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Comparative Study on Nutritional Values and Physicochemical Properties of Industrially Processed and Fresh Natural Milk Consumed in Arba Minch Town, South Ethiopia

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Abstract: **The aim of this study was to evaluate physicochemical quality of industrially processed and fresh natural milk samples found in Arba Minch town, and to compare the nutritional and mineral contents of the processed and raw milks. Physicochemical properties like pH, viscosity, Ash, total solid, acidity and conductivity are examined the results were found to be 6.54 ± 0.32 for Raw, 6.38 ± 0.197 for Anchor and 6.48 ± 0.33 in Harme brand, 1.38 ± 0.14 in raw 1.86 ± 0.22 in anchor and1.32 ± 0.12 in Harme,1.8 ± 0.032 in raw, 1.6 ± 0.001 in anchor, 1.4 ± 0.006 in Harme, 14 ± 0.25 in raw, 9.6 ± 0.183 in anchor and 14.76 ± 0.093 in Harme, 0.81 ± 0.005 in raw, in anchor, and 1.35 ± 0.009 in Harme, 2.10 ± 0.017 for raw, 4.38 ± 0.155 for anchor, 1.72 ± 0.038 for Harme of milk samples respectively. The nutritional values (protein, carbohydrate) of these milk samples were also determined to be 3.989 ± 0.012 for raw, 5.181 ± 0.018 for Anchor and 4.763 ± 0.192 in Harme, 3.3 ± 0.28in raw, 3.988 ± 0.015 in Anchor and 4.83 ± 0.14 in Harme respectively. Finally the micro and macro nutrients (Fe, Zn, Ca, Na and K) were analyzed using FAAS Table 4 and the values of all parameters were compared with the maximum limit of each parameter as cited by WHO (1986) guidelines to see the suitability of the milk for human body.**

Keywords: **Raw milk, Physico-chemical, FAAS, Nutritional Value, Micro and Macro Nutrients.**

1. INTRODUCTION

1.1. Back Ground of study

Throughout the world, milk is used as a food in one or more than one form for human consumption. It is considered as one of the most important diet items of many people because of its high nutritive value, and therefore has nutritionally been defined as "the most nearly perfect food" containing protein, carbohydrate, fat, vitamins and minerals that make it an absolute food to consume (Mehari, 1988; Komorowski and Early,1992).

Huge responsibility therefore lies on dairy producers, retailers and manufacturers to produce and market safe milk and milk products (Adeysin *et al*., 1995; Hahn, 1996; Menanne, 2007). In Ethiopia at large and Arba Minch at small scale, milk produced at smallholders' farm is marketed without any form of pasteurization or quality control measures, which means, hygienic control of milk and milk products are not usually conducted on routine bases. Moreover, door to door raw milk delivery in the urban and per urban areas is commonly practiced with virtually no quality control at all levels (Gudefay and Molla, 2000). The quality of raw milk is a term with a very broad meaning encompassing characteristics such as chemical composition, physical properties; quality, technological suitability and nutritive value (AOAC, 2000, Ali *et al*., 2012). Most of the studies conducted yet concerning the quality of either raw or pasteurized milk were on milk collecting centers and processing plants in and around Addis Ababa (Alehegne, 2004).

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Milk and its products are very common in our food due to their nutritive values of vitamins and lot of mineral constituents which are necessary for proper development and functioning of our organs. Some elements, being essential micro-nutrients have a variety of biochemical functions in all living organisms and form an integral part of several enzymes. However, overdose of these mineral constituents can be harmful to the consumers. Industrially processed milk equivalently provides both basic and additional requirements needed by children especially at their early developmental stage. This milk may be contaminated by heavy metals that at certain levels can cause toxicity to our body. The concentration of micro and macro elements in milk depends upon the concentration of these elements in soil and cattle feed, varying considerably from one to another place in the country (Farid *et al*., 2004). Thus, information about the concentration of dietary minerals (Ca, Mg, Se, Fe, Zn, Co and Cu) in milk is very important.

However, there is no study conducted on quality of fresh natural milk collected from dairy cooperative milk collection centers, hotels, small shops and small scale milk producers in Arba Minch town. In addition, there is no formal quality control system in place to monitor and control the quality of milk produced and sold in the town and as a result, there is no documented information on the safety and quality of raw milk produced and sold in Arba Minch town. Therefore, the objective of this study was to assess physical and chemical quality of raw milk produced and marketed in Arba Minch town and compare with industrially processed milk and further with the WHO (1986).

2. MATERIALS AND METHODS

2.1. Description of Study Area

The study was conducted in Arba Minch University (Abaya campus) which is found in Gamo Gofa zone. It is located at Arba Minch town, 505 km south of Addis Ababa in the vicinity of unique natural and anthropogenic diversity. Arba Minch town is located (situated, in Great Rift Valley) with average temperature and rain fall of 30 °C and 575 mmHg respectively. More over general elevation of the zone ranges from 600 to 3300 meters above sea level. Therefore, the spices sold in this town are expected to be cultivated in this zone's woredas.

2.2. Chemicals and Reagents

The chemicals and reagents which were used in this study were, Phenolphthalein indicator and for acid titration end point indication, NaOH used as titrant for neutralization of pH, buffer solution to control the pH change of the solutions, H_2SO_4used for digesting of organic matter, boric acid used to indicate whether the organic carbon is removed and nitrogen is present during protein determination, methyl red, Bromocresol green indicator, potassium sulphate, selenium catalyst speed up of the reaction, sodium tungstate, Benedict reagents,$HClO_4$, HNO_3,both are used for digestion organic matter during mineral analysis, HCL used as titrant during protein determination deionized water used for preparation of all standard solution and samples during mineral analysis , distilled water were required for sample preparation as well as for the sample analysis.

2.3. Instruments and Apparatus

Instruments and apparatus used are: pipette, oven drying the sample, pH meter used to measure pH of sample, beaker used in containing samples, viscometer for viscosity measurements, conductivity meter measure conductivity of sample, Kjeldahl tube used to distillate digested sample, digester used to digest sample, round bottom flask, conical flask, flat-bottomed aluminum dish, steam bath, thermometer measure temperature/ control temperature, desiccator used to cooling sample, crucible, burette, muffle furnace used for ashing of sample, FAAS, Erlenmeyer flask freezing dryer, volumetric flask, water bath, stirrer, filter were used.

2.4. Experimental Methods

2.4.1. Sample collection and preparation

Two industrially processed brands of popular milk sample (Harme liquid and Anchor powder) which were packed with aluminum sheet and consumed in Arba Minch town were purchased from super markets around Sekela. One litter of raw milk sample was also collected from Arba Minch University Abaya campus by plastic bottle that was first rinsed with acid and repeatedly washed with distilled-deionized water. Immediately after collecting the industrially processed liquid and raw milk samples, drying was done using freeze dryer (ALPH12LDPLUS) in order to change it into powder. And

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then it was labeled and put in ice box (4 ^{o}C for protection of microbial multiplication for subsequent analysis. After sample collection and preparation the step by step analysis was done for each parameter following the standard methods.

2.4.2. Analytical Procedure

2.4.2.1. Physical Analysis

The physical characteristics of various milk samples were determined according to standard methods of AOAC (2000). The pH measurement was made using a digital pH-meter. Acidity was measured by titrimetric method, and expressed as percent of lactic acid. Other parameters like conductivity and viscosity were determined as per the standard methods (Seher *et al*., 2013; Javaid *et al*., 2009; Songgrod and Worawat, 2015).

Determination of acidity in milk

Acidity of milk was determined by titration method given in AOAC (2000).10 g of powdered milk was taken and dissolved in 50 mL of warm distilled water at 28 ^{o}C. Acidity was determined by taking 10ml of milk sample prepared in Erlenmeyer flask and 2-3 drops of phenolphthalein was added and titrated against 0.1N NaOH until formation of pink color persevered. The titratable acidity, defined as percent lactic acid, was calculated by the following formula:

$$\% \text{ acidity} = \frac{\text{Volume of NaOH used(ml) X0.009}}{\text{weight of smaple}} \text{ X } 100$$ (Seher *et al*., 2013)

Determination of Viscosity

Viscosity of milk was determined with viscometer at required temperature according to the standard method of AOAC (1990). The rate of flow of a given volume of milk was compared with the rate of flow of the same amount of water. The viscosity of milk was calculated according to the following formula:

$$\text{Viscosity} = \frac{\eta 1}{\eta 2} = \frac{t1.d1}{t2.d2}$$ (Taiseer and Mohammed, 2012)

Where, $\eta 1$ *and* $\eta 2$ viscosity of milk and water respectively, $t1, d1$ time flow and density of milk and t_2, $d_2$time flow and density of water.

Determination of pH

The pH of milk was measured with digital pH meter. Buffers of pH 7 and 4 were used for the calibration of pH meter. After calibration, 100 mL volume of prepared milk sample was taken in a beaker and then the electrode was immersed and kept in the sample until constant reading is attained (Javaid *et al*., 2009).

Determination of conductivity

The conductivity of milk was measured by an electrical conductivity meter pouring 100 mL of each milk sample into a beaker separately at different temperature. For each sample, the measurements were repeated three times (Songgrod and Worawat, 2015).

2.4.2.2. Chemical Analysis

Different chemical properties of milk such as protein, carbohydrate, ash, and total solid content were estimated by different methods.

Determination of Protein in Milk

5 g of each brand of powder milk samples was transferred into a digestion tube and to each sample and also to an empty digestion tube (blank), the reagents for the digestion such as 2 tablets of potassium sulfate, selenium catalyst and 10 mL of concentrated sulfuric acid (98%) were added into the digestion tube which contain sample and mixed. The mixture was heated for 2:30 hrs at 420 ^{o}C on a Kjeldahl digestion apparatus fitted with reflex condenser.

The digested sample was removed from Kjeldahl digestion apparatus and allowed to cool to 50-60 ^{o}C and filled with 50 mL of distilled water. The cooled digested samples were transferred into Kjeldahl flask and 50 mL of 35% sodium hydroxide was added.

$(NH_4)_2SO_{4\,(aq)} + 2NaOH \rightarrow Na_2SO_4\,(aq) + 2H_2O_{\,(l)} + 2NH_3\,(g)$.

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The sample was distilled and collected into an Erlenmeyer flask containing 25 mL of 4% (w/v) boric acid until 100 mL of distillate received.

$H_3BO_3 + NH_3 \rightarrow NH_4^+ + H_2BO_3$

2-3 drops of indicator (Bromocresol green plus methyl red) was added to the Erlenmeyer flask containing distillated sample and titrated with 0.1 M HCl and green color of end point was obtained. Protein content was calculated as follow:

Nitrogen (%) $= \frac{T \times 0.1 \times 0.014 \times 20}{Weight of sample} \times 100$

Protein (%) =Nitrogen (%) ×6.38 (Taiseer and Mohammed, 2012). Where:

T= Titration figure

0.1= Normality of HCL

0.014= Atomic weight of nitrogen $^2/_{1000}$

20= Dilution factor

Determination of total solids

Total solid content was determined according to the modified method of AOAC (1990). 5 g of each dried and powdered milk sample was placed in a clean and dried flat-bottomed aluminum dish. The weight of each dish with sample was recorded, heated on the steam bath for 10 minutes and placed in an oven at 100 ^oC for 3 hrs. Finally, the dishes were taken out of the oven and transferred to desiccators to cool at room temperature. Heating, cooling and weighting were repeated several times until the difference between successive weighting were less than the sample taken. The total solid content was calculated from the following equation: $\text{Total Solid (\%)} = \frac{\text{Weight of sample after drying}}{\text{Weight of sample before drying}} \text{ X } 100$ (Taiseer and Mohammed, 2012)

Determination of Ash

The ash content of the milk was determined according to standard method of AOAC 1990. Five grams of milk samples were transferred into suitable clean dry crucible and evaporated to dryness in steam bath. The dried milk samples were ignited in muffle furnace at 550 ^oC for 1:30 hrs, and then cooled in desiccators and weigh. Lastly, the ash content was calculated as follows:

$\text{Ash(\%)} = \frac{\text{Wt of ash}}{\text{Wt of sample}} \text{X } 100$ (Taiseer and Mohammed, 2012)

Determination of carbohydrate (lactose)

5 g of milk sample was transferred into 50 mL volumetric flask and then 2.5 mL of sodium tungstate (10%) was added drop by drop with continuous shaking, which was then followed by addition of5 mL of 0.6N H_2SO_4 was added with continuous mixing and. Finally the volumetric flask was filled with distilled water up to the mark and the mixture was rested for 10 minutes and filtered by Whatmann filter paper No. 1. The filtrate was transferred into burette and then used as titrant for the solution that contains 25 mL of Benedict reagent with 30 mL distilled water and 2 g of anhydrous sodium carbonate to increase the alkalinity. The titration was continued drop wise till the reduction of the blue color was observed and the volume of filtrate (R) was recorded. Every 25 mL of benedict solution was reduced by 0.067 g lactose.

Lactose (%)$= \frac{0.067}{R} \times 10 \times 100$ (Seher *et al.*, 2013)

Determination of Mineral

By applying the optimized conditions, 0.5 g of dried, homogenized and representative of each brand of powder milk samples was transferred into 100 mL round bottom flask and mixed with 8 mL of a mixture of HNO_3 (69-72%) and $HClO_4$ (70%) with a volume ratio of 5:3 (v/v) and digested at 210 ^oC for 2 hrs and 30 minutes on a Kjeldahl digestion apparatus fitted with reflex condenser. The digested sample was cooled at room temperature. Digestion was carried out in

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triplicate and a reagent blank was performed in parallel with digestion powder milk samples keeping all digestion parameters constant.

5 mL of deionized water was added into the digested residue and filtered through Whatmann No 1filter paper. The volume of the filtrate was made up to 100 mL using deionized water and the solution was then used to determine the calcium (Ca), sodium (Na), potassium (K), iron (Fe) and Zinc (Zn) content of the digested samples using FAAS. Atomic absorption spectroscopic standard solutions containing 1000 mg/L were used for preparing intermediate standard solutions (10 mg/L) in 100 mL volumetric flask and working standards using deionized water. Working standards of metal solutions were prepared in 100 mL volumetric flask by diluting with deionized water.

Determination of Method Detection Limits

The method detection limit in this study was determined for micro and macro minerals using FAAS in each milk sample with a blank reagent, i.e. 8 mL mixture of 69-72 % $HNO_3$and (70 %) of $HClO_4$ were added to acidify 100 mL of deionized water which was used for the dilution of standard solutions. It was determined for each mineral as three times the standard deviation of the blank solution (3σ blank, n = 7) as indicated in table 1. The results showed that, the method detection limit for each metal was below the instrumental detection indicated on the manual and therefore, it was possible to determine the minerals using the instrument stated above.

Table 1: Instrument and method detection limits for the analysis of milk sample by FAAS

Detection Limits in ppm or mg/L		
Elements	IDL	MDL
Na	0.005	0.0025
Ca	0.05	0.0081
K	0.01	0.0067
Fe	0.05	0.0068
Zn	0.005	0.0032

Statistical analysis: Each experiment was repeated three times. The results are presented with their means, and standard deviation.

$$\text{Mean} = \frac{\sum_{i=1}^{n} xi}{n}$$

$$\text{Standard deviation } (\sigma) = \sqrt{\frac{\sum_{i=1}^{n} (xi - \overline{x})^2}{n-1}}$$

3. RESULT AND DISCUSSION

3.1. Accuracy and precision

Analytical results must be evaluated to decide on the best values to report and attempt to establish the probable limits of errors of the values. In this study the precision (repeatability) of the results was evaluated by the pooled standard deviation and relative standard deviation of the results of three samples with triplicate measurements of each sample (n = 3) were used for the analysis of physical parameter, composition of analysis-proximate analysis, nutritional value, and macro and micro elements of milk sample. The results are summarized in Table 2, 3 and 5 respectively.

3.2. Physical properties

The physical parameters such as pH, conductivity, viscosity and titratable acidity are important parameters in studying the physicochemical compositions and nutritional issues of milk. Table 2 shows the various physical parameters determined for the raw and industrially processed milk sample consumed in Arba Minch town.

Titratable acidity: The values for the titratable acidity determined were in the range from 0.78 ± 0.006 % to 1.35 ± 0.009 % lactic acid (Table 2). In the Anchor milk samples, the trend for the titratable acidity is the lowest value followed by the raw and Harme samples. According to WHO (1986) guidelines, the titratable acidity range from minimum 0.3% to 18% for various packed milk samples. The result of this study for titratable acidity was found within the WHO values.

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pH: The pH is the parameter that determines the sample acidity and alkalinity. The pH of all the milk samples analyzed by pH meter (MP 220) of (Raw, Anchor, and Harme were 6.54 ± 0.32, 6.38 ± 0.197 and 6.48 ± 0.33 respectively. The order of the pH measured for the three samples can be put as, Harme >Raw >Anchor brand. Comparing with the previous investigation (6.38±0.60 to 6.77±0.88) and the WHO guide line recommended values for pH in milk (6.6-6.8) (Javaid *et al*., 2009), the pH range for the current study was also found to comply and fall within the range.

Conductivity: The conductivity for each milk samples was determined by conductivity meter (HACH HQ40d multifunctional portable meter). It is mainly due to the presence of various electrolytes. The variation in conductivity may be due to the different levels of the electrolytes present in the milk samples. The conductivity range of Raw, Anchor, and Harme milk samples determined were: 2.10 ± 0.017 mS, 3.38 ± 0.055 mS and 1.72 ± 0.038 mS, as shown in Table 2. From the table we can see that, Anchor > raw > Harme sample. The WHO guide line Codex Satan 243-2003 conductivity recommended was 3.84-4.06 mS which include the result of the current study.

Viscosity: Viscosity of each sample was determined by viscometer and the value obtained was compared as Anchor > Raw >Harme as shown in Table 2.The standard viscosity of milk was 1.38 ± 0.41 Pa s according to different literature (Javaid *et al*., 2009; Taiseer and Mohammed, 2012). Compared to the standard literature values, raw milk was in the limit, but Anchor and Harme brand not comparable due to cool temperatures in Anchor, whereas denaturation of the protein when the temperature is above 65^{o}c in Harme.

Table 2: Physical analysis of milk samples consumed in Arba Minch town

Sample	PH	Acidity %	Conductivity (ms)	Viscosity (cp)
Raw	6.54±0.32	0.81±0.005	2.10±0.017	1.38±0.14
%RSD	4.94	0.62	0.81	10.14
Anchor (Powder)	6.38±0.197	0.78±0.006	4.38±0.155	1.86±0.22
%RSD	3.08	0.76	3.54	11.82
Harme (Liquid)	6.48±0.33	1.35±0.009	1.72±0.038	1.32±0.12
%RSD	5.09	0.66	2.21	9.09

3.3. Chemical Analysis

Several chemical analyses such as percentages (%) of fat content, protein content, total solid (TS), ash content have been done for this study.

Total solid: Total solid of each milk sample was determined by drying milk sample in oven (Britsh B35) at 100^{o}C for 3 hrs and expressed in terms of %. The determined values of total solids were 14 ± 0.25, 9.6 ± 0.183 and 14.76 ± 0.093 % for Raw, Anchor and Harme respectively as given in Table 3. Anchor had the lowest amount of total solids 9.6 ±0.183 % followed by raw milk (14 ± 0.25 %) and the highest was found in Harme milk (14.76 ±0.093 %. The total solids in the milk from literature ranged from 10 to 17% (Mohammad *et al*., 2008). The results determined show that values for the total solids in all the milk samples were in good agreement with the reported literature value.

Lactose: The carbohydrate (lactose) amount in each sample was determined by titration method and was compared as: Harme (4.83± 0.14) brand > Anchor brand (3.988± 0.015) > Raw (3.3± 0.28) sample. Compared to values reported in the literature (2 to 5 %) (Mohammad *et al*., 2008) the lactose values for the samples selected in this study are in good agreement.

Protein: The protein content of the samples considered in the study was determined using Kjeldahl method and the values were put as Anchor (14.76 ± 0.0093 %) > Harme (4.763 ± 0.192 %) > Raw milk (4.763 ± 0.192 %). The declaration of milk protein content shall be in a manner acceptable in the country of sale and it can be (i) as a percentage by mass or volume, or (ii) in grams per serving as quantified in the label provided that the number of servings was stated. According to WHO /FAO/ Codex Stan 243-2003, the minimum milk protein composition is 2.7 (Slavica *et al*., 1998).

Ash content: The white ash is mainly composed of oxides and chlorides of mineral elements, which include lime, magnesia, soda ash, potash, phosphorus oxides, sulfur trioxide, ferric oxide, etc. The ash in the sample was determined by igniting the sample in to the muffle furnace (FUSEF 1AH, R000047UK) and the values for each were shown in Table 3.

Raw milk ash content was greater than that of Harme and Anchor. According to previous literature guide lines the ash content are, for raw milk (0.64±0.07), powder (0.55±0.06) and liquid (0.53±0.09) (Taiseer and Mohammed, 2012).

Table 3: Compositional analysis of raw and industrial processed milk samples (mean ± SD)

Composition in (%)	Milk Samples		
	Raw (liquid)	Anchor (solid)	Harme (liquid)
Total solid	14 ± 0.25	9.6 ± 0.183	14.76 ± 0.093
Ash content	1.8 ± 0.032	1.6 ± 0.001	1.4 ± 0.006
Lactose content	3.3 ± 0.28	3.988 ± 0.015	4.83 ± 0.14
Protein	3.989 ± 0.012	5.181 ± 0.018	4.763 ± 0.192

Micro and Macro element analysis

Under the micro and macro mineral determination, a calibration curve was established for each metal at five points by running the prepared standard solutions in flame atomic absorption spectrometer. Linear correlation coefficient (>0.993) was obtained for each analyte. After calibration, the sample solutions were fed into the FAAS instrument and absorbance were recorded with three replicate runs. The same analytical procedures were employed for the determination of minerals in blank samples. The concentration of analytes in each sample was determined from the calibration curve drawn through origin and using the absorbance measured. The precision results were evaluated by the mean, standard deviation with triplicate measurements of each sample (n=3) and mentioned in Table 4.

Table 4: Minerals concentration in the samples considered (Mean ± SD) analyzed by FAAS

Samples	**Elements in (ppm)**				
	Na	Ca	K	Fe	Zn
Anchor	9.5 ± 0.44	4.34 ± 0.196	34.86 ± 0.59	0.42 ± 0.49	0.371 ± 0.02
Harme	87 ± 0.379	3.536 ± 0.18	48.74 ± 0.27	0.117 ± 0.01	0.227 ± 0.03
Raw	6.7 ± 0.153	4.786 ± 0.16	28.89 ± 0.69	0.988 ± 0.12	0.321 ± 0.04

Sodium (Na): The determined concentration of Na is 6.5 ± 0.15, 9.5 ± 0.44 and 87 ± 0.37 ppm in raw, Archor and Harme respectively. According to WHO (1989), the concentration of this metal is 4420, 67 and 133 ppm respectively in raw, Anchor and Harme indicating that, the determined value in this study is acceptable.

Calcium (Ca): The concentration of calcium determined in raw, Anchor and Harme were respectively, 4.786 ± 0.164, 4.34 ± 0.196 and 3.536 ± 0.181 ppm. According to WHO (1989) calcium level in milk were reported as: 12900 for raw, 599 for powder and 662 ppm for liquid respectively. Therefore the determined calcium was well below for each milk sample when compared to standard WHO (1989) level.

Potassium (K): The potassium concentration determined in each sample considered in this study is listed in Table 4. Comparing it's concentration among the three samples, the order looks like, Harme > Anchor > Raw milk. According to WHO (1989) standard, the level of potassium concentration was reported as 1722, 129 and 800 ppm in raw, powder and liquid milk respectively indicating that the value in the present study being far less than the WHO (1989) standard.

Zinc (Zn): The zinc amount in this study is tabulated in Table 4, and comparing them among the samples considered, Anchor > Raw > Harme. The average daily intake of zinc determined in these samples is 0.24 ± 0.034 ppm/day while the recommended daily intake of this metal is 1200-1500 ppm/day (Farid *et al*., 2004).

Iron (Fe): The determined concentration of iron in the samples considered in this study were 0.988 ± 0.283, 0.420 ± 0.0699 and 0.117 ± 0.0462 ppm for Raw, Anchor and Harme milk respectively.

4. CONCLUSION

In the present study, preliminary investigations were carried out to ascertain the physicochemical characteristics and nutritional quality of various milk samples marketed in Arba Minch. The results obtained were compared with different literature and international guide lines values showing that except mineral elements, all parameters of the tested milk samples were within the recommended nutritional levels. These findings may be helpful for the concerned governmental

parties to monitor the quality of the milk products in the market of Arba Minch. It would be of great interest if further investigations are carried out to examine other organic and inorganic components in the milk in the markets.

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