Comparative Studies of Macronutrients in Different Processed and Un-Processed Milk Samples

Iqbal Hussain, Farhat Ali Khan, Muhammad Ishaq, Inayat Ur Rehman, Muhammad Siddique Majed Ahmad and Ijaz Ahmad

Department of Chemistry, Kohat University of Science and Technology Kohat 26000, Pakistan
Department of Pharmacy, Sarhad University of Science and Information Technology, Peshawar, Pakistan
PCSIR Laboratories Complex, Jamrud Road, Peshawar-25120, Pakistan
Institute of Chemical Sciences, University of Peshawar, Peshawar-25120, Pakistan

Abstract: Milk is a basic food in the human diet, both in its original form and as various dairy products, is an excretion of the mammary glands. It can carry numerous xenobiotic substances (pesticides, disinfectants, drugs, metals and various environmental contaminants) which constitute a danger for the health of the human. The present study is concerned with different types of milk samples including powder milk, milk packs and unprocessed animal milk. Different parameters including Alkalinity, Hardness, Chloride, Nitrate, Phosphate, Sulphate were carried using the literature methods. The main purpose of the study was to quantify the above inorganic constituents parameters which may help the consumer for their health maintenance. The study is also of particular importance for providing a scientific data base.

Keywords: Milk • Inorganic constituents • Alkalinity

INTRODUCTION

Mineral and trace elements occur in the human body in the form of inorganic ions and salts or as constituents of organic molecule, such as proteins, fats, carbohydrates and nucleic acids [1]. As a food, milk serves various purposes e.g. growth, reproduction, supply of energy, maintenance and repair and appetite satisfaction. The requirements of these categories vary with the individual, e.g. adults no longer require food for growth whereas infants do.

Nutritionally, milk is being considered as perfect food. It provides more essential nutrients in significant amounts than any other single food. Milk is an outstanding source of calcium and phosphorus for bones and teeth and contains riboflavin, vitamins B, A and B, in significant amounts. It also contains B, the antiperiodic anaemia vitamin [2].

Milk is the characteristic secretion of the mammary glands [3], which meets the nutritional needs of the body better than other single food. The functions of a food are served specifically through the various nutritionally important components, comprising proteins, carbohydrates, lipids, minerals, vitamins and water and further more contains these dietary essentials in fairly suitable proportions [4].

Milk is a basic food in the human diet, both in its original form and as various dairy products, is an excretion of the mammary glands. It can carry numerous xenobiotic substances (pesticides, disinfectants, drugs, metals and various environmental contaminants) which constitute a danger for the health of the consumer [5].

Fresh milk also referred to as “full fat milk” or “whole milk” is also a good source of calcium. Calcium and phosphorous are needed at all ages for bone health. It contains different types of minerals and metals, varying with the variety of milk. Minerals present in milk, include calcium, sodium, potassium, magnesium and inorganic phosphates. Trace elements in milk, important in human nutrition, include chromium, zinc, iron, cobalt (in vitamin B), copper (enzyme co-factor) and manganese (hypocholesterol) [6].

Corresponding Author: Dr. Iqbal Hussain, Department of Chemistry, Kohat University of Science and Technology, Kohat 26000, Pakistan.
The present study will deal with all these nutrients to be determined in various types of milk, which people are using as daily intakes.

Experimental
Sampling: During this study, about 24 different milk samples were taken for analysis. Among these milk samples, 14 were dry, 7 were milk packs obtained from local market in Peshawar (Pakistan) while 3 animal milk samples were obtained directly from the animals (Goat, Cow and Buffalo) and were processed for their inorganic constituents.

The samples were then analyzed for different parameters like alkalinity, hardness, chlorides, nitrates, nitrites, phosphates and sulphates.

Chemical Reagents/Method: All the chemical and reagents used were of AR grade purchased from E Merck and BDH and all the solution of standards and samples were prepared in freshly prepared deionized water.

A Hitachi UV-VIS Spectrophotometer (model U-2000 Japan), with a 1.0 cm optical path quartz cell was used for spectrophotometric measurements at a particular wavelength for a particular constituent to be determined. Alkalinity, hardness and sulphates were determined by titrimetric method. Chlorides were determined by the standard argentometric method using potassium chromate indicator [7, 8]. Phosphates and nitrates were determined spectrophotometrically. The nitrate contents were determined by screening method.

Sample Preparation
Dry Milk Sample Preparation: 1g of each milk sample was accurately weighed in a crucible. The sample was charred carefully and then the contents in crucible were placed in an electric Muffle furnace at 600°C for 1 hour, so that the carbon free ash contents were obtained. The sample was cooled in dessiccatior, weighed and % ash was calculated. The ash residue was dissolved in distilled water thoroughly and the final volume was made 1000ml (1litre) with distilled water after filtration. This was the sample stock solution in which different constituents were determined by subsequent procedures.

Liquid Milk Samples Preparation: 1ml of each liquid milk sample was taken accurately with the help of graduated cylinder. It was diluted with distilled water with continuous shaking and the final volume was made 1 litre.

The diluted sample was filtered to remove the suspended fat particles if any. Again this was the sample stock solution and is used for the quantitative determination of $\text{Cl}^-, \text{NO}_2^-, \text{NO}_3^-, \text{SO}_4^{2-}$ and $\text{PO}_4^{3-}$ [9].

Preparation of Different Standard Solutions
Standard Sodium Nitrite: 1, 2, 3, 4, 5 and 6 ml of standard sodium nitrite solution was taken in six separate flasks, then to each flask 1 ml sulphamic acid, 1 ml 1-naphthalmine hydrochloric acid and 1 ml sodium acetate solution was added. The final volume was made 20 ml with distilled water. The analysis was made with the help of UV-spectrophotometer at wavelength of 540 nm. 20 mL distilled water was taken for blank test.

Standard Nitrate Solution: 1, 2, 3, 4, 5 and 6 ml of standard sodium nitrate solution was taken in six separate conical flasks and then 1 ml of (1N) HCl was added to each conical flask respectively and then the final volume was made 20 ml with distilled water. The analysis was made with the help of UV-Spectrophotometer at wavelength of 220 nm. 20 mL distilled water was taken for blank test.

Standard Phosphate Solutions: 1, 2, 3, 4, 5 and 6 ml of standard solution was taken in six separate conical flasks and then 1 drop of phenolphthalein indicator (when colour was produced then sulphuric acid solution was added and if there was no colour then there was no need of sulphuric acid to be added), 4 ml ammonium molybdate and 5 drops of stannous chloride solution were added. The final volume was made 20 ml with distilled water. The analysis were made with the help of UV-spectrophotometer at wavelength of 690 nm. Blank test was performed with twenty ml distilled water.

RESULTS AND DISCUSSION

Alkalinity: Alkalinity, an index of the buffering capacity of water, is closely linked to hardness. For the most part, alkalinity is produced by anions or molecular species of weak acids, mainly hydroxide, bicarbonate and carbonate. Whichever solute species contributes to the alkalinity of water (or solutions of water), it is always expressed in terms of an equivalent quantity of calcium carbonate. [10]. In Table 1, 2 and 3 different standard solutions of nitrate, nitrite and phosphate, in terms of their concentrations and absorbance are given respectively.
The alkalinity study has been presented in Table 4 for dry milk, Table 5 for liquid milk pack and Table 6 for animal fresh milk. It is clear from the data in Table 4 that among the dry milk samples Haleeb ISMP has higher alkalinity value (2.5 mg/L) while Millac King F.C has low alkalinity 0.75 mg/L. Similarly among the liquid milk packs dairy queen has higher alkalinity 1.25 mg/L while Haleeb tea max has zero alkalinity value and the rest of the samples have alkalinity value of 1 mg/L (Table 5).

As far as the animal fresh milk samples are concerned (Table 6) goat and buffalo milk have higher (0.87 mg/L) and lower (0.5 mg/L) alkalinity respectively while cow milk has 0.75 mg/L alkalinity value. Among all the brands Dry milk Haleeb has the highest alkalinity.

**Hardness:** The principal hardness causing ions are calcium and magnesium, strontium, iron, barium and manganese also contribute in causing hardness [11]. In the SI system, it is recommended that hardness be expressed as moles of Ca$^{2+}$ per cubic meter [12]. A hardness level of about 100 mg of CaCO$_3$/L provides an acceptable balance between corrosion and the problems of incrustation, although, an aesthetic consideration, 500 mg/L is recommended as a guideline value [13].

From the results obtained for hardness study on milk it is seen that in dry milk samples (Table 4) Nestle everyday has higher value of 5.75 mg/L while Haleeb ISMP has lower value of hardness 1.3 mg/L. The rest of the samples have hardness between these two limits. Similarly everyday and candida samples, among the

<table>
<thead>
<tr>
<th>S.No</th>
<th>Sample</th>
<th>Alkalinity</th>
<th>Hardness</th>
<th>Chloride mg/L</th>
<th>Nitrate mg/L</th>
<th>Nitrite mg/L</th>
<th>Phosphate mg/L</th>
<th>Sulphate mg/L</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Haleeb ISMP</td>
<td>2.5</td>
<td>1.3</td>
<td>75</td>
<td>0.23</td>
<td>0.0047</td>
<td>3.64</td>
<td>124.80</td>
</tr>
<tr>
<td>2</td>
<td>Nirala Lahore (SMP)</td>
<td>1.5</td>
<td>2.3</td>
<td>50</td>
<td>13.29</td>
<td>0.0199</td>
<td>3.59</td>
<td>350.24</td>
</tr>
<tr>
<td>3</td>
<td>Russian</td>
<td>1.25</td>
<td>2.2</td>
<td>60</td>
<td>13.29</td>
<td>0.0006</td>
<td>2.01</td>
<td>128.64</td>
</tr>
<tr>
<td>4</td>
<td>Skinz Candia</td>
<td>1.5</td>
<td>2.0</td>
<td>50</td>
<td>13.29</td>
<td>0.0567</td>
<td>11.97</td>
<td>167.94</td>
</tr>
<tr>
<td>5</td>
<td>Poland SMP</td>
<td>1.5</td>
<td>4.25</td>
<td>50</td>
<td>13.29</td>
<td>0.022</td>
<td>8.97</td>
<td>465.60</td>
</tr>
<tr>
<td>6</td>
<td>Dairy America</td>
<td>0.75</td>
<td>3.6</td>
<td>55</td>
<td>0.28</td>
<td>0.0035</td>
<td>5.43</td>
<td>378.24</td>
</tr>
<tr>
<td>7</td>
<td>Millac King (F.C)</td>
<td>0.75</td>
<td>4.8</td>
<td>50</td>
<td>13.29</td>
<td>0.0006</td>
<td>1.41</td>
<td>409.92</td>
</tr>
<tr>
<td>8</td>
<td>Russian 2 (SMP)</td>
<td>1</td>
<td>4.7</td>
<td>50</td>
<td>6.277</td>
<td>0.0199</td>
<td>8.62</td>
<td>508.80</td>
</tr>
<tr>
<td>9</td>
<td>Millac Unilac</td>
<td>1.25</td>
<td>4.8</td>
<td>45</td>
<td>0.443</td>
<td>0.0044</td>
<td>6.22</td>
<td>544.32</td>
</tr>
<tr>
<td>10</td>
<td>Dairy Crot (F.C)</td>
<td>1</td>
<td>3.4</td>
<td>40</td>
<td>13.29</td>
<td>0.0024</td>
<td>0.87</td>
<td>505.92</td>
</tr>
<tr>
<td>11</td>
<td>Nestle Everyday</td>
<td>1</td>
<td>5.7</td>
<td>30</td>
<td>13.29</td>
<td>0.0015</td>
<td>11.95</td>
<td>622.40</td>
</tr>
<tr>
<td>12</td>
<td>Russian 1 SMP</td>
<td>1</td>
<td>3.65</td>
<td>45</td>
<td>13.29</td>
<td>0.062</td>
<td>3.18</td>
<td>450.24</td>
</tr>
<tr>
<td>13</td>
<td>Nestle Nido</td>
<td>1.25</td>
<td>4.8</td>
<td>40</td>
<td>13.29</td>
<td>0.0136</td>
<td>2.55</td>
<td>608.64</td>
</tr>
<tr>
<td>14</td>
<td>Green Pak (vegi. Int)</td>
<td>1</td>
<td>4.3</td>
<td>45</td>
<td>13.29</td>
<td>0.0565</td>
<td>0.82</td>
<td>407.40</td>
</tr>
</tbody>
</table>

Average 1.23      3.7     48.93         10.00924      0.019154        5.0868        403.73
Table 5: Concentrations of Inorganic Constituents in Liquid milk (Milk Pack) Samples

<table>
<thead>
<tr>
<th>S.No</th>
<th>Sample</th>
<th>Alkalinity</th>
<th>Hardness</th>
<th>Chloride mg/L</th>
<th>Nitrate mg/L</th>
<th>Nitrite mg/L</th>
<th>Phosphate mg/L</th>
<th>Sulphate mg/L</th>
</tr>
</thead>
<tbody>
<tr>
<td>15</td>
<td>Haleeb Tea Max</td>
<td>0</td>
<td>0.40</td>
<td>15</td>
<td>0.443</td>
<td>0.0034</td>
<td>5.5675</td>
<td>148.8</td>
</tr>
<tr>
<td>16</td>
<td>Candida</td>
<td>1.0</td>
<td>0.45</td>
<td>20</td>
<td>0.282</td>
<td>0.199</td>
<td>4.6850</td>
<td>33.60</td>
</tr>
<tr>
<td>17</td>
<td>Narpar</td>
<td>1.0</td>
<td>0.4</td>
<td>20</td>
<td>5.55</td>
<td>0.072</td>
<td>3.6625</td>
<td>96.00</td>
</tr>
<tr>
<td>18</td>
<td>Milk-Pak Nestle</td>
<td>1.0</td>
<td>0.42</td>
<td>20</td>
<td>0.903</td>
<td>0.057</td>
<td>2.8225</td>
<td>222.72</td>
</tr>
<tr>
<td>19</td>
<td>Haleeb</td>
<td>1.0</td>
<td>0.4</td>
<td>20</td>
<td>13.29</td>
<td>0.022</td>
<td>5.8060</td>
<td>128.40</td>
</tr>
<tr>
<td>20</td>
<td>Dairy Queen</td>
<td>1.25</td>
<td>0.35</td>
<td>15</td>
<td>13.29</td>
<td>0.00592</td>
<td>7.380</td>
<td>134.4</td>
</tr>
<tr>
<td>21</td>
<td>Everyday Nestle</td>
<td>1.0</td>
<td>0.45</td>
<td>65</td>
<td>0.859</td>
<td>0.1529</td>
<td>2.2775</td>
<td>9.6</td>
</tr>
<tr>
<td></td>
<td>Average</td>
<td></td>
<td></td>
<td></td>
<td>0.89</td>
<td>0.41</td>
<td>4.9453</td>
<td>25</td>
</tr>
</tbody>
</table>

Table 6: Concentrations of Inorganic Constituents in Fresh milk (Animal Milk) Samples

<table>
<thead>
<tr>
<th>S.No</th>
<th>Sample</th>
<th>Alkalinity</th>
<th>Hardness</th>
<th>Chloride mg/L</th>
<th>Nitrate mg/L</th>
<th>Nitrite mg/L</th>
<th>Phosphate mg/L</th>
<th>Sulphate mg/L</th>
</tr>
</thead>
<tbody>
<tr>
<td>22</td>
<td>Buffalo</td>
<td>0.5</td>
<td>0.3</td>
<td>5</td>
<td>13.29</td>
<td>0.0809</td>
<td>5.5875</td>
<td>131.52</td>
</tr>
<tr>
<td>23</td>
<td>Cow</td>
<td>0.75</td>
<td>0.25</td>
<td>50</td>
<td>13.29</td>
<td>0.036</td>
<td>3.065</td>
<td>91.2</td>
</tr>
<tr>
<td>24</td>
<td>Goat</td>
<td>0.87</td>
<td>0.35</td>
<td>20</td>
<td>0.1816</td>
<td>0.0738</td>
<td>2.5075</td>
<td>48</td>
</tr>
<tr>
<td></td>
<td>Average</td>
<td>0.71</td>
<td>0.3</td>
<td>25</td>
<td>8.9205</td>
<td>0.0636</td>
<td>3.0833</td>
<td>90.24</td>
</tr>
</tbody>
</table>

liquid milk pack category (Table 5) have 0.45 mg/L hardness value, while dairy queen has lower hardness value of 0.35 mg/L. Buffalo milk has 0.3 mg/L while cow milk has low hardness value of 0.25 mg/L (Table 6). As a whole alkalinity in the milk samples is in the order of: Dry milk (1.23 mg/L) > liquid milk (0.89 mg/L) > animal milk samples (0.71 mg/L), while hardness is in the order of: Liquid milk pack samples (0.4 mg/L) > dry milk (3.7 mg/L) > animal milk (0.3 mg/L).

Chloride: Chloride is the most abundant anion in the human body and contributes significantly, along with its associated cations, to the osmotic activity of the extracellular fluid. 88% of the chloride in the body is extracellular. A normal 70-kg human body contains approximately 81.7 g chloride and 45 litres of water. [14] The additions of salts during processing can markedly increase the chloride level in food. Approximately 600 mg of chloride per day are in gested in a salt-free diet [15, 16]. Because of the addition of salt to food, the daily intake of chloride averages 6 g and may exceed as high as 12 g [17, 18]. From the data given in Table 4 it can be seen that among the dry milk samples greater chloride ion concentration is found in Haleeb ISMP (75 mg/L) while nestle everyday has low chloride content (30 mg/L). The remaining samples have chloride content between these two limits. Similarly, among liquid milk packs (Table 5) everyday nestle has greater chloride contents (65 mg/L). Haleeb tea max and dairy queen both have similar low chloride concentration of 15 mg/L. As for as the animal milk samples are concerned (Table 6) cow milk has 50 mg/L chloride content while buffalo milk has 5 mg/L of chloride. At average dry milk samples have higher chloride contents i.e. 48.93 mg/L than liquid milk samples and animal milk samples both of which contain 25 mg/L of chloride contents.

Nitrate and Nitrite: Nitrites also occur fairly widely, but generally at very much lower levels than nitrates. Considerable quantities of nitrates and lesser amounts of nitrites are present in certain foods and in general the major source of human intake of both nitrites and nitrites is food [19]. In certain crops the nitrate levels may be as high as 100 mg/kg and if that crop is the food of the animals, it transfers in the milk of that animal. [20].

The higher average concentration of nitrates in dry milk samples (Table 4) 10.0 mg/L, which may be due to the addition of preservatives to dry milk. While nitrite is found lower in dry milk i.e. 0.019 mg/L which may be due to, that nitrates addition to dry samples as a preservative is not favoured and in liquid milk packs (Table 5) it is higher i.e. 0.07 mg/L than fresh animal milk (Table 6) samples i.e. 0.06 which supports our conclusion that nitrate preservatives are better for dry milk products like powder milk and cheese etc. while nitrite preservatives are better for liquid milk packs. The nitrite level in fresh milk is lower than packed liquid milk which indicates that nitrite is preservative added during packing.

As infants are the most sensitive group of the population to nitrate, it is important to define their exposure, which for a two-month old infant has been estimated to be approximately 25 mg of nitrate [20].
The most common cause of infantile methaemoglobininaemia is excessive levels of nitrate in water used for the reconstitution of baby food [21].

**Nitrate**: Among the dry milk samples (Table 4) Haleeb ISMP has the lowest nitrates 0.23 mg/L while the highest value is 13.29 mg/L for most of the samples, while the milk packs have also been found in these ranges. But as far as the animal milk sample (Table 6) is concerned goat milk shows lower nitrate concentration (0.181 mg/L) than Haleeb ISMP in dry milk samples (Table 4) 0.23 mg/L taking the lowest concentrations found in these two particular categories. Because buffalo and cow both have 13.29 mg/L nitrate content while goat has 0.1816 mg/L nitrate content in the fresh milk samples.

The average of the animal milk sample is 8.9 mg/L which is lower than the average of the dry milk sample (Table 4) 10.0 mg/L.

**Nitrite**: In dry milk sample (Table 4) the nitrite content is below the permissible level. However in liquid milk pack samples (Table 5) candida has higher content of nitrite 0.1988 mg/L which is above the permissible level for water followed by everyday Nestle 0.1529 mg/L. Dairy queen has shown lesser content of Nitrite in liquid milk. Buffalo milk, among the animal milk (Table 6) have greater nitrite content 0.0809 gm/L followed by goat milk 0.0739 mg/L while cow milk has 0.036 mg/L of nitrite concentration.

**Phosphate**: Phosphorus is present in the body almost exclusively in the form of phosphate. The rest is located primarily inside the cells, where it is involved in energy metabolism. Phosphate is necessary for the formation of bone and teeth. [22] The phosphate causes two types of common diseases i.e. hypophosphatemia and hyperphosphatemia. In the farmer the level of phosphate in the blood is too low, while in the later the level of phosphate in the blood is too high.

From the data given in Table 4, 5 and 6 for phosphate ions in different milk samples, it is clear that skinz candida (Table 4, dry milk) has higher phosphate content 11.97 mg/L followed by nestle everyday 11.95 mg/L while Green Pak vegi.fat has low phosphate concentration 0.82 mg/L. The rest of the samples have phosphate content between these two limits.

Similarly among the liquid milk samples (Table 5) higher and lower phosphate contents were found in dairy queen 7.38 mg/L and Everyday Nestle 2.27 mg/L respectively.

Animal milk sample (Table 6) have shown low phosphate content at an average than dry milk and liquid milk samples i.e. Dry milk (5.086 mg/L) > liquid milk pack sample (4.52 mg/L) > animal milk Table 4 (3.083 mg/L).

**Sulfate**: Majority of the sulfates are soluble in water, the exceptions being the sulfates of lead, barium and strontium. Moreover Sulfate doses of 1.0-2.0 g have a cathartic effect on humans, resulting in the purgation of the alimentary canal [23]. Taste threshold concentrations for the most prevalent sulfate salts are: 200-500 mg/L for sodium sulfate; 250-900 mg/L for calcium sulfate; and 400-600 mg/L for magnesium sulfate [23-24]. High sulfate concentrations in water may contribute to the corrosion of metals in the distribution system, particularly in waters having low alkalinity.

From the results given in the Table 4 for dry milk samples, it can be seen that Nestle everyday has greater content 622.4 mg/L followed by nestle nido i.e. 608.64 mg/L. Haleeb ISMP has low sulphate content 124.8 mg/L.

Among the liquid pack milk samples (Table 5) milk pak nestle has greater sulphate concentration 222.72 mg/L and everyday nestle has lower 9.6 mg/L sulphate concentration. The rest of the samples have sulphate between these two limits.

As far as the animal milk samples (Table 6) are concerned buffalo milk has higher sulphate content while goat milk has 48 mg/L sulphate content and cow milk has 91.2 mg/L of sulphate concentration. As a whole sulphate content in all the milk samples is in the order dry milk (403.73 mg/L > liquid milk (109.99 mg/L) > animal milk (90.24 mg/L). However liquid milk and animal milk samples have low sulphate content than the permissible limit which is 250 mg/L except dry milk samples (403.73 mg/L).

**REFERENCE**


