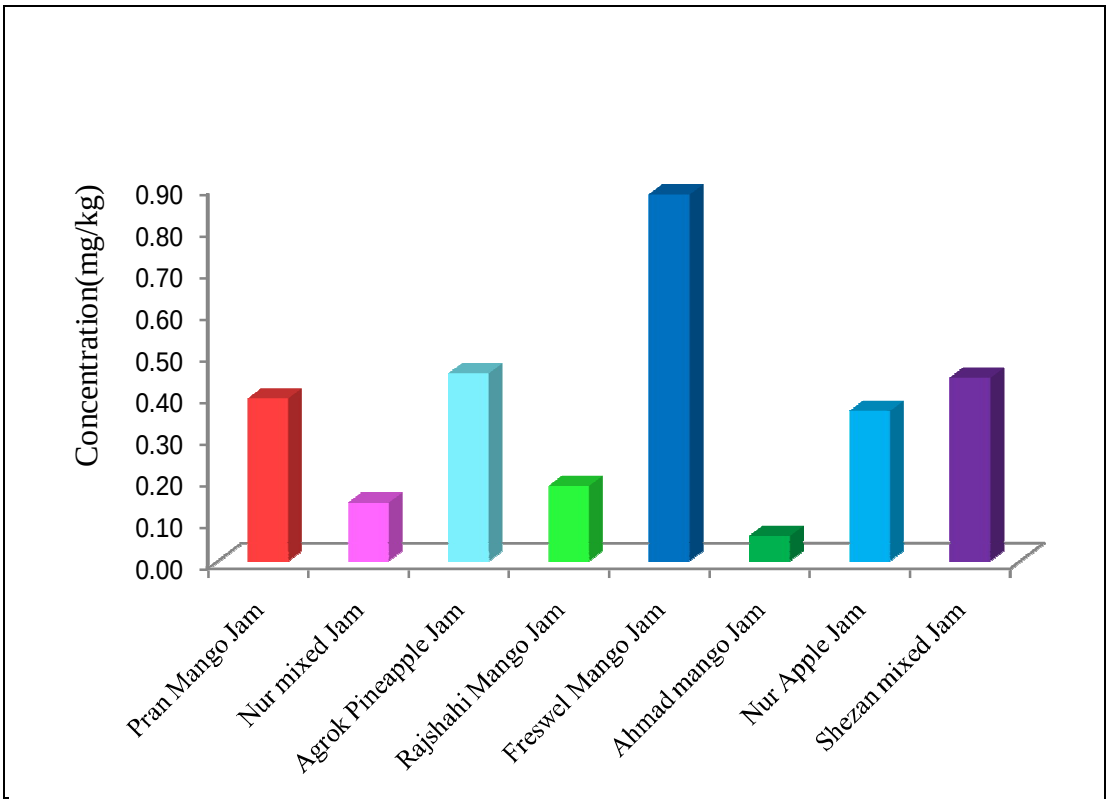

Figure 4.36: The variation of concentration of arsenic in jams of different brands.

4.3.8.b *Estimation of the amount of trace and toxic metals in jellies.*

Table 4.27: Amount of trace and toxic metals in jelly of selected brands (mg/kg)

SI. No	Sample	Co	Zn	Pb	Cd	Cr	As	Ni
1	Pran Apple Jelly	0.01	0.22	0.21	0.02	0.15	.008	0.12
2	BD Orange Jelly	0.05	0.34	0.16	0.03	0.14	.007	0.13
3	Pran Orange Jelly	0.03	0.18	0.25	0.02	0.16	.006	0.11
4	Ahmed Guava Jelly	0.07	0.46	0.17	0.01	0.15	.005	0.12
5	Friends Mango Jelly	0.03	0.35	0.30	0.02	0.17	.007	0.14
6	Ahmed Orange Jelly	0.04	0.27	0.28	0.02	0.15	.007	0.12
7	Friends Orange Jelly	0.06	0.16	0.23	0.02	0.16	.008	0.08
8	Agro. Mango Jelly	0.02	0.23	0.26	0.02	0.16	.006	0.11

From the Table 4.27, it is found that the highest concentration of Co is seen in Ahmed Guava Jelly (0.07 mg/kg) and the lowest in Pran Apple Jelly (0.01 mg/kg).

The amount of zinc found in jellies ranges from 0.16 to 0.46 mg/kg. Ahmed Guava Jelly contains the highest amount (0.46 mg/kg) and Friends Orange Jelly contains the lowest amount of Zn (0.16 mg/kg).

Table 4.27 indicates that the highest concentration of Pb is found in Friends Mango Jelly (0.30 mg/kg) and the lowest in BD Orange Jelly (0.16 mg/kg).

The concentration of cadmium in jellies ranges from 0.01 to 0.03 mg/kg. The lowest concentration of Cd is found in Ahmed Guava Jelly (0.01 mg/kg) and the highest in BD Orange Jelly (0.03 mg/kg).

The concentration of chromium in jellies of different brands available in the market is in the range of 0.14−0.17 mg/kg. The lowest concentration is present in BD Orange Jelly (0.14 mg/kg) and the highest amount in Friends Mango Jelly (0.17 mg/kg).

The concentration of As in jellies varies between 8×10^{-3} and 5×10^{-3} mg/kg. The higher concentration is present in Pran Apple Jelly and Friends Orange Jelly (8×10^{-3} mg/kg) and the lowest amount of As is found in Ahmed Guava Jelly (5×10^{-3} mg/kg).

It is seen that the concentration of nickel in jellies of selected brands varies between 0.08−0.15 mg/kg. The highest concentration is present in Friends Mango Jelly (0.15 mg/kg) and the lowest in Friends Orange Jelly (0.08 mg/kg).

The recommended values of Co, Zn, Pb, Cd, Cr, As and Ni are 0.029 mg/day, 12 mg/day, 0.0 mg/kg, 0.025 mg/kg, 0.033 mg/day, 0.0 mg/kg and 3−7 mg/kg respectively [8−13].

From the above study it is observed that all the jellies contain the higher amount of Pb and Cr than the recommended value. Hence these jellies are not safe for us.

The variations of concentrations (mg/kg) of metals in jellies of different brands are shown in Figures 4.44, 4.45, 4.46, 4.47, 4.48, 4.49 and 4.50.

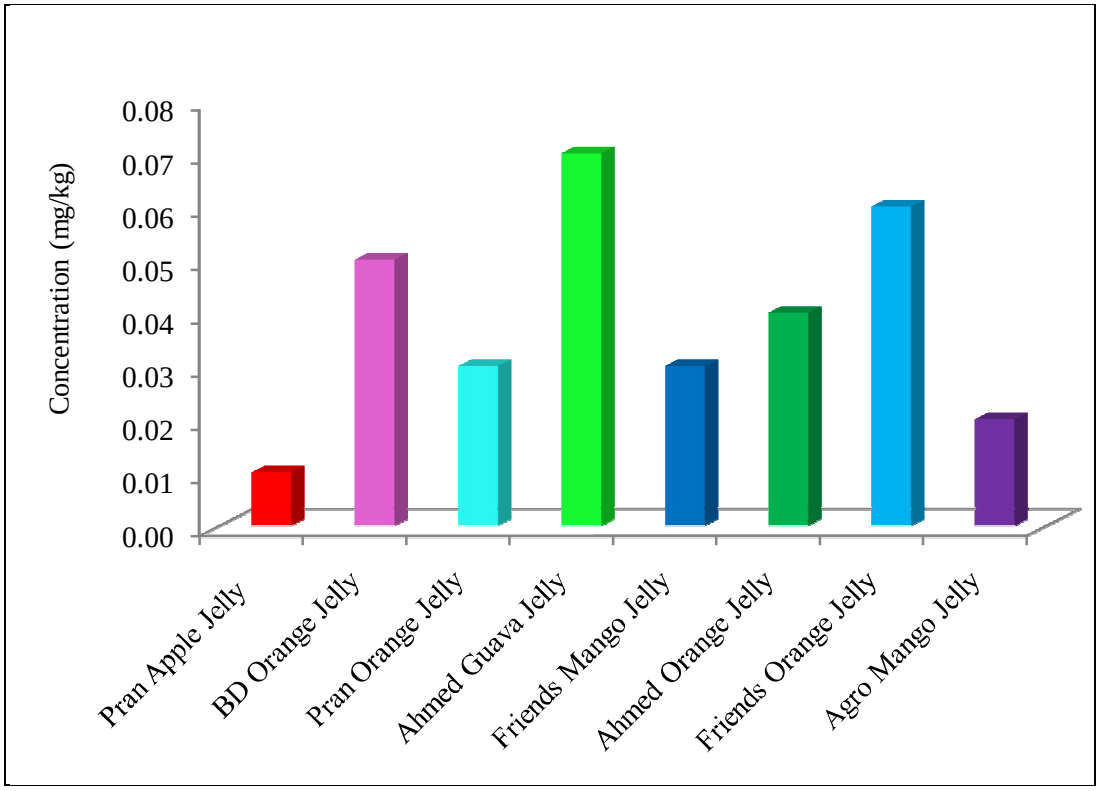

Figure 4.37: The variation of concentration of cobalt in jellies of different brands.

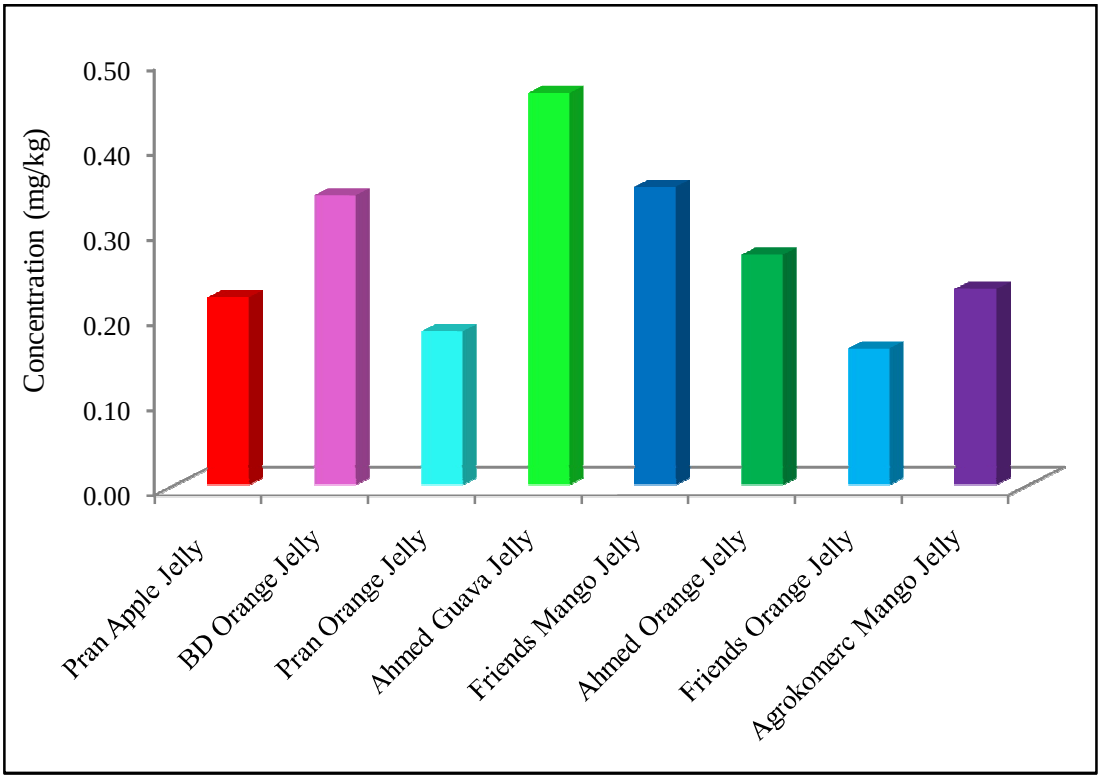

Figure 4.38: The variation of concentration of zinc in jellies of different brands.

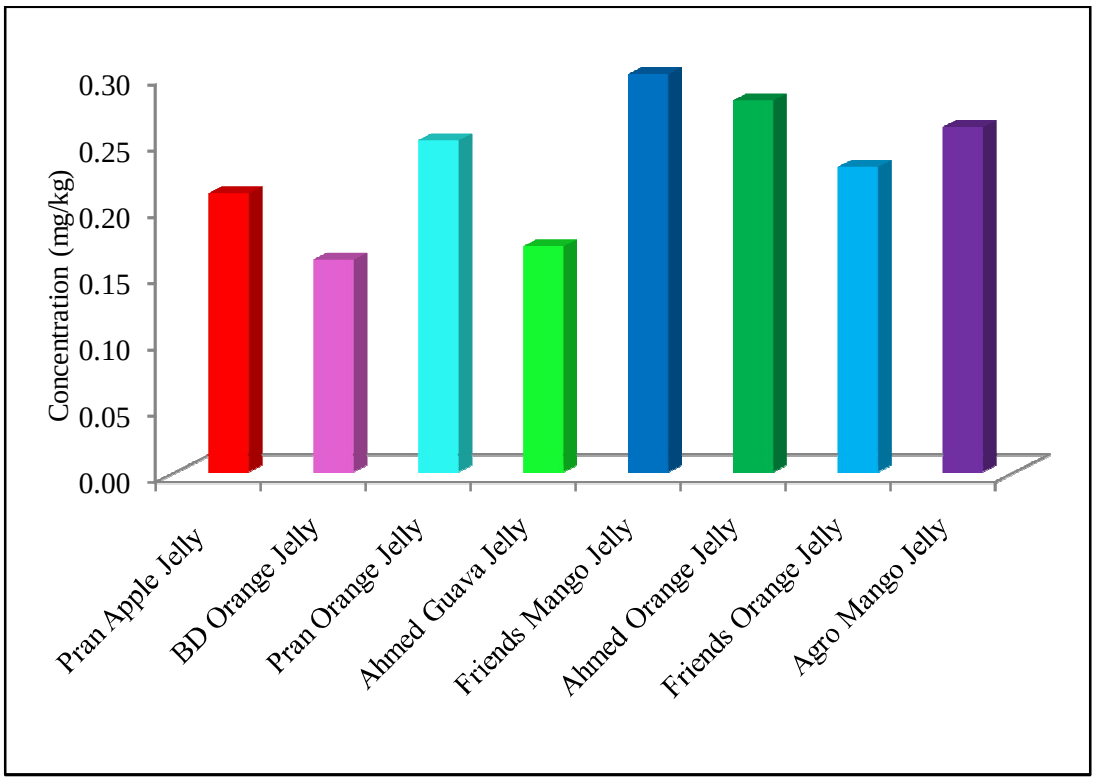

Figure 4.39: The variation of concentration of lead in jellies of different brands.

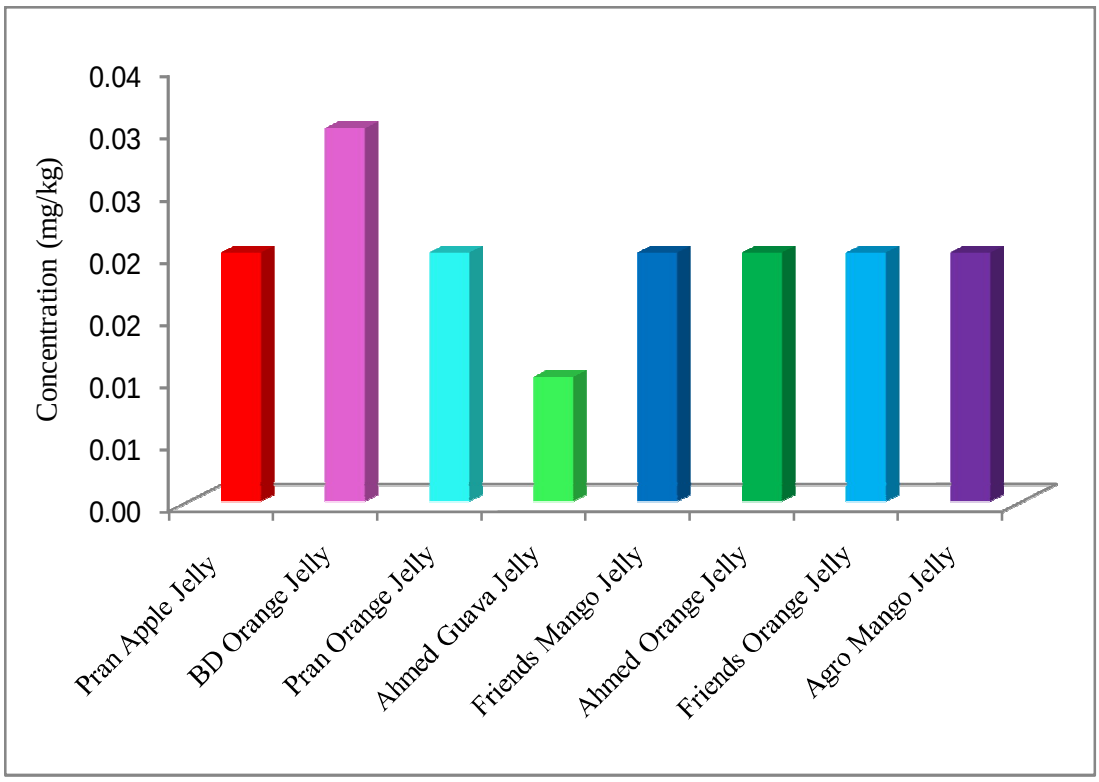

Figure 4.40: The variation of concentration of cadmium in jellies of different brands.

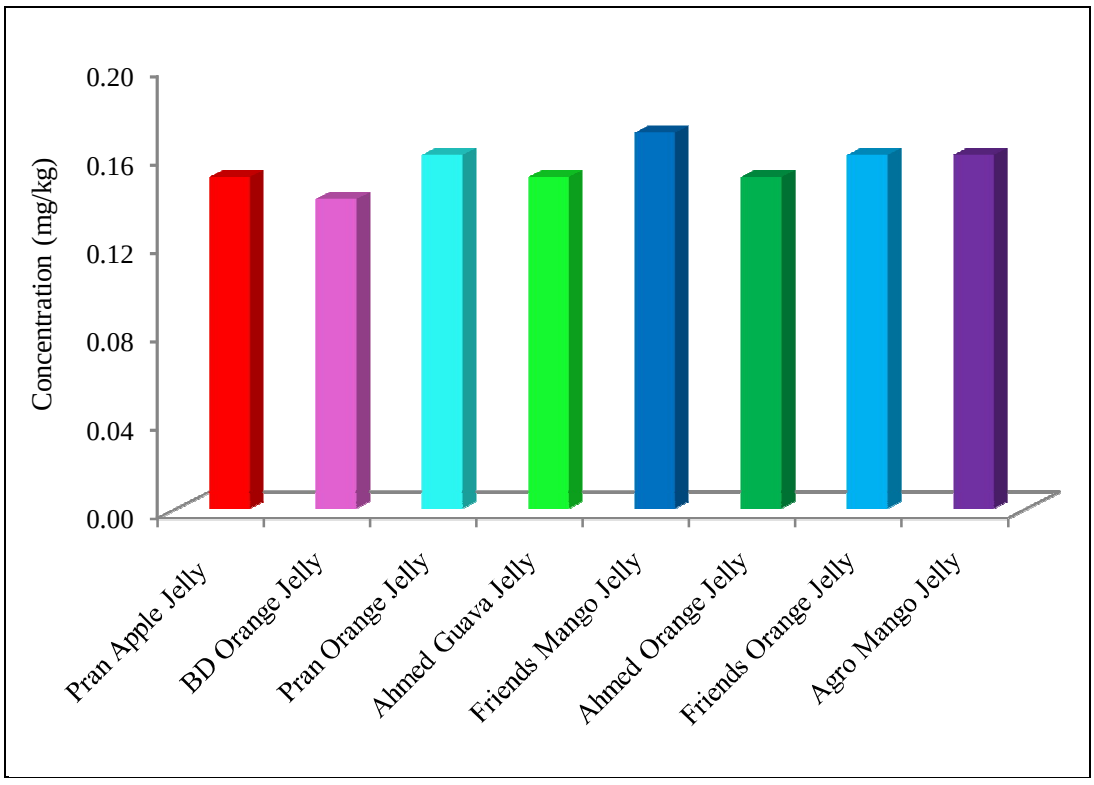

Figure 4.41: The variation of concentration of chromium in jellies of different brand

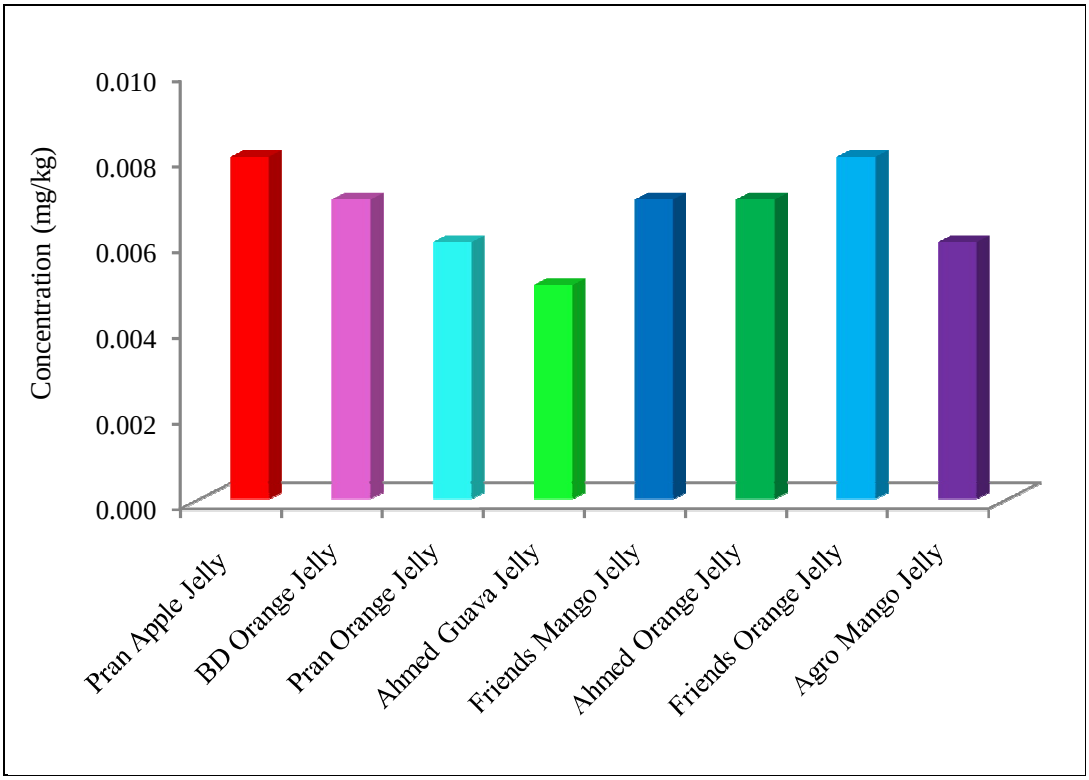

Figure 4.42: The variation of concentration of arsenic in jellies of different brands.

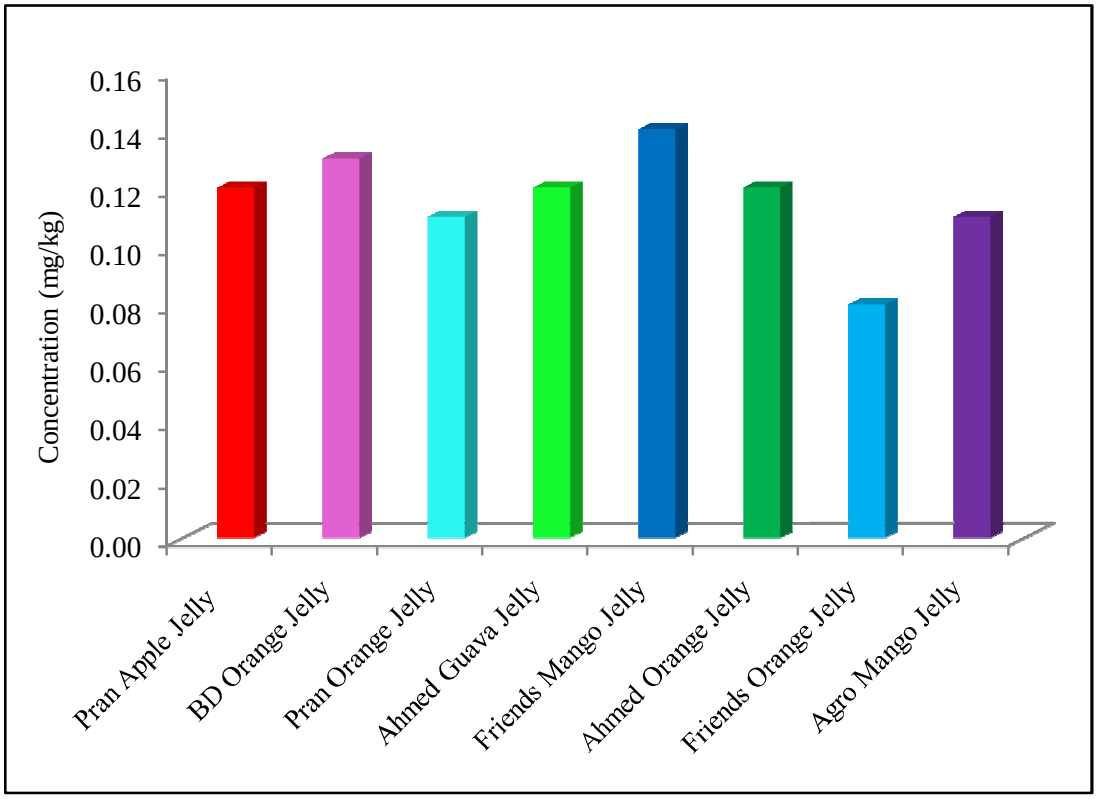

Figure 4.43: The variation of concentration of nickel in jellies of different brands.

4.3.8.c Estimation of the amount of trace and toxic metals in juices

The Table 4.28 shows that Frutika Red Grape Juice contains the highest amount of Co (0.09 mg/kg) and Danish Mango Juice contains the lowest amount (0.03 mg/kg).

The amount of zinc found in juices ranges from 0.12 to 0.28 mg/kg. Aarong Orange Flavor contains the highest amount of Zn (0.28 mg/kg) and Aarong Tamarind Juice contains the lowest amount (0.14 mg/kg). The recommended values of Co, Zn, Pb, Cd, Cr, As and Ni are 0.029 mg/day, 12 mg/day, 0.0 mg/kg, 0.025 mg/kg, 0.033 mg/day, 0.0 mg/kg and 3−7 mg/kg respectively [8−13].

Table 4.28: Amount of trace and toxic metals in juice of selected brands (mg/kg)

SI. No	Sample	Co	Zn	Pb	Cd	Cr	As	Ni
1	Danish Mango Juice	0.03	0.20	0.11	0.02	0.08	.004	0.02
2	Shezan Juicepac (Mango)	0.05	0.18	0.10	0.01	0.18	.005	0.01
3	Starship (Mango)	0.05	0.16	0.16	0.01	0.17	.004	0.05
4	ACME Pr. Mango Juice	0.04	0.23	0.18	0.03	0.12	.006	0.04
5	Pran Pr. Mango Juice	0.05	0.23	0.20	0.01	0.14	.005	0.08
6	Aarong Orange Flavor	0.04	0.28	0.14	0.01	0.14	.006	0.05
7	Aarong Tamarind Juice	0.06	0.12	0.23	0.02	0.12	.004	0.12
8	Frutika Red Grape Juice	0.09	0.27	0.15	0.02	0.13	.004	0.07
9	Pran Jr. Juice (Mango)	0.08	0.18	0.11	0.04	0.13	.007	0.11
10	Pran Frooto Mango Juice	0.05	0.14	0.13	0.01	0.14	.003	0.08

Pr = Premium, Jr = Junior

Table 4.28 Indicates that Aarong Tamarind Juice contains the highest concentration of Pb (0.23 mg/kg) and Shezan Juicepac (Mango) contains the lowest amount (0.10 mg/kg). All the juices samples contain the higher amount of Pb than the recommended value. So these juices are harmful and should be avoided.

The concentration of cadmium (Cd) in juices ranges from 0.01 to 0.04 mg/kg. It is seen that Shezan Juicepac (Mango), Pran Premium Mango Juice, Aarong Orange Flavor, Pran Frooto Mango Juice and Starship (Mango) contain the lowest

concentration of Cd and that is 0.01 mg/kg. Pran Junior Juice (Mango) contains the highest amount of Cd which is 0.04 mg/kg.

The concentration of chromium in juices of different brands available in the market ranges from 0.08 to 0.18 mg/kg. The lowest concentration was found in Danish Mango Juice (0.08 mg/kg) and the highest amount was found in Shezan Juicepac (Mango) (0.18 mg/kg).

The concentration of As in juices varies between 7×10^{-3} and 3×10^{-3} mg/kg. The highest concentration is present in Pran Junior Juice (7×10^{-3} mg/kg) and the lowest amount in Pran Frooto Mango Juice (3×10^{-3} mg/kg). The concentration of Ni in juices of selected brands varies between 0.01 and 0.12 mg/kg. The highest concentration was found in Aarong Tamarind Juice (0.12 mg/kg) and the lowest amount in Shezan Juicepac (90.01 mg/kg).

The effects of heavy metal toxicity studies confirm that it can directly influence the behavior by impairing mental and neurological function. Influence may take place in neurotransmitter production and utilization, altering numerous metabolic body processes etc. Toxic metals can hamper the normal function of different organs of our body, such as blood vessel and cardiovascular system, detoxification pathways, endocrine glands, energy production pathways, enzymatic systems, gastrointestinal tracts, immune, nervous, urinary and reproductive system [14].

The variations of concentration of metals in juices of different brands are shown in Figures 4.44, 4.45, 4.46, 4.47, 4.48, 4.49 and 4.50.

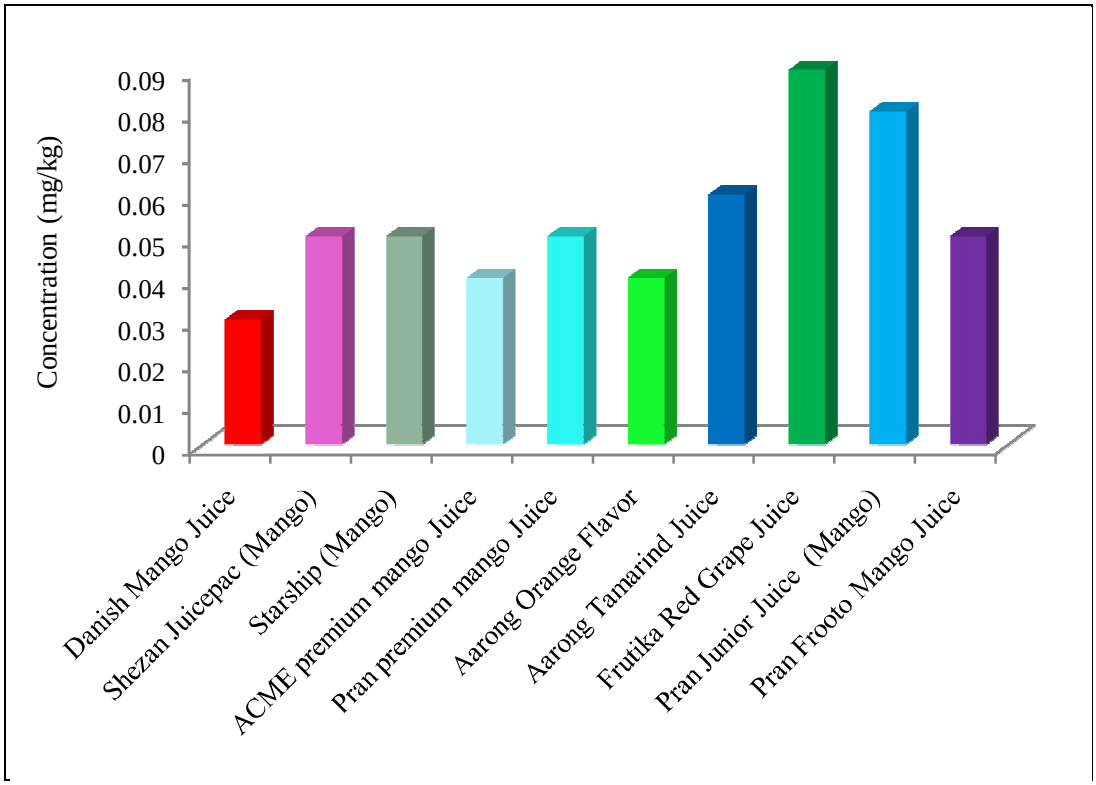

Figure 4.44: The variation of concentration of cobalt in juice of different brands.

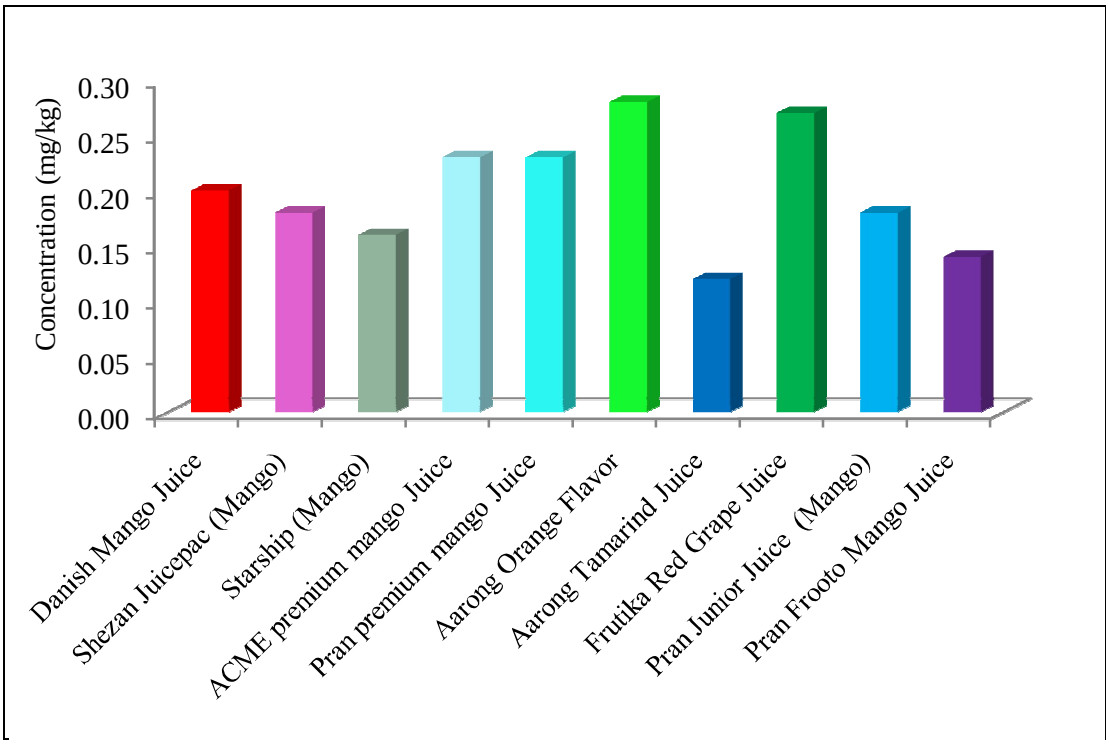

Figure 4.45: The variation of concentration of zinc in juice of different brands.

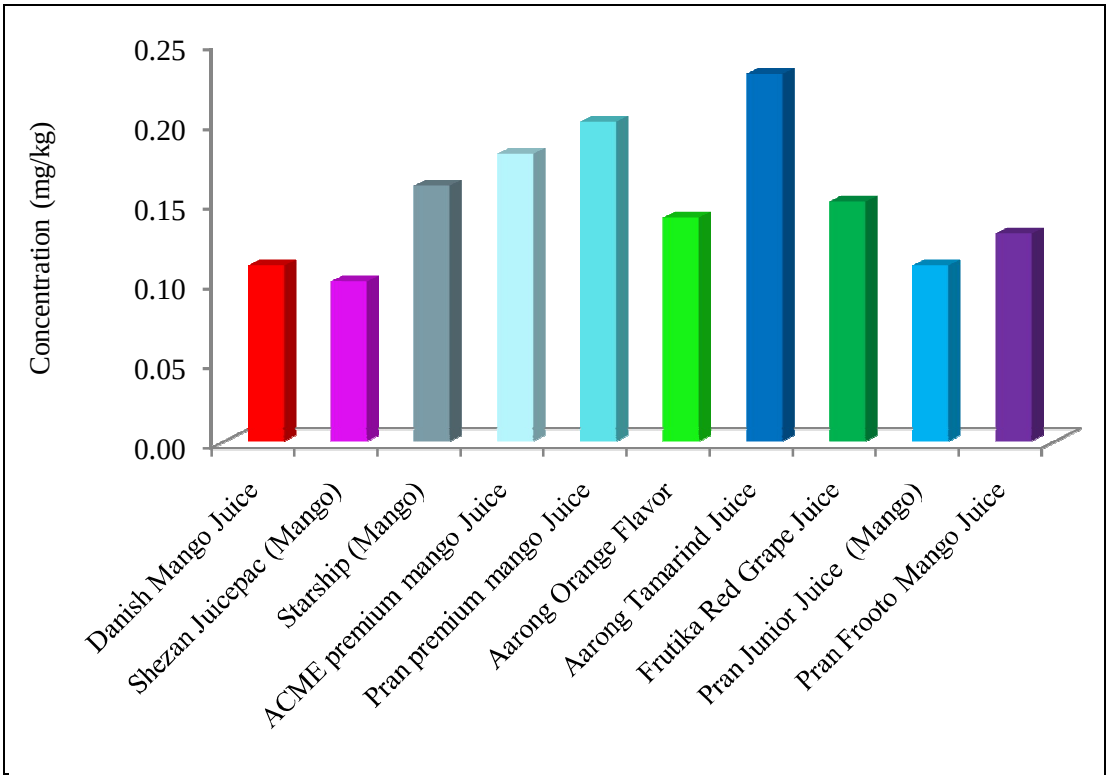

Figure 4.46. The variation of concentration of lead in juice of different brands.

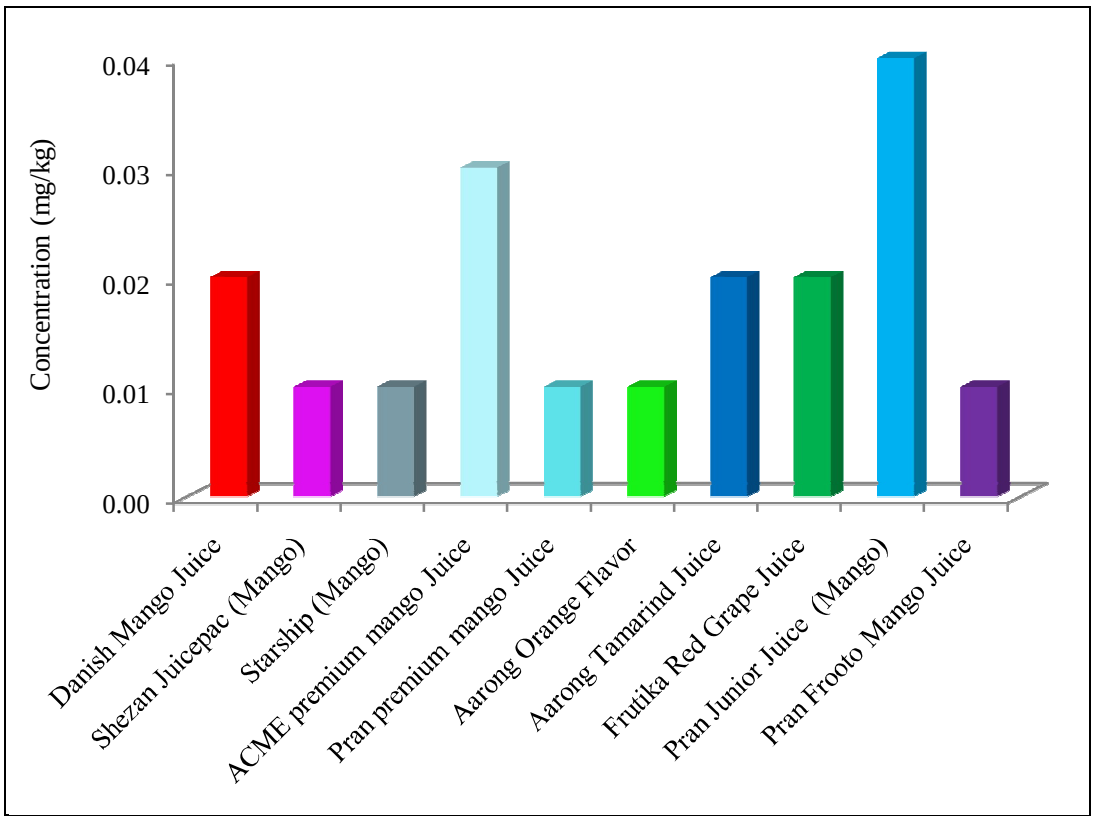

Figure 4.47: The variation of concentration of cadmium in juice of different brands.

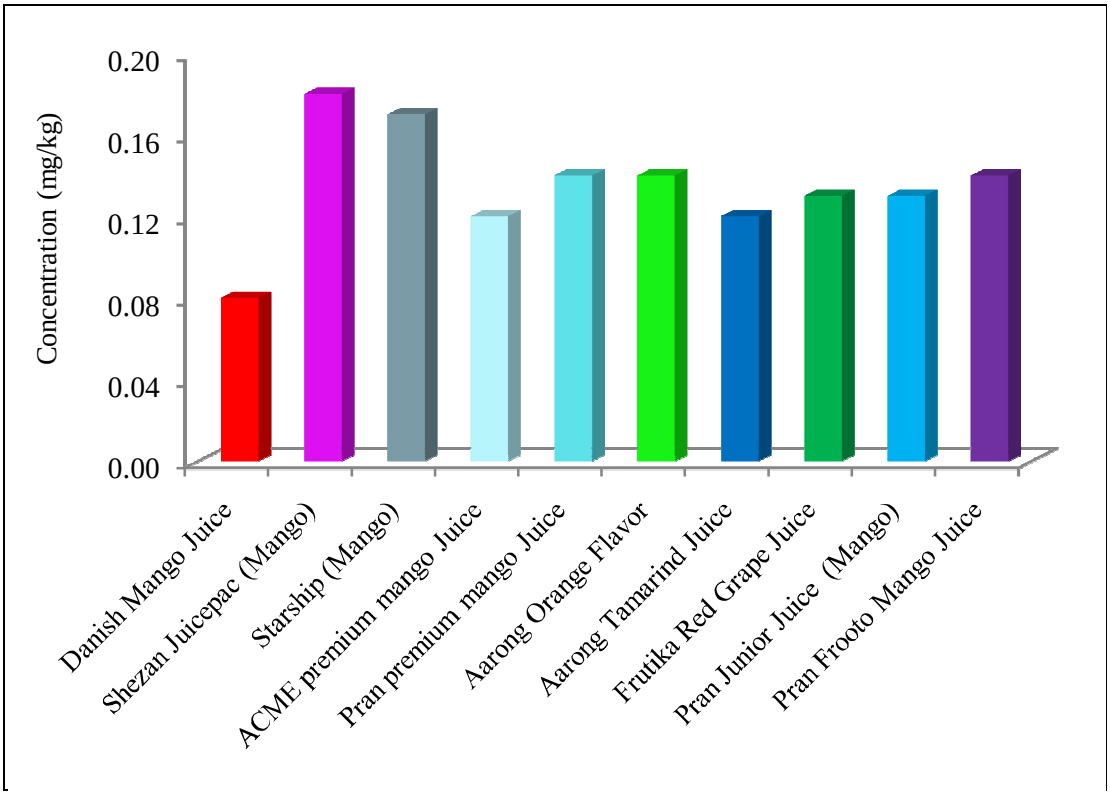

Figure 4.48: The variation of concentration of chromium in juice of different brands.

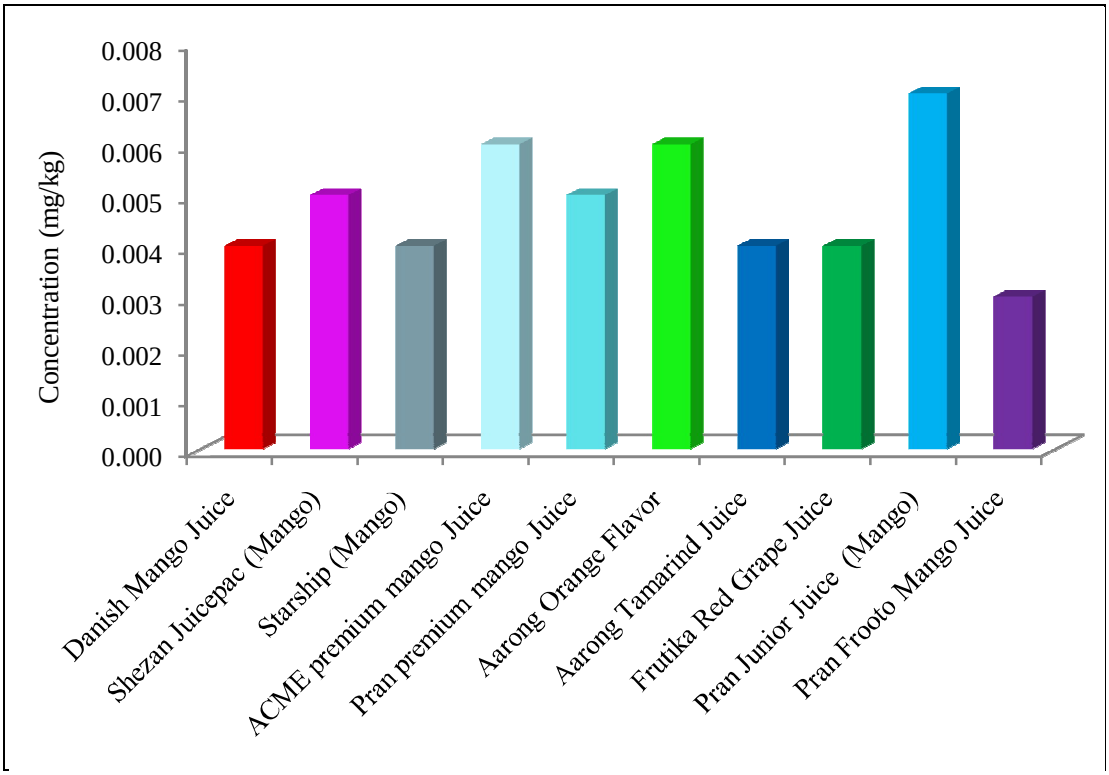

Figure 4.49: The variation of concentration of arsenics in juice of different brands.

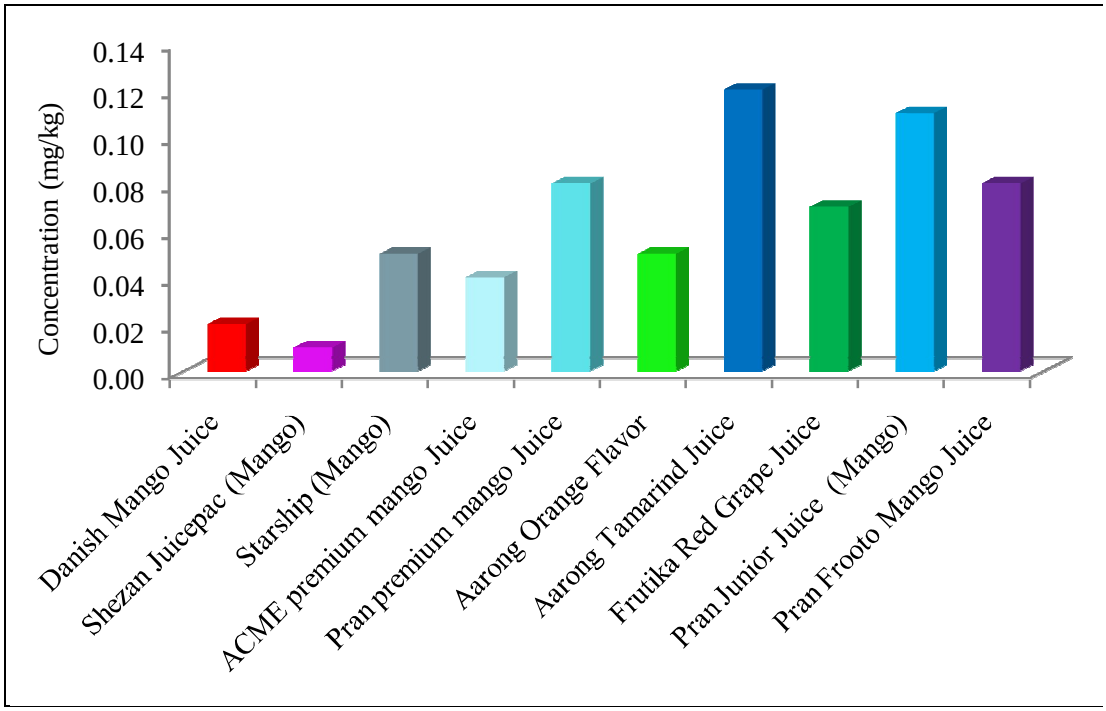

Figure 4.50: The variation of concentration of nickel in juice of different brands.

4.3.9. Estimation of the amount of essential metals (Na, K, Ca and Mg) in jams, jellies and juices of different brands.

The concentrations of Na, K, Ca and Mg in jams, jellies and juices of different brands are estimated by atomic absorption spectrophotometer and given in Table 4.29, 4.30 and 4.31.

Table 4.29: Estimation of the amounts of sodium, potassium, calcium and magnesium in different jams (mg/kg)

SI. No	Sample	Na	K	Ca	Mg
1	Pran Mango Jam	2.3	11.4	28.2	0.02
2	Nur Mixed Jam	3.7	24.3	15.8	7.6
3	Agro Pineapple Jam	1.9	9.9	8.6	4.3
4	Rajshahi Mango Jam	3.0	21.6	10.0	0.02
5	Freswel Mango Jam	5.2	200.2	14.2	29.1

mg/kg = milligram per kilogram of edible portion

Table 4.29 indicates the amounts of Na, K, Ca and Mg in jams of different brands. Five jams of different brands have been analyzed and the metals were estimated.

Sodium found in jams of different brands ranges from 1.9 to 5.2 mg/kg. The highest amount of Na (5.2 mg/kg) is present in Freswel Mango Jam and the lowest amount (1.9 mg/kg) in Agrokomerc Pineapple Jam. Sodium in the other three jams is as follows: Pran Mango Jam is 2.3 mg/kg, Nur Mixed Jam is 3.7 mg/kg and Rajshahi Mango Jam is 3.0 mg/ kg. Recommended Daily Intake of sodium is 1500 mg [8]. Hence the amount of Na in jams is within the recommended level and shows no adverse effect in human body.

Potassium found in jams ranges from 9.9 to 200.2 mg/kg. The highest amount of K (200.2 mg/kg) is found in Freswel Mango Jam and the lowest amount (9.9 mg/kg) is in Agrokomere Pineapple Jam. Pran Mango Jam, Nur Mixed Jam and Rajshahi Mango Jam contain 11.4, 24.3 and 21.6 mg/kg of potassium respectively. Recommended Daily Intake of K is 3800 mg [8]. The amount of K in all of the jams is within the recommended level except Freswel Mango Jam. So other four jams show no adverse effect in human body.

Recommended Daily Intake of Ca is 600 mg [9]. The amount of calcium present in jams ranges from 8.6 to 28.2 mg/kg. The highest amount of Ca was found in Pran Mango Jam (28.2 mg/kg) and the lowest amount in Agrokomerc Pineapple Jam (8.6 mg/kg). Ca found in Nur Mixed Jam, Rajshahi Mango Jam and Freswel Mango Jam is 15.8, 10.0 and 14.2 mg/kg respectively. All the brands are safe except Freswel Mango Jam.

Recommended Daily Intake of Mg is 340 mg [9]. The amount of magnesium present in jams under study ranges from 0.02 to 29.1 mg/kg. The highest amount of magnesium is detected in Freswel Mango Jam (29.1 mg/kg) and the lowest amount (0.02 mg/kg) in Rajshahi Mango Jam and Pran Mango Jam. Magnesium found in Nur Mixed Jam and Agrokomerc Pineapple Jam is 7.6 and 4.3 mg/kg respectively. Hence all brands are safe.

Table 4.30: Amount of sodium, potassium, calcium and magnesium content in jellies (mg/kg)

SI. No	Sample	Na	K	Ca	Mg
1	Pran Apple Jelly	2.2	19.3	11.4	9.7
2	Ahmed Orange Jelly	5.5	99.1	21.2	0.02
3	Friends Orange Jelly	4.2	12.1	09.8	0.6
4	Agro. Mango Jelly	2.5	42.1	11.3	0.02
5	Shezan Sw.Orange Jelly	4.1	38.2	62.2	03.1

Sw. = sweet, Agro = Agrokomerc, mg/kg = milligram per kilogram of edible portion.

The amount of Na, K, Ca and Mg in jellies of different brands has been tabulated in the Table 4.30. The analyzed values of sodium, potassium, calcium and magnesium in mg/kg have been given in the Table 4.30.

Table 4.31 contains the determined values of Na, K, Ca and Mg present in juices.

The amount of Na found in jelly and juice samples is less than the Recommended Daily Intake level. So these brands are not harmful. The determined value of K in Ahmed Orange Jelly is quite higher and the same metal present in all the samples (jelly and juice) are within the permissible limit. Mg content in jelly and juice samples are almost within the recommended level.

Table 4.31: Amount of sodium, potassium, calcium and magnesium found in juices (mg/kg)

SI. No	Sample	Na	K	Ca	Mg
1	Danish Mango Juice	2.2	25.2	30.3	1.7
2	Shezan Juicepac (Mango)	2.1	19.8	48.3	0.8
3	Starship (Mango)	1.3	19.8	4.3	01.5
4	Acme Pr Mango Juice	1.1	8.0	8.9	0.5
5	Pran Pr Mango Juice	1.3	28.7	20.3	2.0
6	Aarong Orange Flavor	4.2	38.0	63.0	1.0
7	Aarong Tamarind Juice	4.5	16.0	25.3	1.1
8	Frutika Red Grape Juice	2.3	11. 7	31.5	1.5
9	Pran Jr Juice (mango)	2.2	15.3	76.3	1.7
10	Pran Frooto Mango Juice	1.9	26.1	85.3	2.1

mg/kg = milligram per kilogram of edible portion, Jr = Junior, Pr = Premium

Shezan Sw. Orange Jelly contains the amount of Ca is more than the Recommended Daily Intake value. From the Table 4.31 it is seen that the higher amount of Ca is present in eight juices samples than the recommended value [9]. Starship (Mango) and Acme Pr Mango Juice contain the lower amount of Ca than the recommended value. Continuous intake of these juices (brand no. 1, 2 and 5−10) may cause the accumulation of Ca in the human body. So the excess intake of Ca in human body exhibits various types of adverse effect.

The variations of concentration (mg/kg) of essential metals in jams, jellies and juices of different brands are shown in Figures from 4.51 to 4.62.

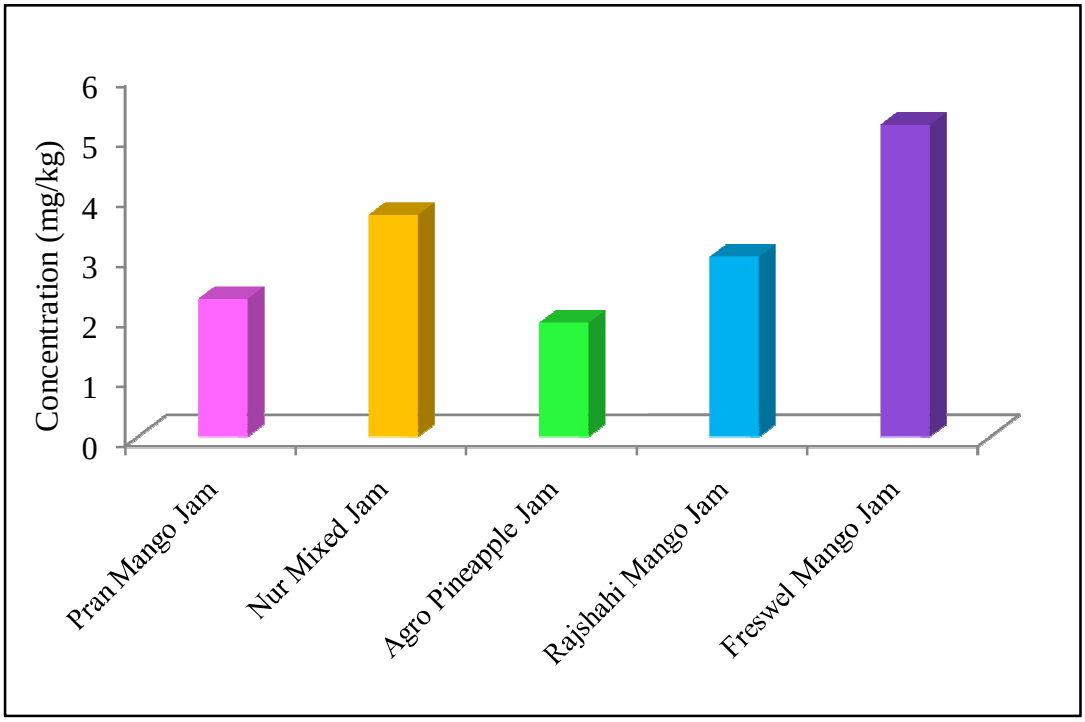

Figure 4.51: The variation of concentrations of sodium in jams of different brands.

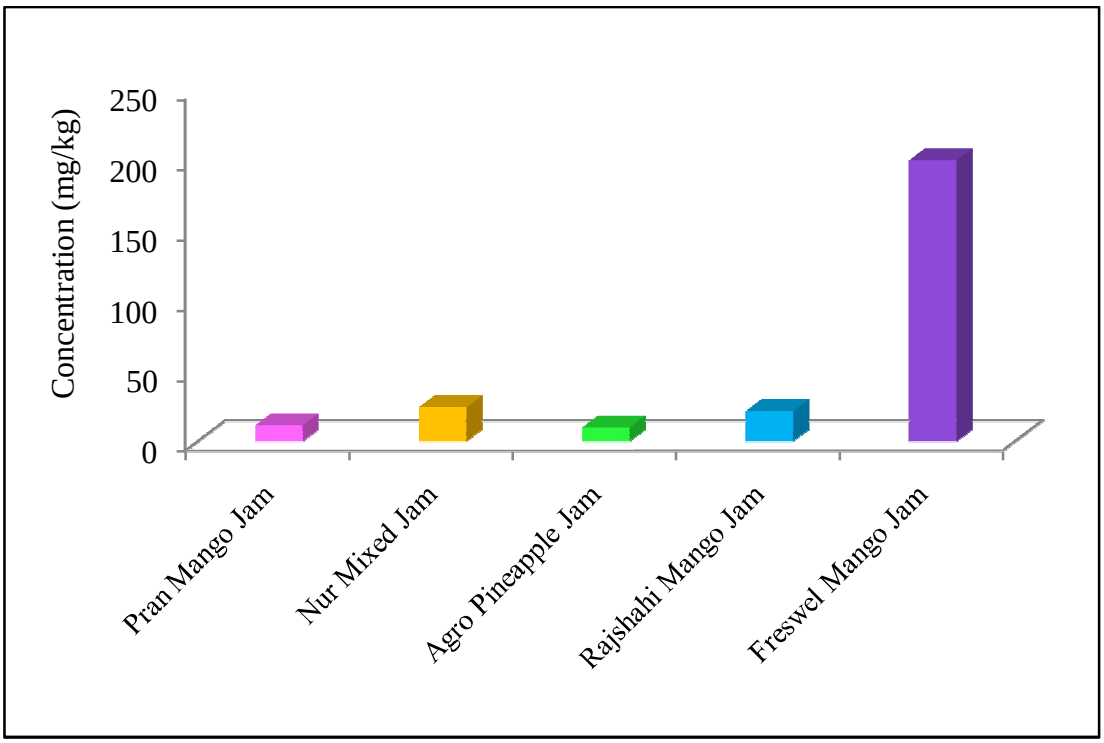

Figure 4.52: The variation of concentrations of potassium in jams of different brands.

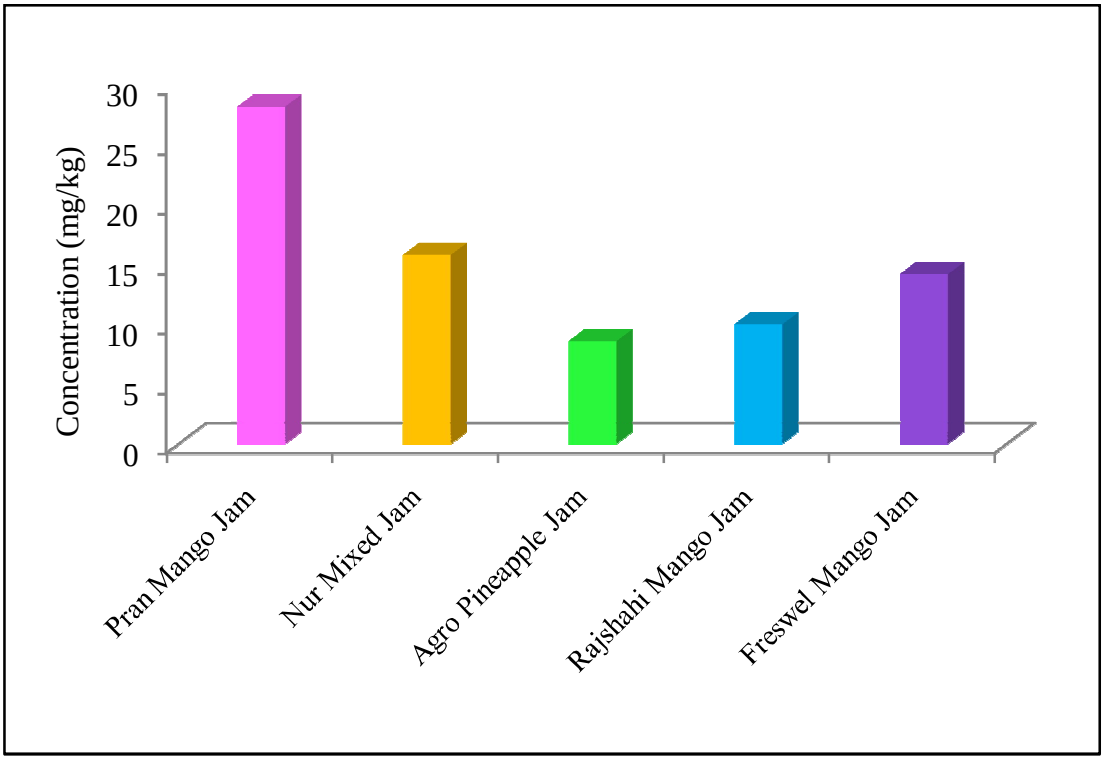

Figure 4.53: The variation of concentrations of calcium in jams of different brands.

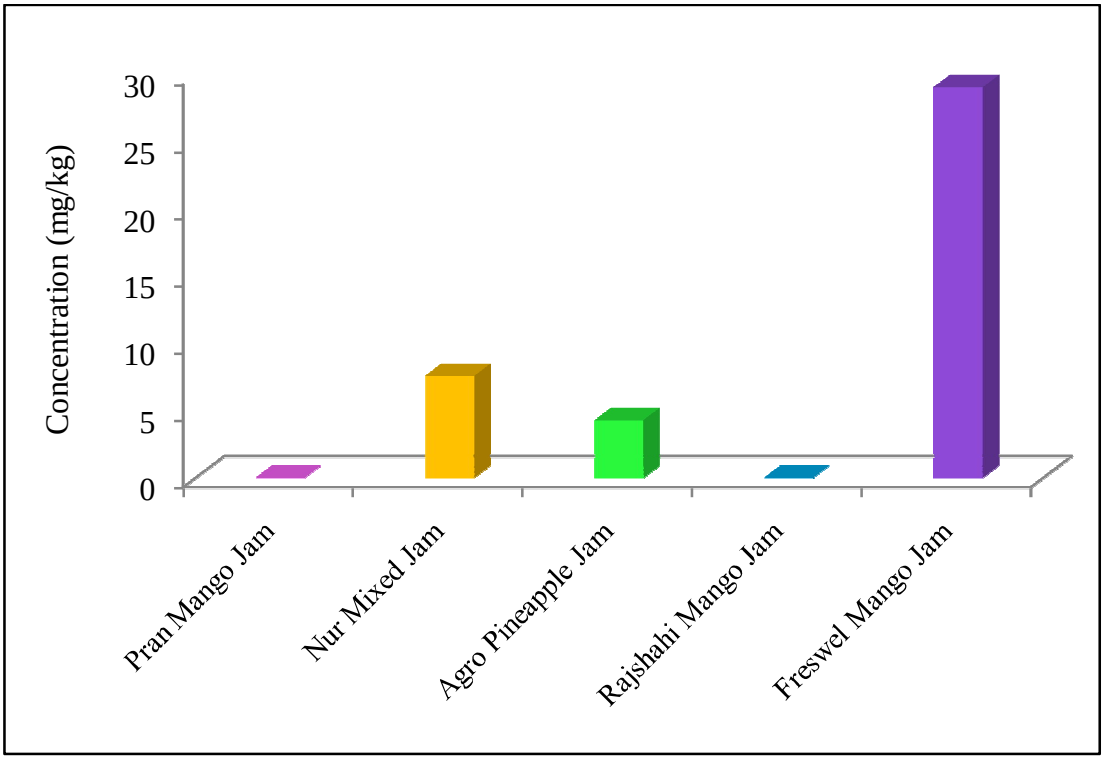

Figure 4.54: The variation of concentrations of magnesium in jams of different brands.

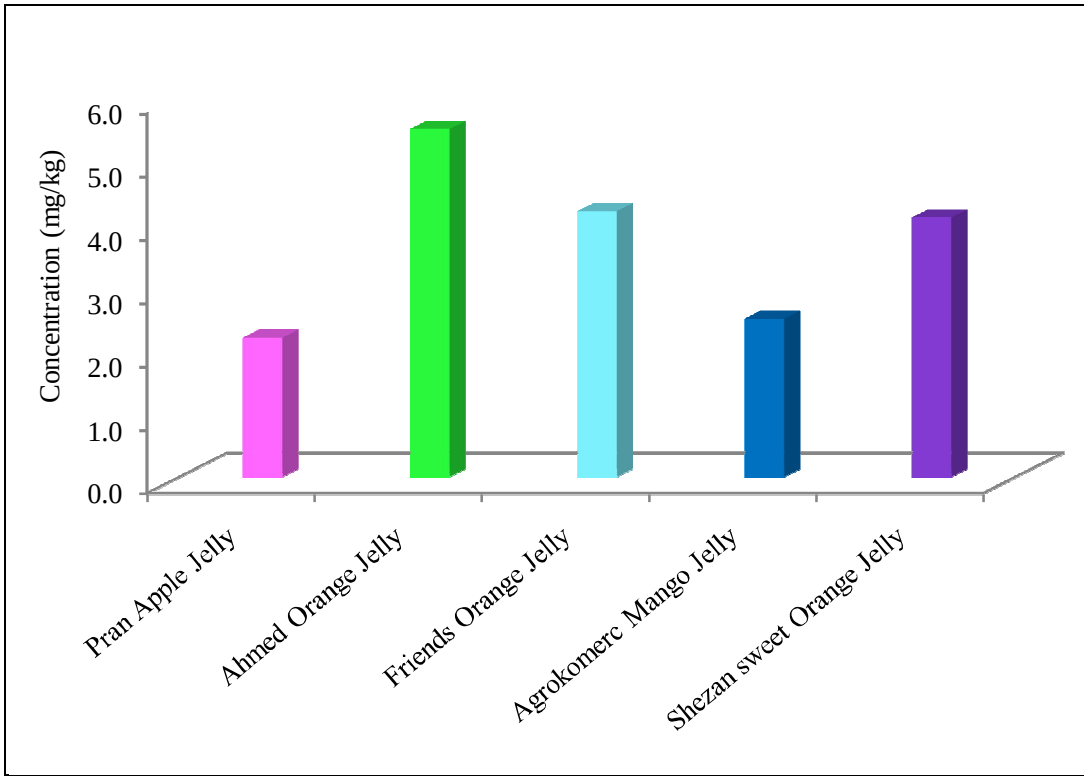

Figure 4.55: The variation of concentrations of sodium in jellies of different brands.

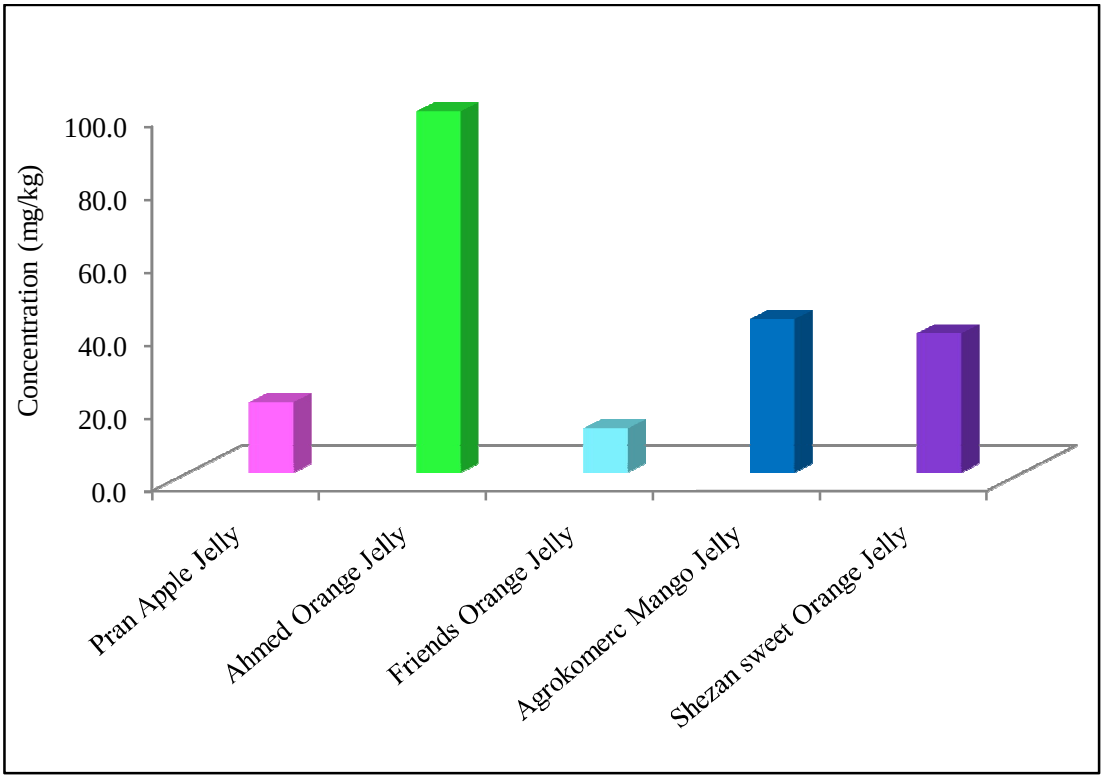

Figure 4.56: The variation of concentrations of potassium in jellies of different brands.

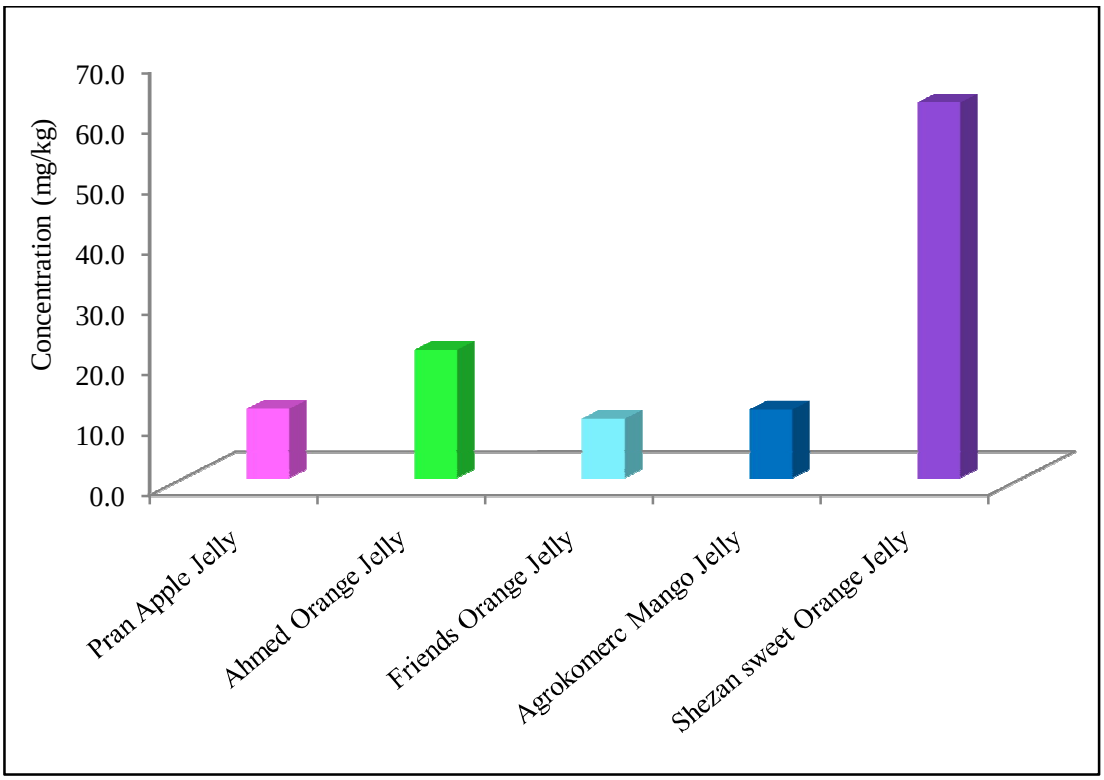

Figure 4.57: The variation of concentrations of calcium in jellies of different brands.

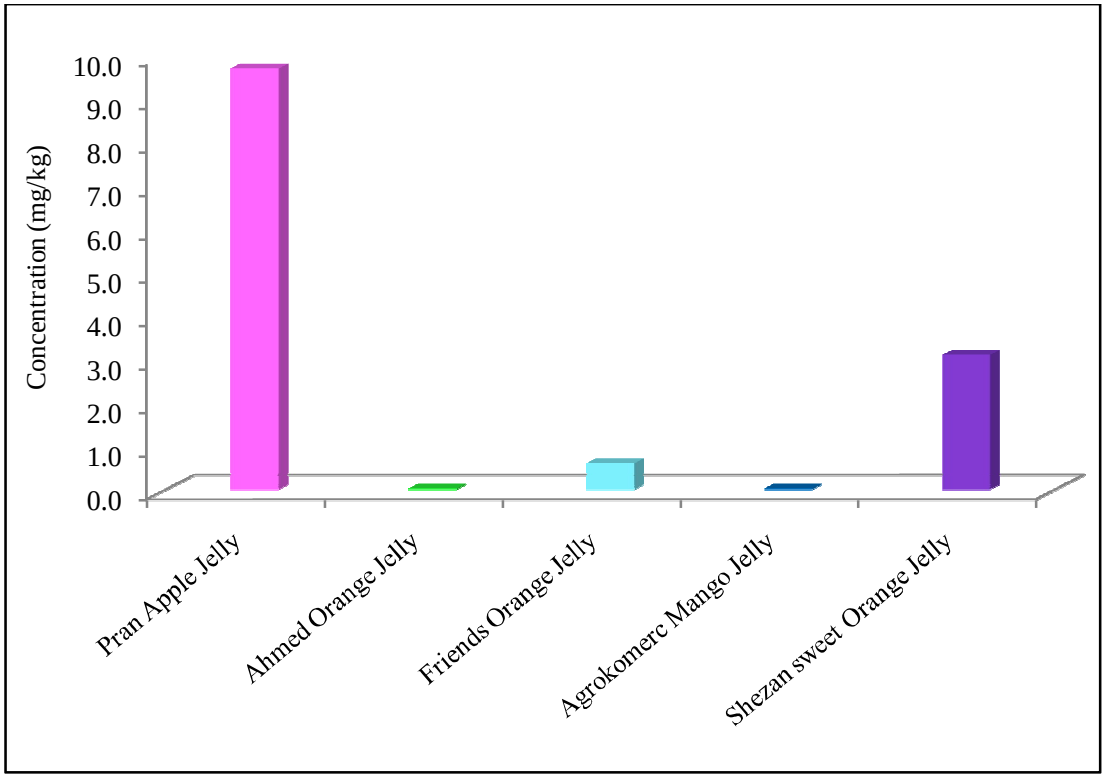

Figure 4.58: The variation of concentrations of magnesium in jellies of different brands.

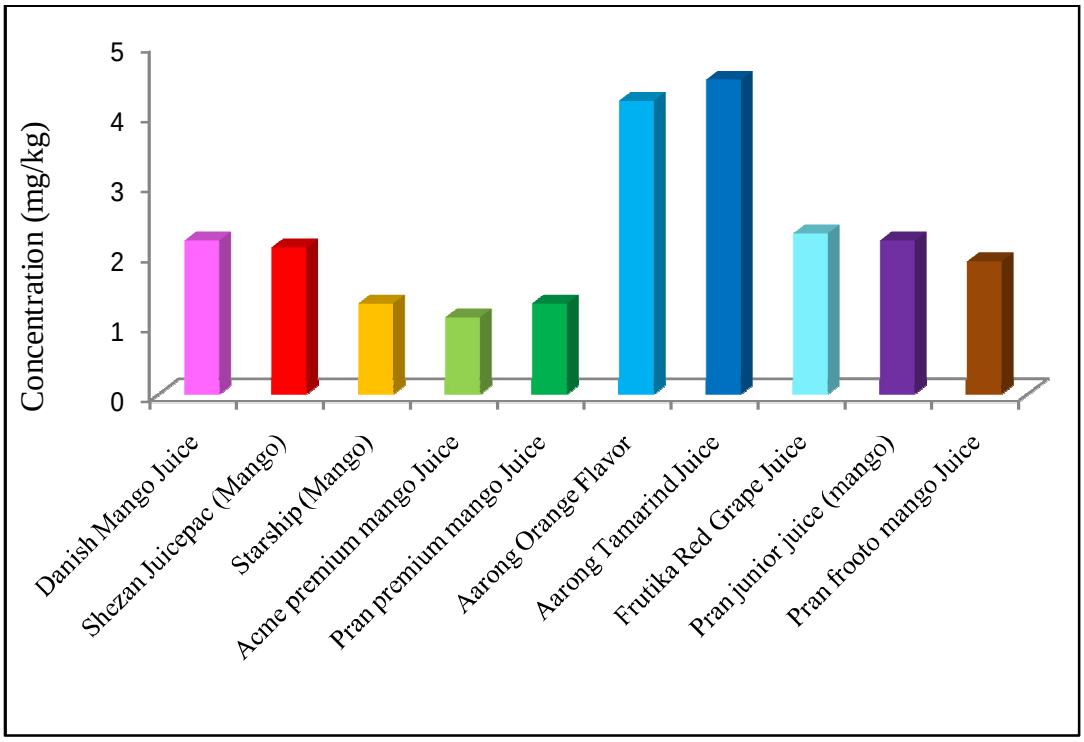

Figure 4.59: The variation of concentrations of sodium in juices of different brands.

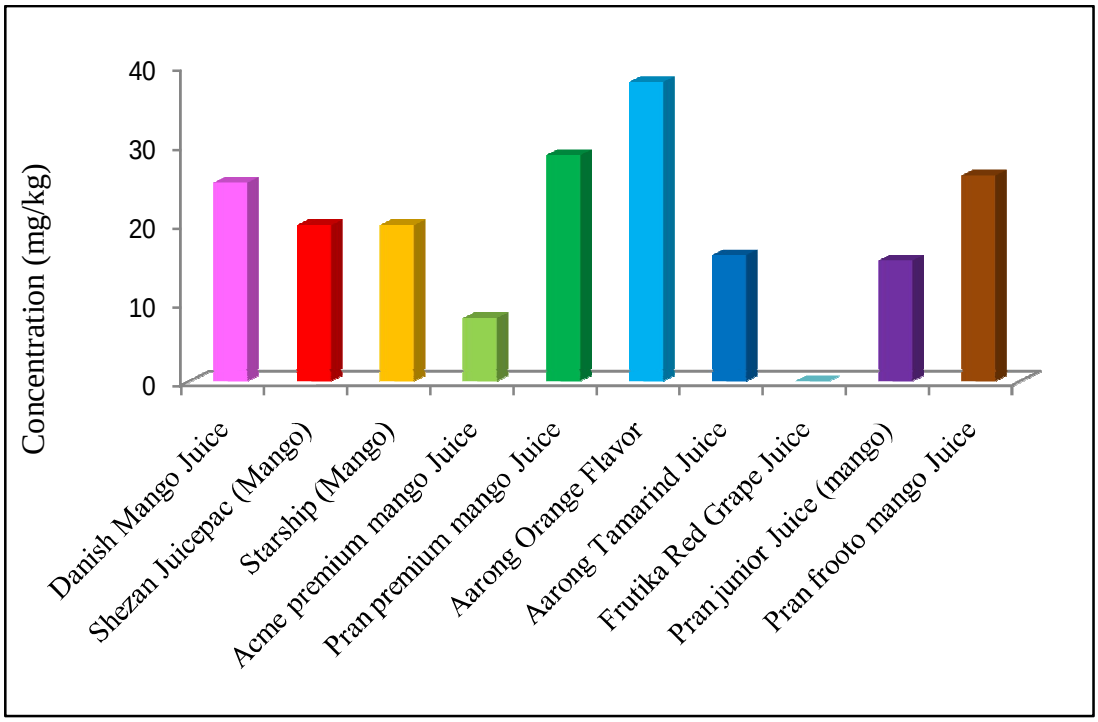

Figure 4.60: The variation of concentrations of potassium in juices of different brands.

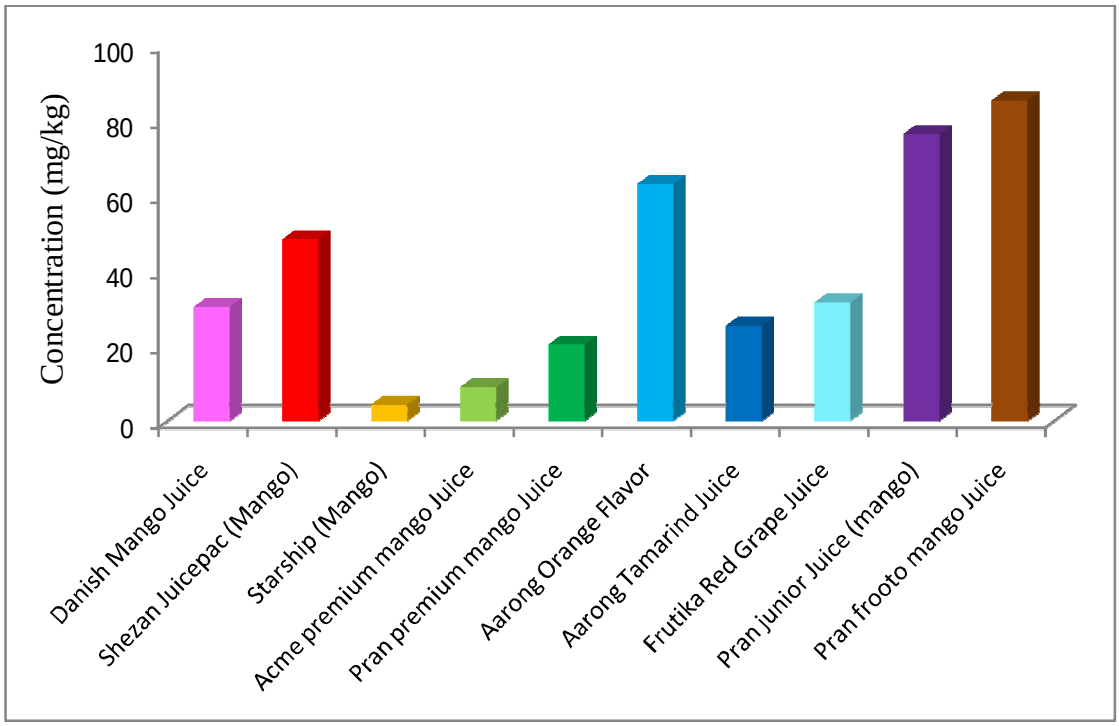

Figure 4.61: The variation of concentrations of calcium in juices of different brands.

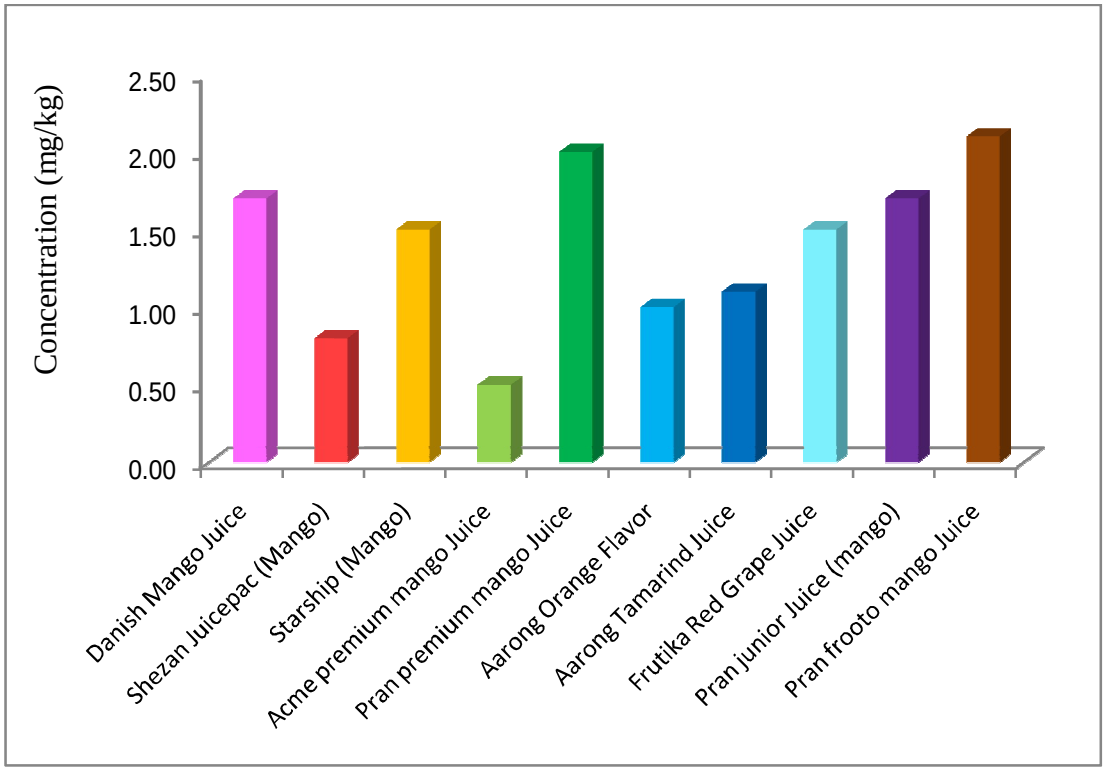

Figure 4.62: The variation of concentrations of magnesium in juices of different brands.

The average values are shown and compared in the graph (Figure 4.63) for Na, K, Ca and Mg of various types of jams, jellies and juices at a glance

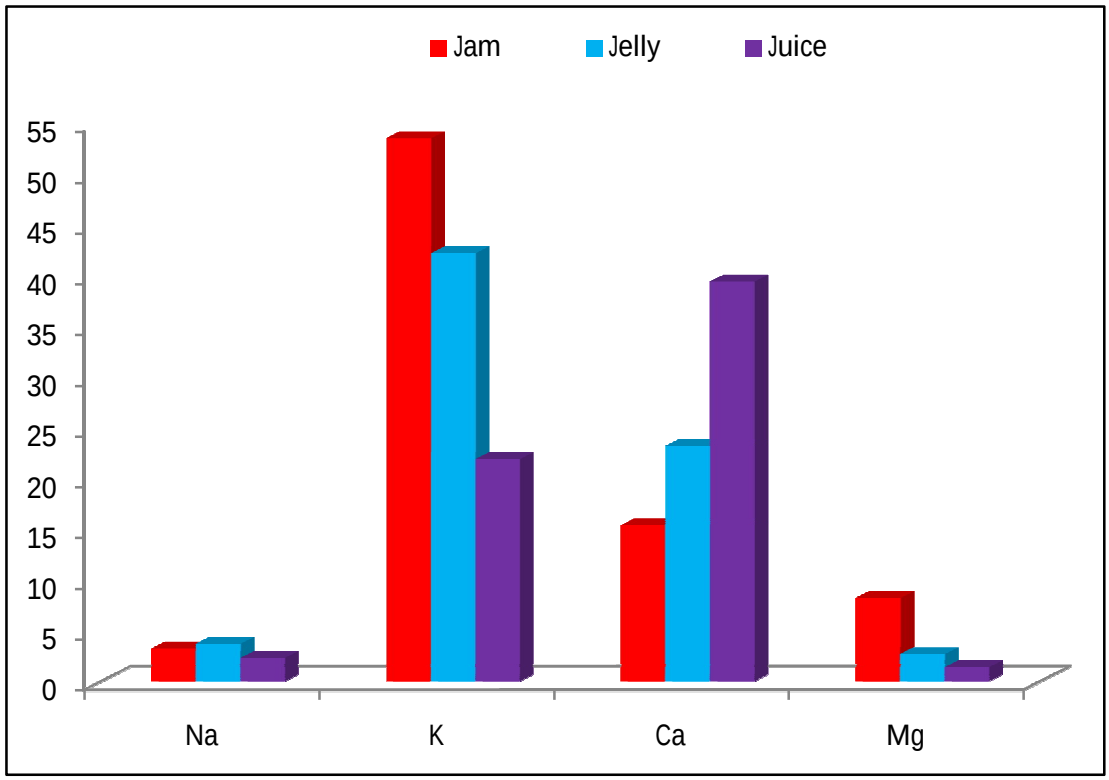

Figure 4.63: Comparison of the variation of concentration of essential metals (Na, K, Ca and Mg) in jams, jellies and juices of different brands.

4.4. References

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CHAPTER FIVE

Overall Conclusion

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The study aims at testing the presence of important bio-chemical parameters, trace elements (Co and Zn) and toxic metals especifically lead, cadmium, chromium, arsenic and nickel in infant formula for infants aged 0−6 months and 6−24 months and try to establish whether this amount are within the standard limit of the World Heath Association.

Fourteen types of infant formula milk and ten types of baby cereals were tested. In view of the experimental results of the biochemical analysis of different milk, it could be concluded that the investigated milk shows good results with a few exceptions. Excess protein in all these milks may be due to the presence of non-protein nitrogenous substances such as urea, nitrogen, amino nitrogen, creatinine, uric acid, adenine and guanine.

The percentage of lactose varies from 50 to 61% and 51 to 59% in 0−6 months and 6−24 months of baby powder milk respectively. The lactose is found in some samples exceed their given value and some of them contain comparatively lesser amount than their given value. The decreased value indicates that some lactose may be lost during processing of the raw milk or other carbohydrate, such as sucrose (non-reducing) may be added which give the higher value of total carbohydrate. The reason for decrease of the lactose containing may be due to either decomposition of lactose during processing of milk or some conversion of lactose to lactic acid by bacteria.

The results of the study provide information about the concentration of trace and toxic metals in different baby powder milks, baby cereals, jams, jellies and juices. The higher amount of As, Pb, Cd, Cr and Ni are found in these studied samples but other baby powder milk, jam, jelly, juice and baby cereal samples are safe considering the recommended daily allowance of the concerned metals. Since the study shows the significant concentration of As, Pb, Cd, Cr and Ni in baby powder milk, baby cereal, jam, jelly and juice samples available in Bangladesh, it is recommended to carry out extensive research in future.

The information gained from these measurements will provide a baseline level of toxicity for baby powder milk, baby cereals, jams, jellies and juices available in Bangladesh. The data obtained from this study will help to make a food list according to the presence of estimated metals. This research will also help consumers, manufacturers and professionals to realize about the possible direct or cumulative effects of the toxic metals to health care system. This study can be used as a reference for future studies.

Single monitoring is not sufficient to assess the quality of food on the basis of the presence of toxic metals. Concentration of toxic metals increases gradually in the environment due to mainly human activities and there is a greater possibility of entrance of toxic metals into the human body through the food chain. So continuous monitoring at given intervals should be carried out to judge the quality of the investigated food items.

Pollution of water and soil by the presence of toxic metals is a major environmental problem. Rapid growth in population and massive industrialization in recent years have resulted in pollution with toxic metals. The possible sources of toxic metals are untreated industrial wastes, dyes, chemicals, automobile emissions, agrochemicals, disposable batteries, paints, etc. If the sources of the contamination are not controlled by the concerned public agency or authority, we will not achieve the goal in lessening the metal concentration in foods. The environment and health department of the government should take some stern policies to reduce the discharge of toxic metals especially from various industries (tannery, textile, chemicals, fertilizers etc.). The government can make it compulsory for the industries to treat their waste products by ETP (effluent treatment plant) before releasing them to the environment to minimize the spread-out of the contaminants. The government can take steps to convert the petrol and diesel engines into gas engines in order to reduce emissions.

Fruits of Bangladesh are valuable sources of fiber, some of the essential minerals and vitamins. Around fifty different kinds of fruits are available in Bangladesh. From the analyses of different parameters of jams, jellies and juices, it is evident that the most of the results are in good agreement with the given results (marked on the bottle or packed) with a few exceptions.

There is no sufficient information about the concentration of essential metals in different jams, jellies and juices of Bangladesh. The results of the study also provide information about the concentration of reducing sugar, non reducing sugar and total sugar in different jams, jellies and juices. Jams, jellies and juices of different brands contain small amount of protein. Jams and jellies are not good sources of potassium.

Very poor amount of calcium and magnesium are present in jams, jellies and juices. The information gained from these measurements will provide a baseline level of nutrition for jams, jellies and juices. The data obtained from this study will help to make a food list according to the presence of determined metals.

Concentration of essential trace and toxic metals changes in the environment with time due to human activities mainly. So continuous monitoring at a given interval should be carried out to judge the quality of the investigated food items.

The per capita income in Bangladesh is recorded to be one of the lowest in the world. People have no idea about the role of balanced diet. Less than 5% of the population consumes an adequate quantity of food comprising of balanced diet. About 95% of the population is suffering from malnutrition. The nutrients studied in the present work have an important specific role in human metabolism and their deficiency can be removed through intake of sufficient quantity of fruit products with the traditional food.

List of Publication

1. Lokonuzzaman Ahmmed, Md. Nazrul Islam, M. Saidul Islam . **A Quantitative Estimation of the Amount of Sugar in Fruits Jam Available in Bangladesh.** Inorganic Research Laboratory, Department of Chemistry, University of Rajshahi, Rajshahi, Bangladesh. *Science Journal of Analytical Chemistry* **2015**; *3*(5): 52−55.

2. Lokonuzzaman Ahmmed, M. Nazrul Islam, M. Saidul Islam, Md. Sher Ali. Inorganic Research Laboratory, Department of Chemistry, University of Rajshahi, Rajshahi, Bangladesh. **Estimation of Protein in Jams, Jellies and Juices Available in Bangladesh.** *Science Journal of Analytical Chemistry* **2015**; *3*(4): 43−46.

3. Lokonuzzaman Ahmmed, Md. Nazrul Islam, M. Saidul Islam, Md. Ruhul Amin, Md. Sher Ali. Inorganic Research Laboratory, Department of Chemistry, University of Rajshahi, Rajshahi, Bangladesh. **Estimation of the Biochemical Parameters in Baby Powder Milk.** *Science Journal of Chemistry* **2015**; *3*(4): 67−71.