Project Report
On
"Quantitative assay of major constituents and determination of heavy metals in commercially available milk in Bangladesh"

Submitted To

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LETTER OF TRANSMITTAL

20th December 2018

Professor Dr. Md. Bellal Hossain

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Subject: Submission of Project Report

Dear Sir,

I would like to take this opportunity to thank you for the guidance and support that you have provided me during the course of this report. Without your help, this report would have been impossible to complete.

To prepare the report I collected what I believe to be most relevant information to make my report as analytical and reliable as possible. I have concentrated my best effort to achieve the objectives of the report and experience gathered during report preparation will immeasurably help in my future professional life. I request you to excuse me for any mistake that may occur in the report despite of my best effort.

I would really appreciate it you enlighten me with your thoughts and views regarding the report.

I therefore, would like to place this report for your judgement and suggestion. Your kind advice will encourage me to perform better planning in future.

Sincerely yours

Md. Rayhan Asif Khan Shuvo

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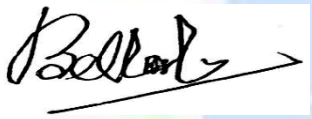
CERTIFICATE OF APPROVAL

I am pleased to certify that the project report on **"Quantitative assay of major constituents and determination of heavy metals in commercially available milk in Bangladesh"** conducted by **Md. Rayhan Asif Khan Shuvo**, bearing ID **151-34-366** student of the Department of Nutrition and Food Engineering has been approved for presentation and defense/ viva-voce.

I am pleased to hereby certify that the data and findings presented in the report are the authentic work of **Md. Rayhan Asif Khan Shuvo**. I strongly recommended the report presented by **Md. Rayhan Asif Khan Shuvo** for further academic recommendations and defense/viva-voce. **Md. Rayhan Asif Khan Shuvo** bears a strong moral character and a very pleasant personality. It has indeed a great pleasure working with him. I wish him all success in life.



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Dedicated to my beloved parents, my honorable teachers and to my well-wishers.

ACKNOWLEDGEMENT

During the preparation of this project report, I would like to acknowledge the encouragement and assistance given to me by a number of people. At first, I would like express my gratitude to my creator the Almighty Allah for enabling me the strength and opportunity to complete the report in time successfully. I am grateful to each and every person who are involved with me in every phase of my life.

I am grateful to my parents without whom I cannot be here. Without the support of my parents, I could not be able to achieve my objectives and goals.

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My gratitude goes to entire Nutrition and Food Engineering Department for arranging project work that facilitates integration of theoretical knowledge with real life situation.

Abstract:

Present study aimed to determine the amount of protein, fat, and the level of heavy metals present in pasteurized milk those are locally available in the market in Bangladesh. Protein was determined by the kjeldahl method, fat was determined by Garber method, and pH was determined by the Hannah pH meter by following AOAC. Study taken to the mentioned contents are in standard range or not.

It was observed that sample M contained the higher percentage of protein (4.5%), where sample P and F contain lower percentage of protein (2.2%). Standard range given by food regulatory body is 3.3% to 3.8%. Protein content only sample A showed protein content is in the range.

Fat percentage of the samples P and F are within range 3.5% (3.5% to 3.7% as FDA). But for sample A is found to be slightly higher and for sample M it is of slightly low value.

pH value of the samples also varied within 6.43 to 6.68 which consistent with the guided value available. (6.5 to 6.9 as FDA)

Lead and cadmium were determined by using Flame Atomic Absorption Spectroscopy, where lead and cadmium detected in all samples. Cadmium were detected within acceptable range but lead were found in higher amount than acceptable range.

1. Introduction

1.1 Milk

Milk is nutrient rich and best balance diet for human health. Milk is helpful to reduce many diseases, as it is good sources of many nutrients like macro and micronutrients such as protein, fat and calcium, magnesium, zinc. Because of its nutritional value, people are consuming this milk [1]. Milk is known all over world for its beneficial impact on human health. Milk is very expensive liquid food that secreted from animal. Therefore, it is necessary to provide good quality of milk to the consumer and reduce the price. The white color of milk is due to dispersion of light from fat globule and casein micelles. Milk, which is lower amount of fat, is blueish [2].

Milk composition, analysis on 100g milk [3]

Constituents	**Amounts**
Water	88g
Protein	3.3g
Fat	3.9g
Carbohydrate (lactose)	4.8g
Minerals	0.7%
Energy	66kcal
	275kj

This table is useful for pasteurized milk.

In raw milk, the amount of these constituents can be varied due to feeding pattern of animals or the sources of food sources that animals are consume. So that pasteurization process had introduced to maintain this amount.

1.2 Pasteurization

Pasteurization is a heat treatment process which is use to destroy or inactive the pathogenic bacteria, organism which are responsible for spoilage. Milk is an excellent source for the growth of microorganisms. This temperature in under 100°C. This process helps to increase the shelf life of product [4].

In 1880 scientist LUIS PASTEUR found heating process is able to inactive the harmful microorganisms in wine [5-6]. Now pasteurization is widely used in dairy industries for increase the shelf life, food quality and safety also. This process also inactivates spoilage enzyme [6] .

Milk is a highly perishable food so when milk is stored in room temperature bacteria and other microorganisms are grow rapidly [7]. Pasteurization process on food items can protect human from various diseases. This protect from **tuberculosis, brucellosis, Q-fever.**

The killing of some bacteria protects human from these diseases. The killing bacteria's are **Salmonella, Listeria,** and **E.coli** [8]**.**

Types of pasteurization

- **Low Temperature Long Time (LTLT)** in milk industry milk is pasteurized for long time in low temperature. At 62.8°C for 30minutes.
- **High Temperature Short Time (HTST)** normally milk is pasteurize for short time at high temperature. At 72°C for 15seconds.
- **Ultra High Temperature** it is also known as ultra-heat temperature. Milk is pasteurize for few seconds at very high temperature. At 135°C for 1-2 seconds [9].

Pasteurization process

Pasteurization is a heat treatment process. It allows both heating and cooling. The heating temperature is below 100°C and depends on types of pasteurization. Mainly the acidity of the food is determine that which type of pasteurization have to apply. Pasteurization can be applied for both

packaged and unpackaged food items. Both heating and cooling process are designed to prevent the change of foods size, shape, texture and phase.

Some acidic foods like drink which has pH<4.6, are considered under pasteurization for increase the shelf life and quality by inactivate the enzyme. This process is apply for destroy pathogens and spoilage microorganisms. Some other food whose pH>4.6 such as milk is pasteurize for destroy pathogens and spoilage organisms such as yeast, molds. For pH>4.6 are need to store in refrigerator after pasteurization. If it is pasteurize by following the Ultra High Temperature then milk can be stored at room temperature [4].

Many bacteria are kill by pasteurization process. Some of them with their pH value and pasteurization temperature [10]:

Bacteria	**pH**	**Pasteurization Temperature (°C)**
Escherichia coli	>6.8	65
Staphylococcus aureus	>6.7	66.5
Cronobacter sarazakii	>6.7	67.5
Salmonella	>6.9	61.5
Listeria monocytogenes	>6.9	65.5
Yersinia enterocoliticia	>6.8	62.5

All of these bacteria are killed within 15seconds of pasteurization at these different degrees of temperature.

1.3 Protein

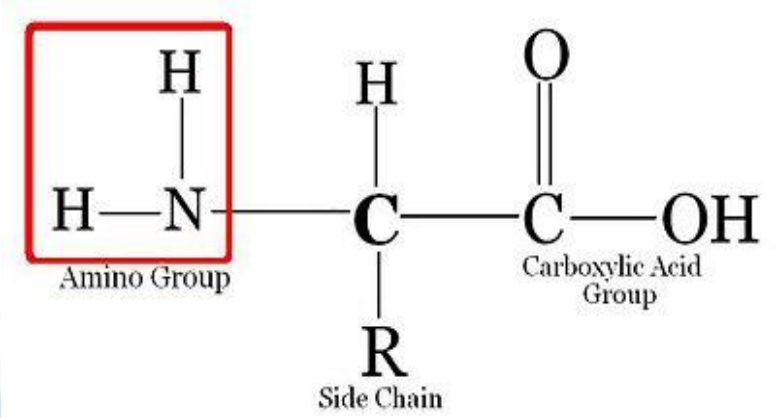
Protein is a macronutrient. An organic compound consist of amino acid. This amino acid is made by peptide bond. Amino acids are two types. Some are essentials and some are not essential. Our body cannot produce essential amino acid. First, we eat food, after digestion protein convert into the amino acid. Animal source food are good source for the essential amino acid than plant source amino acid. Our body can produce non-essential amino acid from the essential amino acids.

Types of amino acid [11]**:**

Essential amino acid	Non-essential amino acid
Arginine	Alanine
Histidine	Asparagine
Valine	Aspartate
Leucine	Cysteine
Isoleucine	Glutanimc
Lysine	Glycine
Methionine	Ornithine
Phenylalanie	Proline
Threonine	Serine
Tryptophane	Tyrosine

First two (arginine and histidine) essential amino acids are for the babies. Only they can produce those amino acids including the rest of the amino acids. However, for adult, they cannot produce (arginine and histidine). But rest of the amino acids they can produce through the consumption of food. Excess amount of consumption of protein is harmful. Because protein inside in our body are not useful, they can produce urea, uric acid.

Intake should be 0.8gram of protein per kg. For athletes or other condition, this can be changed.



1.4 Fat

Fat is one of the major nutrients among the macronutrients. It is consist of **glycerin** molecule with three fatty acid. Functional group –COOH is attached to unbranched hydrocarbon which is connected by single bond is known as saturated fatty acid. If this functional group –COOH is attach with branch or unbranched hydrocarbon that are connected to single bond or double bond or the both bond is called unsaturated fatty acid. Fats are helpful for construction and maintenance the cell membrane. Fats are also needed to regulate body temperature and sustain the health of skin and hair.

Fats should come from the diet. Our body cannot produce the essential fatty acid. So, when consume the food, after digestion this fat part convert into the essential fatty acid [12].

Two essential fatty acids for human are [13]:

- Alpha-linolenic acid (omega-3 fatty acid)
- Linoleic acid (omega-6 fatty acid)

Each gram of fat burn inside the body it releases 9 calories that is similar to 38kj (8.8kcal) [14].

1.5 Heavy metal

Heavy metals are those metals that has density more than 5g/cm^3 and atomic weight from 63.546 to 200.590 and their specific gravity should greater than four. Heavy metals are toxic and poisonous. Trace amount of heavy metals are required for body metabolism but excess amount can is harmful. Water, air and environment can be contaminate by heavy metals due to presence of heavy metals in these sources. These heavy metals can enter human body by ingestion and inhalation. These heavy metals are responsible for the sickness of human being. When lactating animal exposed to the heavy metals then they collect these metals into their. By this way, heavy metals are found in milk. Heavy metals are in the food, environment or in water are increases due human activities. Heavy metal affected water, food and environment are the source for the sickness of human health. This can damage nervous system, cancer and ultimate death [15]. Heavy metals such as lead (Pb) and cadmium (Cd) are found in the environment that are coming through the different wastage of different factories. As milk is a unique food for every age people and in contain both macro and micronutrients. Therefore, it is need to reduce the heavy metals from milk [16].

1.6 Literature review

From the different articles we can summaries that milk is very much unique food product for every age people. It contain both macronutrients and micronutrients. Pasteurize milk is with better quality and longer shelf life. However, due to the air, environment and water milk are also polluted. Milk is also polluted. When lactating cows are exposed to the heavy metal then theses heavy metals are enter in their milk.

From this information, this report is design to find out macronutrients and heavy metals in commercially available pasteurize milk.

2. Materials and Methodology [17]

2.1Determination of protein by kjeldhal method

Kjeldahl method means the measuring of amount of nitrogen in sample. Johan kjeldahl discover this method in 1883. This kjeldahl method is done in three stages. The stages are:

Digestion

The organic nitrogen in samples are utilize the acid solution and this is perform as homogenous sample at boiling temperature in presence of concentrated sulfuric acid. The final product in this digestion flask is ammonium sulfate solution. Only sulfuric acid in this digestion process will take long time. Therefore, that digestion mixture is use that is responsible for increasing the temperature and complete the digestion quickly. This digestion mixture is use as catalyst.

Fig: no fume inside.

Distillation

Addition of excess base to the digestion solution is convert NH_4 to NH_3 followed by boiling and condensation of the NH_3 gas in trapping conical flask.

Titration

To quantify the amount of ammonia ion in the receiving flask. Pink color solution will turn into light yellow color.

Reaction occurring in digestion flask

Organic N $\rightarrow NH_4^+ + H_2SO_4 \rightarrow (NH_4)_2SO_4 + H_2O + CO_2$

Protein $\xrightarrow[\text{Heat, catalyst}]{H_2SO_4}$ $(NH_4)_2SO_4$ (ammonium sulfate)

Reaction occurring in distillation flask

$(NH_4)_2SO_4 + 2NaOH \rightarrow 2NH_3 + Na_2SO_4 + 2H_2O$

Apparatus/chemicals

- H_2SO_4
- Digestion mixture (2g $CuSO_4$ + 98g K_2SO_4)
- 40% NaOH
- 0.1N NaOH
- 0.1N HCl
- 1% Methyl red indicator
- Distilled water

Preparation of 0.1N HCl

- First take 1ml of HCl (concentrated) (merk, Germany)
- Then make it upto 250ml using distilled water in a volumetric flask.

Preparation of 0.1N NaOH

- Take 1g NaOH (merk, germany)
- Then make it upto 250 ml using distilled water in a volumetric flask.

Preparation of 1% Methyl Red

- First measure 0.01g of methyl red powder in a conical flask..
- Then take 30ml of ethanol.
- Add 50ml of distilled water.

Preparation of 40% NaOH

- Take 40g NaOH in a volumetric flask
- Then make it 100ml using distilled water.

Procedure

1. **Digestion**
   - Take 0.4g sample (milk) then add 10ml H_2SO_4 and 2g digestion mixture.
   - Put this into the digestion flask.
   - Take two-digestion flask for each sample.
   - When digestion was started, the heat was slowly increase.
   - First temperature was 50°C, after 5hours the temperature was increased to 80°C and after the 6hours increased to 100°C, heated for 24 hours.
   - At the end point there is no white smoke of H_2SO_4 and the solution is clear
   - Finally cool it for some time.

2. **Distillation**
   - ❖ After digestion, the solution was poured volumetric flask and make it 100ml using distilled water.
   - ❖ Then 10ml of the solution from the volumetric flask into the distillation flask.
   - ❖ Then add 150ml of distilled and 10ml of 40%NaOH into the distillation flask.
   - ❖ Use three distillation flask where one flask of them will blank, i.e. take 150ml distilled water and 10ml of 40%NaOH.
   - ❖ In trapping conical flask, take 10ml of 0.1N HCL and 3drops 1% methyl red indicator.
   - ❖ Then the distillation unit was ran for 30mintues.

3. **Titration**
   - ❖ The burette was filled by 0.1N NaOH
   - ❖ Bring the trapping conical for titration.
   - ❖ The end point was color changed from pink to light yellow.

Calculation

$$\frac{(B-S)\times 1.4\ (N)\times 10\times 6.38\times 0.1(NaOH)}{sample\ weight}$$

$$\frac{(B-S)\times 1.4\times 10\times 6.38\times 0.1}{sample\ weight}$$

2.2 Determination of Fat by Gerber method

Swedish scientist Dr. Niklaus GERBER developed the Gerber method in 1891. For fat determination in milk or dairy product Gerber method is one of the best method to apply. In this method, fat is separated from the solution by centrifugal force. Sulfuric is use to dissolve the protein and it create membrane beside the fat globule. Amyl alcohol is use to separate the fat from the sample.

Apparatus/chemicals

- Sample
- Butyrometer
- H_2SO_4
- Isoamyl alcohol
- Centrifuge machine
- Pipette and pump (10ml pipette, 1ml pipette and 10.75ml).

Procedure

- Take 10ml of H_2SO_4 into the butyrometer.
- Then add 1ml of isoamyl alcohol.
- Finale add 10.75 ml milk sample.
- Then close the butyrometer by rubber lid.
- Then place the butyrometer into the centrifuge machine.
- Butyrometers are placed inside the centrifuge machine opposite of each butyrometer by inverting. More than one butyrometer are required to maintain the balance inside the centrifuge machine.
- Run the centrifuge machine at 1100 rotation per minutes (rpm) for 5minutes.
- Stop the centrifuge machine and keep the butyormeter inside the centrifuge machine for few moments then brought that out.
- After that, take reading of the butyrometer.

2.3Determination of pH

Determination of pH means the quantify the amount of acid or base in the sample. The idea of pH first introduced by Danish scientist Søren Peder Lauritz Sørensen at the Carlsberg Laboratory in 1909.

- pH is the negative log of hydrogen ion concentration.
- pH is measured by the pH meter.
- pH ranges are start from 0 and end at 14. The point 7 means the sample pH is neutral. When this pH value decreases from 7 means it is acidic. And when pH value increases from 7 means sample is alkaline.

To determine pH value by the pH meter it is need to calibrate by two-buffer solution one is buffer solution seven and another is buffer solution ten.

pH 7 preparation

- Take 100ml 0.1M tris (hydroxymethyl) aminomethane
- Add 93.2ml of 0.1M HCl

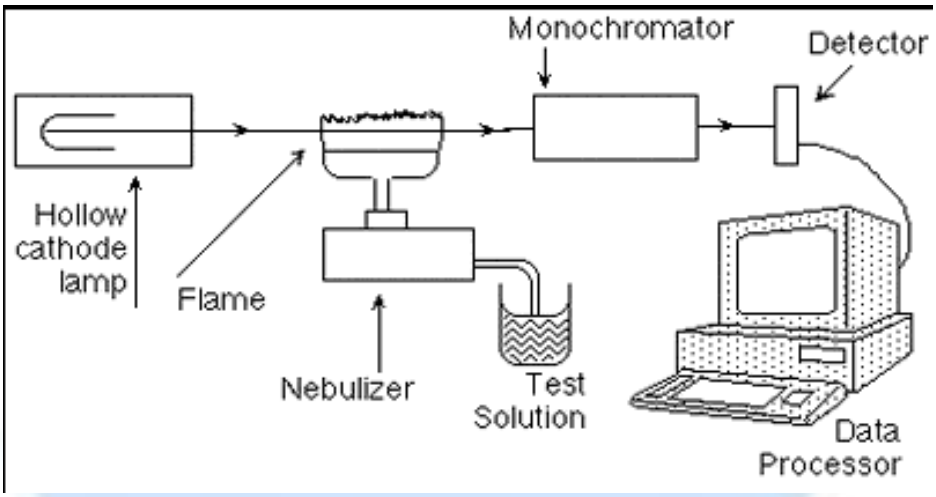
pH 10 preparation

- Take 100ml 0.05M $NaHCO_3$
- Add 21.4ml of 0.1M NaOH

2.4 Determination of Heavy metal by Flame Atomic Absorption Spectrophotometry

Atomic absorption spectroscopy was first used in second half of the 19th century by Robert Wilhelm Bunsen and Gustav Robert Kirchhoff, both professors at the University of Heidelberg, Germany.

- Flame atomic absorption is very common method to detect the metal ion present in the sample.
- The technique is, the metal absorb the light on a specific wavelength.
- The metal ion converted in atomic ion into the flame.
- When the light is pass through the sample the absorbed light is measure and the concentration is also obtain.
- This flame atomic absorption technique provide better result to detect metals in sample.
- For this reason, this technique is widely use.

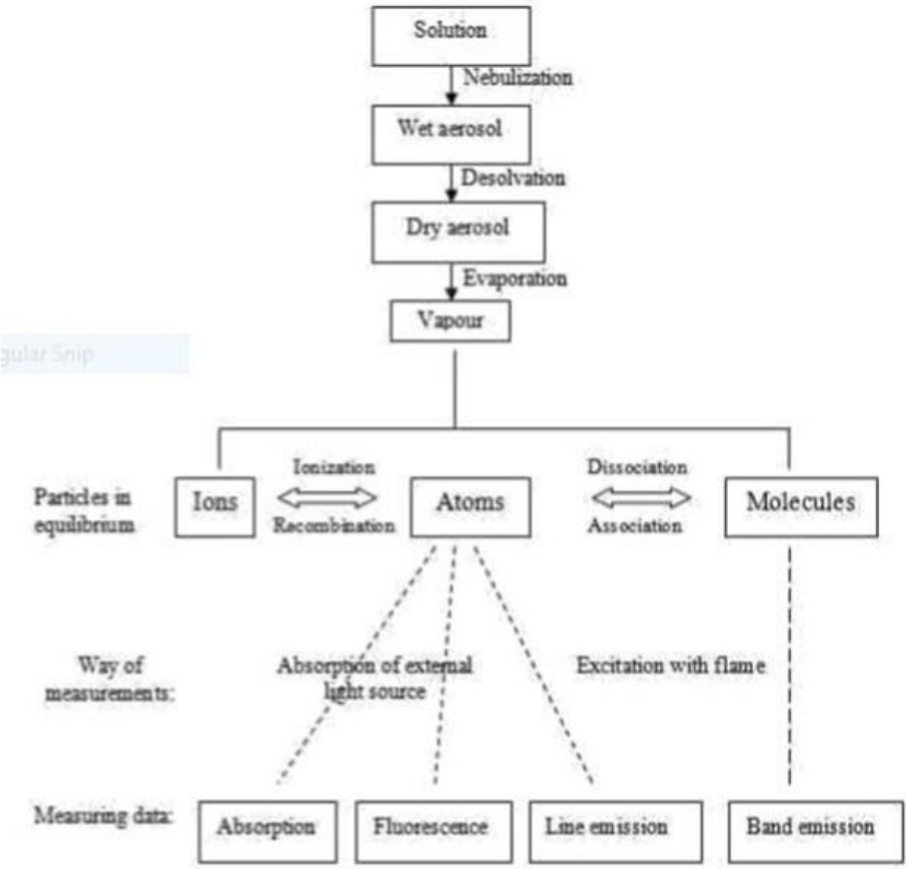


Parts of Flame Atomic Absorption Spectroscopy

- **Capillary tube**
  It suck the sample solution from the vial.
- **Nebulizer**
  It convert the sample in mist or aerosol.
- **Hollow Cathode Lamp (HCL)**
  Light source of FAAS.
- **Flame**
  Combination of air acetylene (C_2H_2). Operating range between 2125°C to 2400°C
- **Monochromator**
  it is a device that isolate, separate and control the intensity of narrow region of light which transmit the light to detector.
- **Detector**
  This device is capable to convert the radiant intensity into the electronic signal whish measure the transmittance of sample.

Working process of Flame Atomic Absorption Spectroscopy

- First prepared samples are taken into vials.
- Then capillary tube dipped into the vial and it sucked the sample solution.
- Then nebulizer convert the sample into aerosol and spray this to the flame.
- Then released light which in specific wavelength pass through the flame and atomic ion absorb the light.
- Absorb light is then enter to the monochromator. Which isolates the line interest.
- Detector then measures the absorbed light.
- After completing the process computer shows the result. Light that absorbed and the concentration obtain.



Standard solution preparation

To run sample in this Flame Atomic Absorption Spectroscopy first the machine need to calibrate by standard solution. Pb solution and Cd solution was used to calibrate.

Procedure of standard solution preparation (Pb)

- First, take 2ml of Pb into volumetric flask.
- Then add 98ml of distilled water to make it 100ml.
- This solution is 20ppm.
- From this 100ml solution of 20ppm prepare 16pmm, 10ppm, 8ppm, 5ppm, 2ppm and 1ppm

This solution was prepared following the formula, $S_1V_1=S_2V_2$

For example: preparation 25ml of 16ppm from 20ppm by following the following formula.

Here S_1= 20ppm

S_2= 16ppm

V_2= 25ml

V_1=?

From the formula $S_1V_1= S_2V_2$

$20 \times V_1 = 16 \times 25$

$V_1 = 20$

So 20ml should take from that 100ml of 20ppm and add distill water 5ml.

Fig: standard solution of Pb.

Serial no	Concentration ppm	absorbance
1	0.00	0.0004
2	1.00	0.0079
3	2.00	0.0147
4	4.00	0.0310
5	8.00	0.0619
6	10.00	0.0748
7	16.00	0.1144

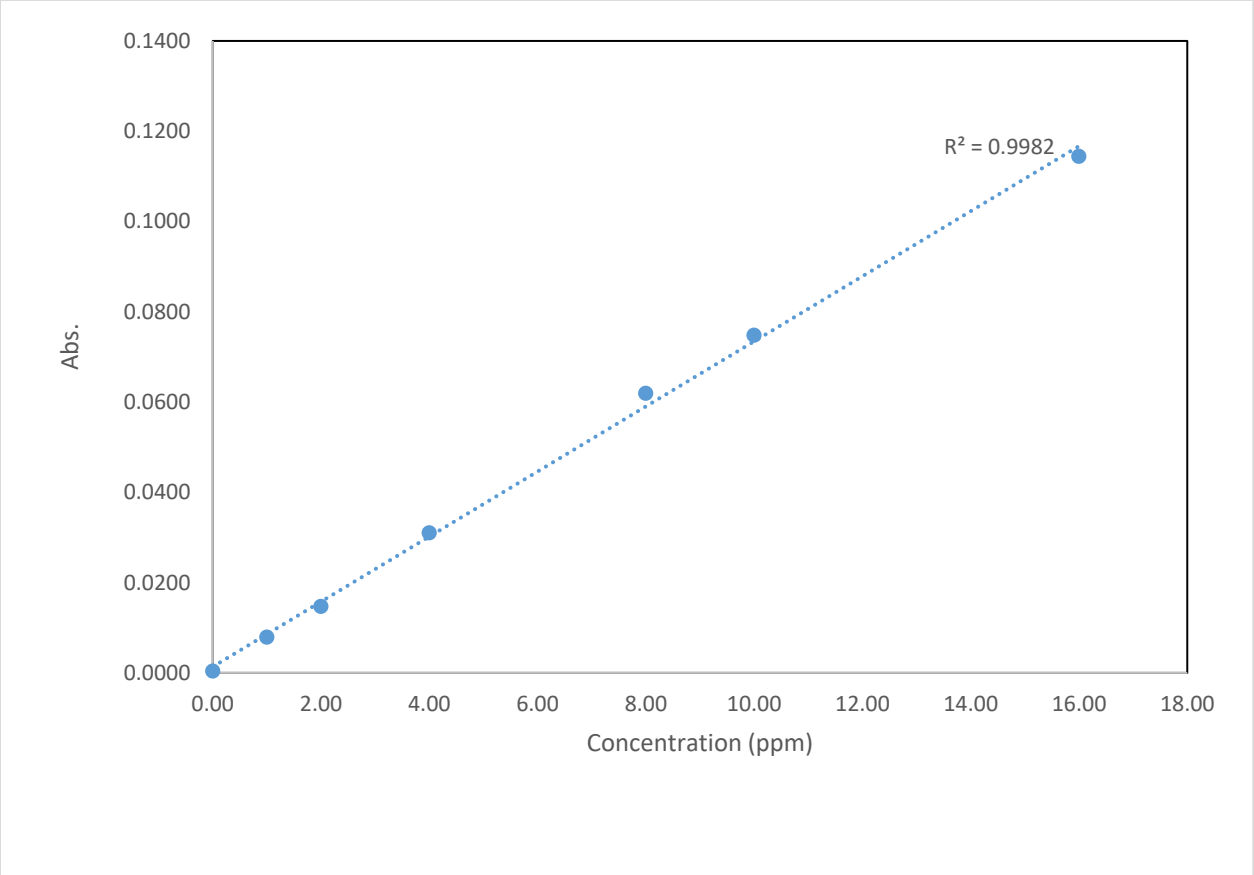


Fig: curve for standard solution of Pb.

Standard solution preparation (Cd)

Standard solution of Cd was prepared in the same manner of preparation of standard solution Pb. This time Cd in 10times more diluted. Standard solution was prepared in 0.2ppm, 0.5ppm, 0.8ppm and in 1.0ppm.

Serial no	Concentration ppm	Absorbance
1	0.00	-0.0005
2	0.2	0.0385
3	0.5	0.0996
4	0.8	0.1835
5	1.0	0.2096

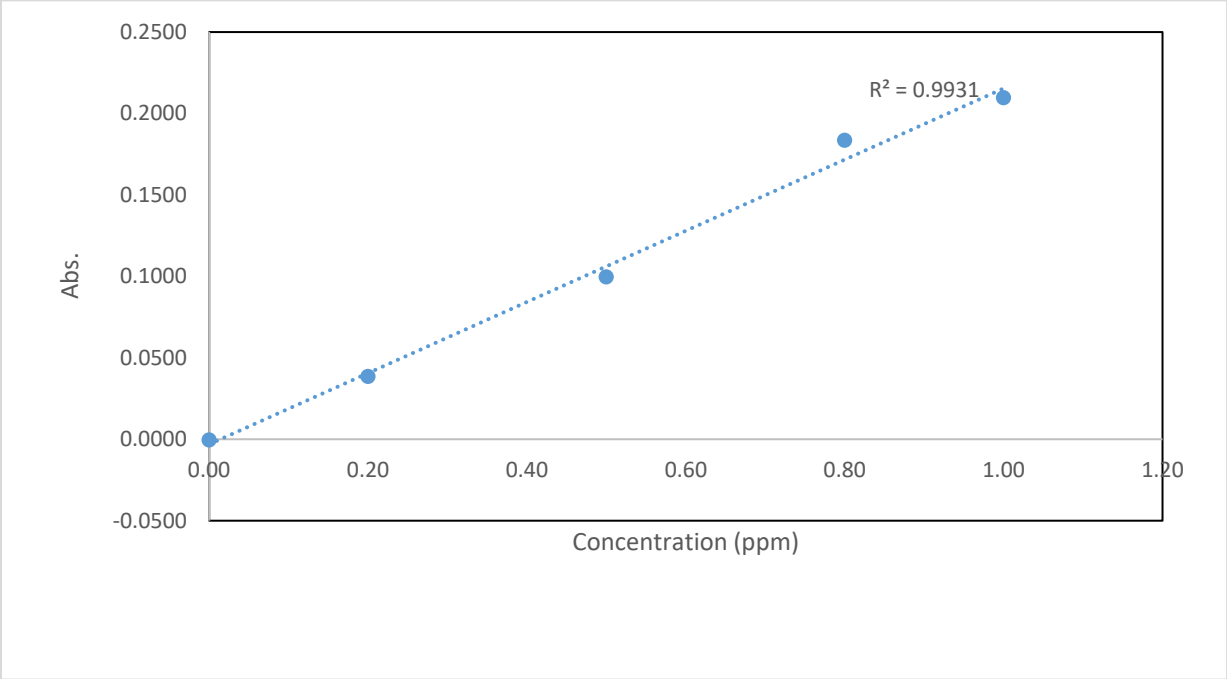


Fig: curve for standard solution of Cd

Sample preparation

- First all the vial was rinsed by tap water and the again rinsed by deionized water.
- Then these vials were warm at 105°C for 20minutes.
- Take 2ml of pasteurized milk in every vials.
- Then dilute the milk sample with 4ml of concentrated HNO_3 (65%, Merck, Germany).
- Incubate in for 29hours at 70°C.
- After incubation, add 4ml 30% hydrogen peroxide (Merck, Germany).
- Then incubate the solution in a semi closed glass digestion apparatus at 60°C for 30minutes.
- Then add 10ml of 1% HNO_3.
- Before transfer this solution into the vial, the solution was filtrated by glass wool and filter paper.
- Then solution transfer into the vial and the solution is ready for the experiment.

Preparation of 1% HNO_3

- Take 1ml of concentrated HNO_3
- Add distilled water upto 100ml.

Why incubation?

Milk sample was incubated with HNO_3. Incubation of milk means that in a certain temperature and also in certain period of time it was kept to prevent the coagulation.

Instrument

Model of the Atomic Absorption Spectrophotometer is Atomic Absorption 7000, Shimadzu Corporation, Japan.

3. Result and Result Discussion

3.1Protein (%) in pasteurized milk

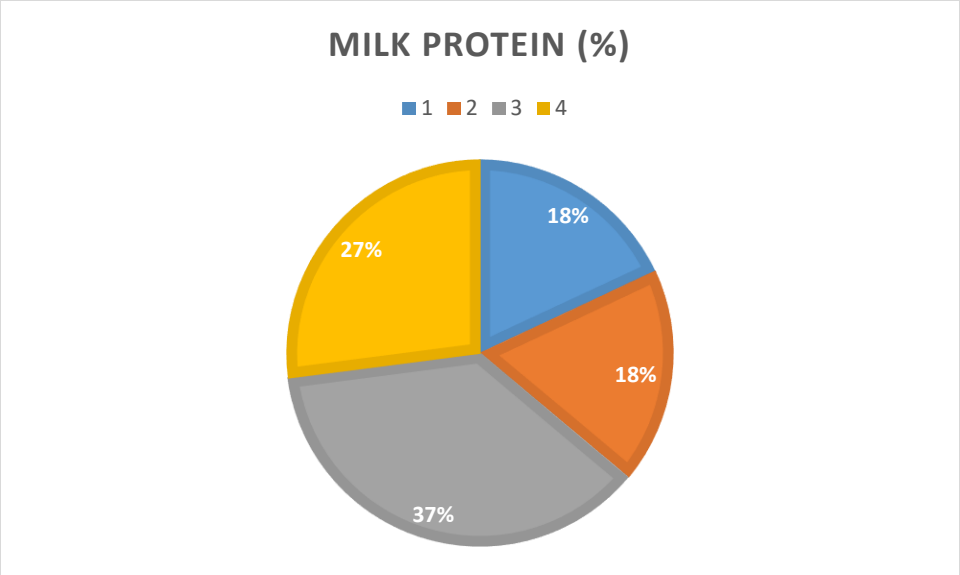
$$M = \frac{(10-9.8)\times 1.4\times 10\times 6.38\times 0.1}{0.4} = 4.5\%$$

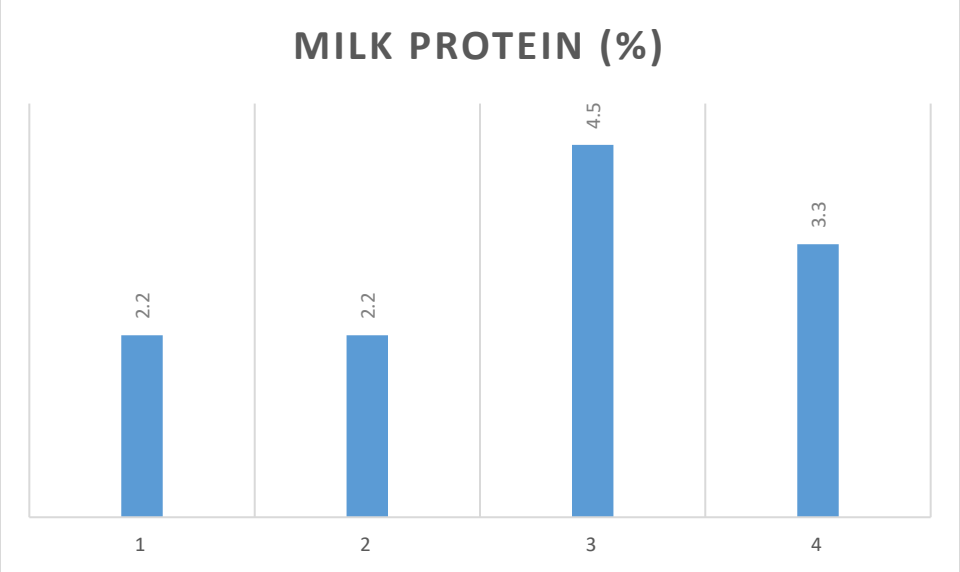
$$F = \frac{(10-9.9)\times 1.4\times 10\times 6.38\times 0.1}{0.4} = 2.2\%$$

$$A = \frac{(10-9.85)\times 1.4\times 10\times 6.38\times 0.1}{0.4} = 3.3\%$$

$$P = \frac{(10-9.9)\times 1.4\times 10\times 6.38\times 0.1}{0.4} = 2.2\%$$

Pasteurization type	Sample name	Protein %	Standard range
UHT	P	2.2%	3.3%
	F	2.2%	3.3%
HTST	M	4.5%	3.3%
	A	3.3%	3.3%





This table shows that only sample A is in the standard range. But others samples are not in the standard range. Protein percemt till 4 is excepted. So here only HTST milk are showing the result in the standard range.

3.2 Fat in pasteurized milk

Pasteurization type	Sample name	Fat %	Standard range
UHT	P	3.5%	3.5-3.7%
	F	3.5%	3.5-3.7%
HTST	M	3.2%	3.5-3.7%
	A	3.8%	3.5-3.7%

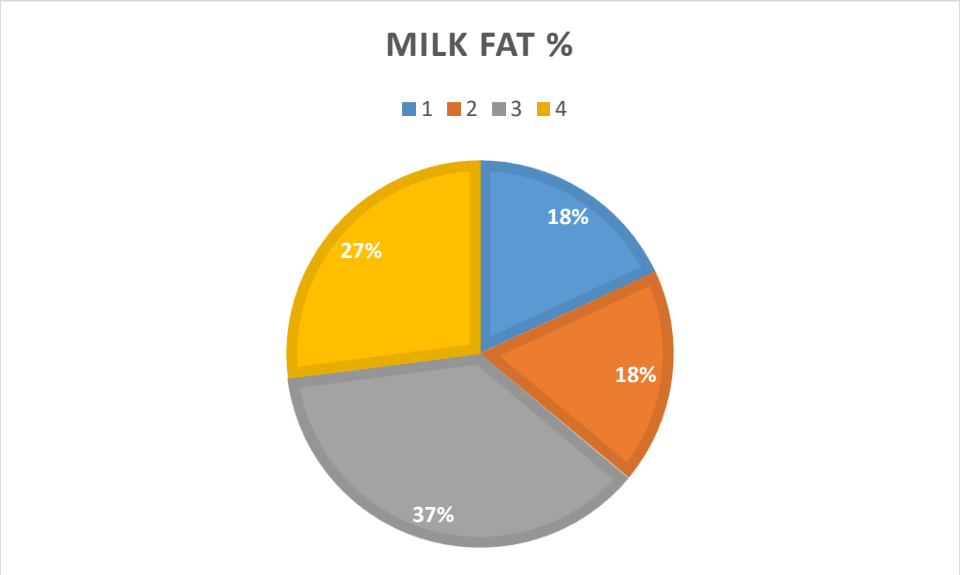
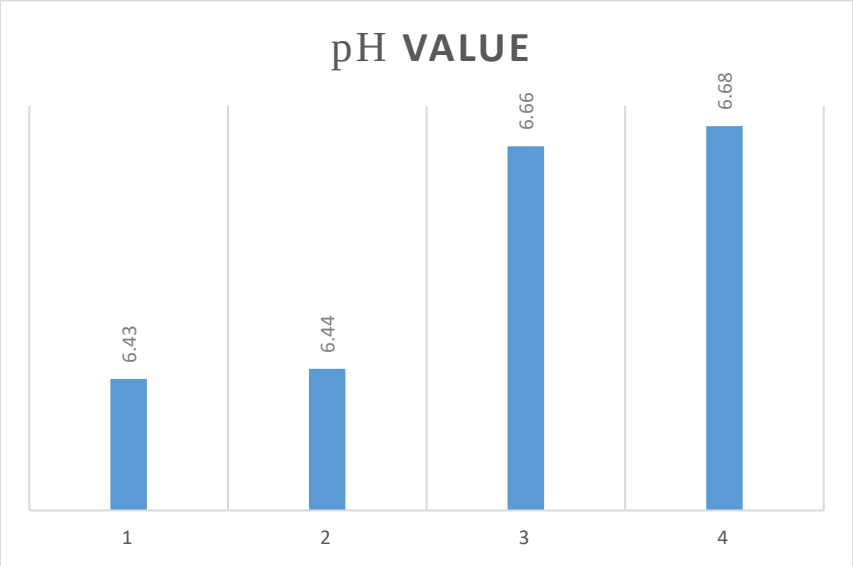
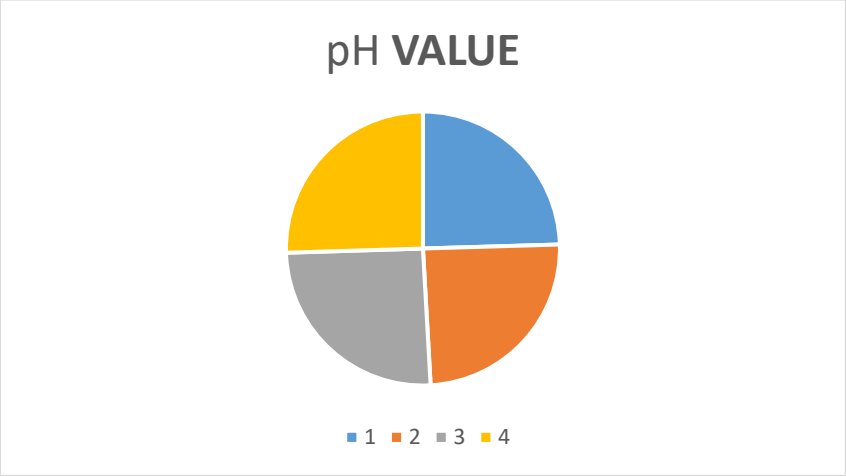


Table shows that fat (%) in every milk sample are in the standard range. If in any milk sample has low fat percent then it is advised to add more cream. And if milk sample with high in fat then skim milk should add.

3.3pH value in milk sample

Pasteurized type	Sample name	pH value	Standard range
UHT	P	6.43	6.5-6.9
	F	6.44	6.5-6.9
HTST	M	6.66	6.5-6.9
	A	6.68	6.5-6.9





From this table we can say that UHT are low in pH. Where HTST are in standard range,

3.4 Heavy metal in pasteurize milk

Pasteurization type	sample	abs.	concentration ppm	actual concentration	Who limit[15]
	Blank	0.0052	0.0411		
HTST	M	0.0072	0.0502	0.0091	0.58
UHT	P	0.0059	0.0443	0.0032	0.58
UHT	F	0.0056	0.0430	0.0019	0.58
HTST	A	0.0063	0.0461	0.0050	0.58

Fig: Cd in pasteurized milk

The table shows that cadmium concentration are below than standard range. However, HTST pasteurized milk contain more cadmium than UHT pasteurized milk.

serial no	sample id	Abs	concentration ppm	actual concentration	Standard range [16]
1	blank	0.0416	5.5822		
2	M	0.1076	14.7217	9.1395	0.00287-0.014
3	P	0.1270	17.4082	11.8260	0.00287-0.014
4	F	0.0743	10.1104	4.5282	0.00287-0.014
5	A	0.0888	12.1182	6.5362	0.00287-0.014

Fig: Pb in pasteurized milk.

This table showing that lead content is abnormally higher than standard range. Where UHT pasteurized milk sample P contain more lead than UHT pasteurized milk sample F. HTST pasteurized milk sample M is contain more lead than HTST pasteurized milk sample A.

4 Conclusion

Milk is a unique food product for every age of people. It is a good source of macronutrients and micronutrients. Some people who are vegetarian. They do not get protein from animal source. Therefore, this milk is also a good source of protein for them.

The present study shows the important information on protein, fat, pH and heavy metals in the different pasteurized milk sample.

From the study, it was found that only HTST pasteurized milk sample A (3.3%) contain the protein in the accepted range. Rest of the milk sample (UHT pasteurized milk sample P and sample F 2.2%, and HTST pasteurized milk sample M 4.5%) were not in accepted range.

Fat content are in guided range. However, HTST pasteurized milk sample M containing slightly higher amount 3.8% and sample A containing slightly lower amount 3.2% of fat. When milk containing lower amount of fat that time cream can be added to increase the fat percentage. Moreover, when fat in lower amount that time skim milk can be added. These things may occurred in these two milk samples.

pH value are in the guided range. UHT pasteurized milk samples are slightly low pH. For UHT pasteurized milk sample P and F has pH 6.43 and 6.44. HTST pasteurized milk samples M and A are in the acceptable range.

Heavy metal lead and cadmium are also detect in the milk sample. Cadmium is found in the acceptable range which given by WHO. However, lead is abnormally high in the entire sample. This may be arise from the background interference.

Although every experiment were done for three times. However, in some samples the result is not acceptable. Therefore, it is need to further investigate of these samples by following the same instruments in the same sampling method.

5 Reference

1. Mehar Ali. Kazi, 2015. 'Mineral analysis of milk through atomic absorption spectroscopy and their biological role in human life'. International journal of advances in chemical engineering.
2. J.J. havranek and Maja Dutour Sikrik, 2003. Determination of metal in cow's milk by flame atomic spectrophotometer.
3. Dr. philipjohn, august 28 2010. 'milk contains traces of ash'. https://www.thehindu.com/todays-paper/tp-features/tp-sci-tech-and-agri/Colour-of-milk/article15411280.ece
4. P.J (2017). Food Processing Technology Principles and Practice. Woodhead Publishing Series in Food Science, Technology and Nutrition. pp. 563–78
5. Tewari, Gaurav; Juneja, Vijay K. (2007). Advances in Thermal and Non-Thermal Food Preservation. Blackwell Publishing.
6. "Heat Treatments and Pasteurization MilkFacts.info". milkfacts.info. Retrieved 2016-12-12
7. "Harold Eddleman, Making Milk Media, 2014-03-19 Indiana Biolab". Disknet.com.
8. Smith, P.W., (August 1981), "Milk Pasteurization" Fact Sheet Number 57, U.S. Department of Agriculture Research Service, Washington, DC
9. Chavan, Rupesh S.; Chavan, Shraddha Rupesh; Khedkar, Chandrashekar D.; Jana, Atanu H. (2011-08-22). "UHT Milk Processing and Effect of Plasmin Activity on Shelf Life: A Review". Comprehensive Reviews in Food Science and Food Safety. 10 (5): 251–68
10. Pearce, L.E.; Smythe, B.W.; Crawford, R.A.; Oakley, E.; Hathaway, S.C.; Shepherd, J.M. (2012). "Pasteurization of milk: The heat inactivation kinetics of milk-borne dairy pathogens under commercial-type conditions of turbulent flow". Journal of Dairy Science. 95 (1): 20–35.
11. Young VR (1994). "Adult amino acid requirements: the case for a major revision in current recommendations".
12. Robert S. Goodhart; Maurice E. Shils (1980). Modern Nutrition in Health and Disease (6th ed.). Philadelphia: Lea and Febinger. pp. 134–138.
13. Whitney Ellie; Rolfes SR (2008). Understanding Nutrition (11th ed.). California: Thomson Wadsworth. p. 154
14. Mozaffarian, Dariush; Katan, Martijn B.; Ascherio, Alberto; Stampfer, Meir J.; Willett, Walter C. (2006-04-13). "Trans Fatty Acids and Cardiovascular Disease". New England Journal of Medicine. **354** (15).
15. Ahmad et al. 2017. Atomic absorption spectrophotometry detection of heavy metal in milk of camel, cattle, buffalo and goat from various areas of Khyber-Pakhtunkhwa (KPK) Pakistan. J anal bioanal tech.
16. Hanieh Moallem Bandani, et al: 2016. Determination of leadand cadmium from cow milk. Nutrition food sciences research.
17. All the materials and method are established by AOAC.