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Chemical Composition and Heavy Metals Analysis of Raw Cow's Milk

Endale Tesfaye Damtew[1]* and Aregay Berhe Gebre[2]

[1]Department of Chemistry, Gambella University, P.O. Box 126, Ethiopia
[2]Department of Food Process Engineering, Gambella University, P.O. Box 126, Ethiopia

Abstract

The study was primarily aimed at determining physicochemical quality, minerals and nutrient composition of cow's milk samples collected from different areas of Nuer Zone and Gambella town, Ethiopia. A total of 6 cow's milk samples from farmers and vendors (dairy cooperative milk collection centers and small shops) were collected. All samples were collected using proportional random sampling method. The presence and concentration of various minerals (such as Na, Ca, Mg, P and N_2) and heavy metals (Cd, Cr and Pb) were determined. Different physicochemical parameters of milk samples such as pH, titratable acidity and density were examined. The proximate chemical compositions of milk samples such as moisture, protein, fat, solids-not-fat (SNF), lactose, total solid and ash contents also were determined. The mean values of pH, moisture, titratable acidity, protein, fat, solids-not-fat, ash, total solid, density and lactose contents of milk samples collected from farmers are 5.22, 86.99%, 1.01%, 3.46%, 4.80%, 6.23%, 0.04%, 11.04%, 1.01 g/cm^3 and 4.43%, respectively. On the other hand, the respective mean values of physicochemical parameters of vendors' milk samples are 5.09, 88.85%, 2.10%, 3.76%, 4.88%, 7.07%, 0.591%, 11.91%, 1.02 g/cm^3 and 4.70%. The results of the study showed that mineral compositions of all collected milk samples are adequate. The contents of heavy metals are below maximum permissible limit as compared with standard levels. However, Most of the milk samples have poor physiochemical quality; they have high titratable acidity values and low pH values which are beyond the permissible ranges. This indicated that milk samples, in particular, samples from vendors are not followed good milk handling practices, and they need improvement. The obtained proximate analysis results revealed that all the collected milk samples fulfilled the WHO and other national and international standards except lactose and protein contents.

Keywords: Cow's milk; Heavy metals; Milk quality; Minerals; Physiochemical analysis

Introduction

Milk has a positive influence on human health. It is considered as nearly complete food since it is good source of proteins, carbohydrates, fats, vitamin supplements and major minerals [1]. There are about 38 micro and trace elements reported to be found in raw milk from different regions around the world. These minerals content in raw cow's milk may vary depending on several factors i.e., lactation period of cows, health conditions, seasonal variations, climatic conditions, annual feed composition and environmental contamination [2]. The milk processing conditions may also have effective influence on the contents and retains of minerals in total composition of milk [3]. Milk quality is important consumers' requirement. The quality of raw milk in conformity with consumers' requirements is determined by chemical composition, physicochemical properties, microbiological quality, sensory properties, technological suitability and nutritive value. The safety of dairy products with respect to food borne diseases is a great concern around the world. This is especially true in developing countries where production of milk and various dairy products take place under unsanitary conditions and poor production practices [4].

Increased environmental pollution has accelerated the problems of milk contamination and uncertainties about milk qualities. Milk contamination via environmental pollutants and xenobiotic compounds through cattle feeds like toxic metals, mycotoxin, dioxin and other pollutants are considered to have great influence on public health [5]. The main sources of metal contamination to humans are industrial or domestic effluents, combustion, bushfires, decomposition of l fertilizers, pesticides etc. Abdominal pain, hepatotoxicity, neurotoxicity, vomiting, decreasing of intelligence quotient level, Alzheimer's disease, behavioral disorders, tissue injury, irritation of lungs, cancer etc. could be generated due to over exposure of heavy metals [6]. A typical schematic diagram of contaminations entering into food chain through milk is shown in Figure 1.

Due to the presence of a plenty number of cattle, cow's milk is widely produced and used in Gambella region particularly in the study areas. Thus, the determination of cow milk quality in the study areas is very important as far as its quality on human health is concerned. Therefore, the purpose of this study was to determine physiochemical parameters, nutrient composition

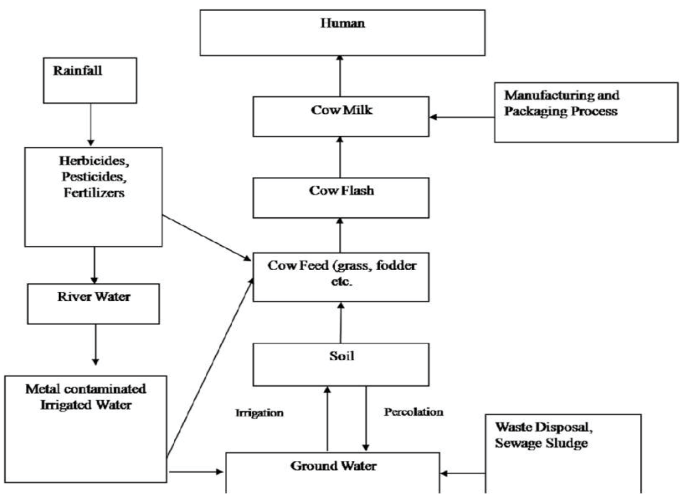

Figure 1. Possible food chain pathways through which humans may be exposed to trace contaminations (such as heavy metals) [7].

***Address for Correspondence:** Endale Tesfaye Damtew, Department of Chemistry, Gambella University, P.O. Box 126, Ethiopia, Email: endesh027@gmail.com*

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and the concentration of selected elements in cow's milk samples in Nuer Zone and Gambella town, Ethiopia. Compressions of the obtained results with the corresponding recommended dietary allowance (RDA) values set by international and national organizations also were performed in the study.

Materials and Methods

Description of the study areas

Gambella town is the capital of Gambella region, which is one of the nine regional states of Ethiopia. The town is situated 766 km south west of Addis Ababa, the capital city of Ethiopia. It is located within Latitude 6028'38" to 8034' North Latitude and 330 to 35011'11" East Longitude. Whereas Nuer Zone is one the zones of Gambella region. The zone is within the Ethiopian low lands and is flat an elevation between 400 - 430 m (Figure 2). The zone consists of grasslands, marshes and swamps with some forests. The economy is predominantly based on livestock. The study areas are selected based on the potential of cow's milk production (Nuer zone) and the accessibility of milk vendors (Gambella town).

Samples collection

The polyethylene sampling bottles were soaked in 20% HNO_3 for 24 hours and rinsed with deionized water before collection of raw milk in order to avoid possible contamination. Fresh cow's milk was preliminarily mixed to disperse the milk fat homogeneously before collection for physicochemical analysis. Raw milk samples were collected directly from bulk milk containers used by the dairy farm and the milk vendors. Dippers were used in sampling from milk containers.

A total of 6 raw milk samples from farmers and vendors (dairy cooperative milk collect- ion centers and small shops) were collected in the study areas. Samples had labels on each indicating the date and place of their receipt. Each container contains 100 ml milk sample. All samples were collected during morning milking time, homogenized and stored in an ice box. The samples then, transported to laboratory and immediately kept in a deep freeze (-20℃) until microwave digestion carried out [8].

Samples treatment

The milk samples were removed from the ice box and dried at 110 °C on oven. After oven drying, the milk samples were grinned into a fine powder and then digested using concentrated nitric acid. After digestion, each sample was diluted with distilled water in 25 ml volumetric flask [9].

Physicochemical analyses of milk determination of pH: The pH of the milk samples was measured in the laboratory using a digital pH-meter.

Density: The density of each raw caw milk sample was measured using density meter at 25°C.

Titratable acidity (TA): Titratable acidity of the milk samples was determined according to the method of the Association of Official Analytical Chemists (AOAC) [10]; 10 ml of milk sample was pipetted into a beaker and 3 to 5 drops of 1% phenolphthalein indicator was added to it. The milk sample was then titrated with 0.1 N NaOH solutions until a faint pink color was persisted. The titratable acidity, expressed as % lactic acid, was finally calculated using equation 1.

$$\text{Titratable acidity (\%)} = \frac{0.1N\ N \quad (m)\ x\ 0.0 \quad x1}{W \quad o\ m \quad sa} \text{ .... Equation (1)}$$

Proximate analysis

Total solids (TS): For the determination of total solids content, fresh cow's milk sample was thoroughly mixed and 5 g was transferred to a pre weighed in flat bottom crucible [10]. The milk sample was dried in a hot air oven at 102°C for 3 hours. Then after, the dried sample was taken out of the oven

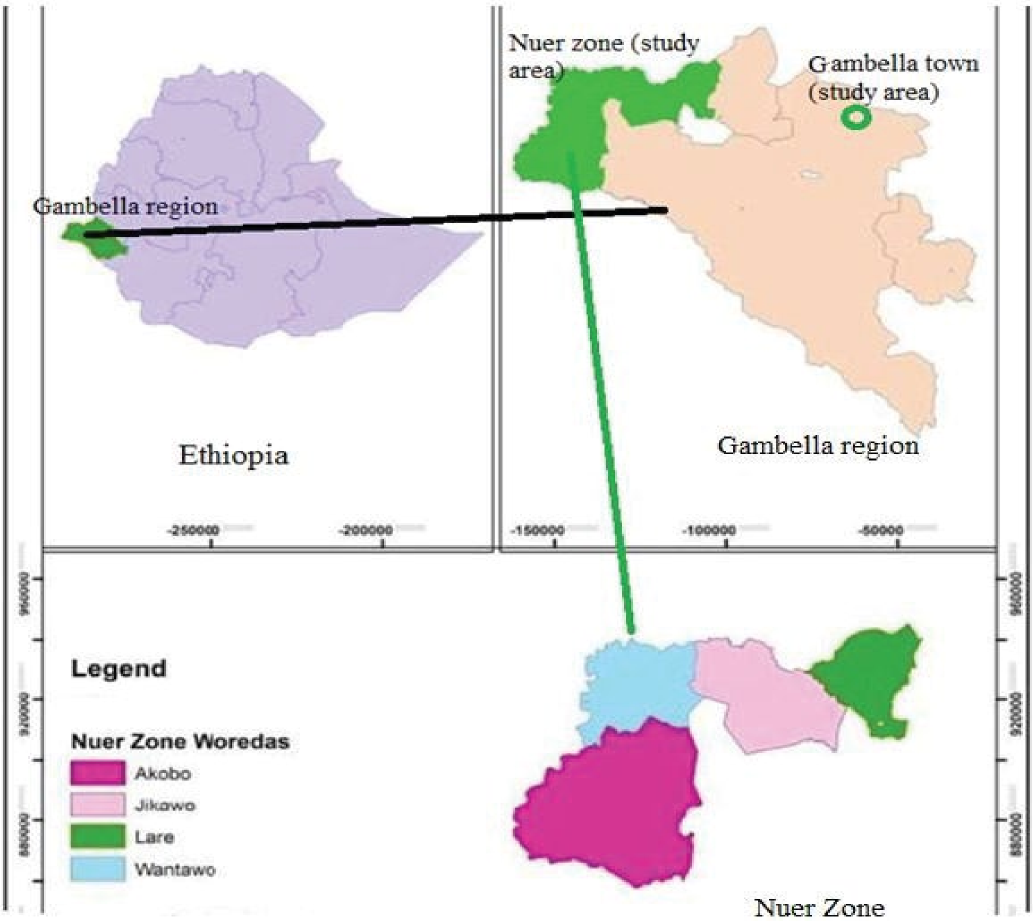

Figure 2. Geographic location of the study areas (Nuer Zone and Gambella town).

and placed in desiccators (Figure 3). The sample was then weighed again, and total solids content was calculated by using Equation 2.

Fat content: The fat content was determined by the Gerber method. A 10 ml of sulfuric acid (density 1.815 gm/ml at 20°C) was pipetted into a butyrometer. Then 10 ml of milk sample was added into the butyrometer and mixed with the sulphuric acid.

This was followed by addition of 1 ml amyl alcohol into the butyrometer which was then closed with a lock stopper. Then the mixture was shaken and inverted several times until the milk was completely digested by the acid. Then after, the butyrometer was kept in water bath for 5 minutes at 65°C and centrifuged in a Gerber centrifuge for 5 minutes. The butyrometer was placed in water bath again at 65°C for 5 minutes. At the end, the butyrometer reading was recorded (Figure 4). Solids not-fat (SNF): Solids-not-fat content was determined by difference using equation 3.

SNF content (%) = TS (%) – Fat (%) (3) Where TS: Total solid

Other proximate parameters were determined using the AOAC method. Moisture, ash, crude protein and lactose contents were determined by oven drying, furnace, micro Kjeldah and titrimetric methods, respectively.

Elemental analysis

Sodium, calcium and magnesium concentrations of the samples were determined using EDTA titration while the total phosphorus and total nitrogen were determined using spectrophotometric method. The heavy metals (lead, chromium and cadmium) levels in the digested samples were measured using flame atomic absorption spectrophotometer.

Statistical analysis

Microsoft Excel was used for statistical analysis. Statistical evaluations such as mean, interval, standard deviation, median, variance, skewness and kurtosis have done for all parameters of milk samples collected from both farmers and vendors.

Results and Discussion

Milk handling system in the study area

The majority of the milk producers (farmers) are used gourd, while the remaining farmers are used clay pot and plastic utensils. In the town, higher numbers of vendors are used plastic utensils and few are used aluminum utensils, gourd and clay pot utensils. This indicated that there is a little variation on milk handing system between farmers and vendors, and it needs to improve the quality sampling materials and hence the milk samples.

Analyses of milk samples

Physiochemical analyses (such as pH, acidity, density); proximate analysis (protein, fat, lactose, moisture, total solid, SNF, ash); concentration of heavy metals(Cd, Cr and Pb) and nutrients (P, N, Ca, Mg and Na) were performed for milk samples collected from six different cites of Nuer Zone and Gambella town (Table 1).

Physiochemical analysis

Physiochemical analysis is important parameter to monitor the quality of milk and its products. In this study, density, pH and titrateble acidity were analyzed for all samples collected from the study areas. Their results are recorded in Table 2. Based on world health organization (WHO) standards and other scientific works, quality milk contains 1.02 to 1.03 g/cm^3 density, 6.6 pH, and 0.17% titratable acidity (TA). From Table 3, the results of the densities of all milk samples are within the required range of 1.02 to 1.03 g/cm^3. The titratable acidity values are between 0.85% and 2.21%, which are beyond WHO permissible limit for fresh milk (0.17%), particularly samples collected from vendors are more acidic compared with samples collected from farmers. The pH values for all milk samples are below permissible limit of WHO and they are slightly acidic. Therefore, physiochemical analysis revealed that the milk samples didn't fulfilled the WHO standard in terms of TA and pH, whereas their density values are in agreement with WHO standard.

Proximate analysis

The proximate chemical compositions of milk samples are shown in Table 4. From the table, it found that the total solids values are between 10.89% and 12.22%. The SNF content of all milk samples are ranged from 6.02% to 7.21%. This value is within the maximum permissible SNF content of WHO standard for quality milk which is 7.71%. It also observed that there is no significant difference between samples collected from farmers and vendors in terms of the contents of SNF.

The results exhibited an average moisture content ranged from 86.32% to 87.58% for milk samples collected from farmers; while the moisture values are between 88.41% and 89.57% for milk samples collected from farmers. This showed that there is some increment of moisture content of milk samples from vendors; this is may be due to the addition moisture in the storage material or adulteration and preservation processes. The study showed that the fat contents are between 4.51% and 5.24% for milk samples collected from both vendors and farmers, which are good enough and above the minimum

Figure 3. Total solids of milk samples.

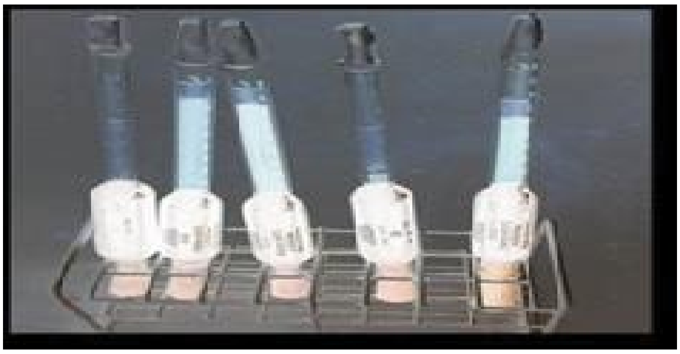

Figure 4. Fat readings of milk samples.

Table 1. Milk samples.

Sample name	Milk sample collected from
S_1	Itang, Nuer Zone (from farmers)
S_2	Lare, Nuer Zone (from farmers)
S_3	Metehar, Nuer Zone (from farmers)
S_4	Kebele 01, Gambella town (from vendors)
S_5	Kebele 03, Gambella town (from vendors)
S_6	Kebele 05, Gambella town (from vendors)

Table 2. Physiochemical analysis.

Milk samples	Titratable acidity (%)	Density(g/cm^3)	pH
S_1	1.23	1.027	5.03
S_2	0.85	1.028	5.12
S_3	0.96	1.030	5.52
S_4	2.21	1.023	4.98
S_5	1.98	1.029	5.05
S_6	2.10	1.020	5.23

standard of WHO which is 3.5%. According to our results the protein contents (3.02% - 4.21%) and lactose contents (3.91% -5.01%) are slightly higher as compared with the permissible standards of WHO and other scientific works, which are 2.6% and 2.8%, respectively. The ash content values are found between 0.41% and 0.63%; these values are lower than that of the maximum permissible limit (0.65%) as reported by Enb et al. [9]. However, the ash contents in milk samples collected from vendors are slightly greater when compared with milk samples collected from farmers.

Generally, the results obtained by the proximate analysis revealed that all milk samples fulfilled the WHO and other national and international standards except lactose and protein contents.

Nutrients analysis

Table 5 shows that all the samples comprised the best quality milk with respect to all nutrients as recommended by WHO standard. Both inorganic and organic nutrients concentration of all milk samples are within the maximum allowable concentrations. This indicated that the milk samples from all sites have optimum amount of nutrients.

Heavy metals analysis

From the nutritional point of view, metal contents of milk and dairy products can be grouped into essential elements (e.g. iron, copper and zinc) at low concentrations and non-essential or toxic ones (e.g. lead, cadmium, etc). The presence of the latter, even in low concentrations, is invaluable and leads to metabolic disorders with extremely serious consequences.

Dairy animals ingest metals while grazing on the pasture and when fed on contaminated concentrate feeds. Toxic metals such as lead, chromium and cadmium are common air pollutants and are emitted into the air as a result of various industrial activities, domestic effluents and combustion [11].

Various environmental contaminations such as soil, water, food and plants with these metals cause their incorporation into the food chain and impose a great threat to human and animal health [12]. Lead and cadmium are considered as potential carcinogens and are associated with etiology of a number of diseases in the cardiovascular system, kidneys, nervous system, blood and skeletal system. Chromium is indispensable for the structure and the activity of more than 300 enzymes responsible for nucleic acid and protein synthesis, cellular differentiation and replication, insulin, secretion, sexual maturation and it may also be involved in the functional performance of the immune system and other physiological processes [13].

In this study, the concentration of cadmium (Cd), lead (Pb), and chromium (Cr)) were determined in the milk samples collected from farmers and vendors,

Table 3. Proximate analysis.

Milk samples	Moisture (%)	Protein (%)	Fat (%)	Total solids (%)	Lactose (%)	Solids-not- fat (SNF) (%)	Ash (%)
S_1	87.58	3.34	4.83	10.95	4.51	6.12	0.41
S_2	86.32	3.17	4.71	11.27	4.87	6.56	0.52
S_3	87.08	3.87	4.87	10.89	3.91	6.02	0.47
S_4	88.65	4.21	4.51	11.40	4.55	6.89	0.60
S_5	88.41	3.02	5.24	12.22	4.86	7.21	0.56
S_6	89.57	4.04	5.12	12.11	5.01	7.09	0.63

Values are means of at least three measurements ± standard deviation.

Table 4. Some inorganic and organic nutrients levels in fresh milk.

Parameters	Na (mg/L)	Ca (mg/L)	Mg (mg/L)	Total phosphors (mg/L)	Total nitrogen (%)
S_1	1951.31	758.86	1852.32	59.02	0.46
S_2	1834.87	812.75	1675.87	64.43	0.54
S_3	1900.21	769.01	2050.65	80.12	0.42
S_4	2002.65	755.15	2031.76	73.89	0.64
S_5	1997.76	801.13	1892.63	55.05	0.55
S_6	1993.32	757.26	1790.21	48.34	0.42

Table 5. Heavy metals levels in raw cow's milk.

Parameters	Pb (mg/L)	Cd (mg/L)	Cr (mg/L)
S_1	1.24	ND	1.85
S_2	1.53	ND	1.67
S_3	1.37	ND	2.05
S_4	2.03	ND	2.03
S_5	1.21	ND	1.89
S_6	1.98	ND	1.88

ND: Not Detected

Table 6. Statistical evaluation of milk samples.

Parameters	Mean	Median	Interval	St Dev.	Variance	Skewness	Kurtosis
Fat	4.84	4.85	4.51-5.12	0.216	0.047	-0.367	-0.116
Protein	3.61	3.60	3.17-4.04	0.495	0.245	0.022	-2.387
SNF	6.65	6.72	6.02 -7.21	0.502	0.252	-0.282	-2.067
Density	1.02	1.02	1.02 -1.03	0.009	8E-05	0.000	-1.875
Acidity	1.55	1.60	0.85 - 2.21	0.61	0.373	-0.099	-2.780
Lactose	4.62	4.70	3.91- 5.01	0.398	0.159	-1.266	1.615
Ash	0.53	0.54	0.41 - 0.63	0.082	0.007	-0.400	-0.922
Moisture	87.92	87.95	86.32 -89.58	1.169	1.366	-0.635	1.366
pH	5.15	5.08	4.80 -6.00	0.199	0.039	1.550	2.326

in order to establish their baseline levels. The concentrations of heavy metals (Cd, Cr, and Pb) in the analyzed milk samples are presented in Table 6 and the results indicated that all analyzed heavy metals levels are trace and- below detection limits.

However, the levels of Cr and Pb in the milk samples, collected from vendors are slightly higher as compared with samples from farmers; this is may be due to some contaminations are occurred during storage and selling processes. These results suggested that various controlling mechanisms are necessary to avoid heavy metal contaminations of primary food products for enhanced quality and safety of vendors' milk samples.

Statistical Analysis

Statistical evaluations such as mean, interval standard deviation, median, variance, skewness and kurtosis for all physicochemical and proximate parameters of milk samples collected from both farmers and vendors are calculated and recorded in Table 6. Statistical results indicated that there are no significant variations among the samples and within a sample of replicate measurements.

Conclusion and Recommendations

Milk is a rich source of major and minor components which are essential to provide the nutritional requirements to human body. However, some commercial milk products contain comparatively higher concentrations of heavy metals and other contaminations. It suggests that the quality control for milk processing should be improved. This study showed that the quality of milk samples from different sources (dairy farms and vendors) is characterized by poor physiochemical quality; in terms of their titratable acidity and pH values since the values are beyond the permissible rang. However, they have good proximate chemical and mineral compositions except fat and lactose contents.

The milk samples have high moisture contents, pH values, acidity, and lactose and fat contents compared with WHO permissible standard levels. This indicated that the milk samples are not properly standardized based on their standards and routinely examined immediately before the products are released into the market. Thus, frequent inspection of cow's milk by responsible bodies is vital to check whether the products meet the minimum legal standards, and should monitored the overall hygienic process and handling of milk samples.

Furthermore, realistic standards for cow's milk need to be devised and appropriate training should be given to producers in hygienic and handling of processes of milk samples. It is also recommended that adequate sanitary measures should be taken at all stages of the processes of consumption of milk in order to protect the milk from spoil- age which eventually be poisoning a serious health risk to the consumers.

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Conflict of Interests

The authors declare that there is no conflict of interests on this paper.

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