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Research paper

Biochemical Analysis of Buffalo Milk Available in Karachi, Pakistan

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ABSTRACT

A comparative analysis was carried out on 25 fresh buffalo milk samples collected from various locations in Karachi, Pakistan and 6 processed milk samples of different brands. An array of biochemical tests were conducted to detect different adulterants in milk samples. These tests included urea, pulverized soap, salt, starch, sugar, formalin, skim milk, catalase, and acidity or lactic acid tests. The processed milk samples showed the presence of urea, sugar, formalin, and skim milk, whereas these substances were either absent or present in insignificant amounts in the fresh milk samples. The lactic acid test was used to determine the bacterial load in the milk, and a value greater than 0.14% was deemed unhealthy. All 6 processed milk samples had lactic acid greater than 0.14%, whereas 6 out of 25 fresh milk samples exceeded this limit, with the remaining 20 samples falling within the range of 0.12-0.14%. The presence of salt, specifically chlorides, was also examined, with inconclusive results. The remaining tests for starch, catalase, and pulverized soap were negative in both raw and processed milk samples.

KEYWORDS: Raw milk, Ultra high treated, Food analysis, Food security, Food safety

INTRODUCTION

Milk is considered a nutritional liquid which is produced by the mammary glands of the mammals to feed their young ones when they are unable to process solid food. Production of milk is a specific mammalian adaptation and milk is unique in being an almost completely natural food. Milk contains the key ingredients of natural importance required for growth. The milk fed by mothers to their young ones helps in developing the immune system. It provides immunity as it is nutrient rich components composed of water, fat, proteins, vitamins, and minerals. So far more than 250 nutrients have been identified in milk and still the researchers are working on milk to find out more nutritional quality in different milk samples.

Naturally the color of milk is white as it reflects all the lights and does not absorb any light because of its high reflectance properties, and it is a well-known fact that when all the lights combine, they make white color. The other chemical compounds present in the milk also contribute to the white color such as carbohydrates, fats, and proteins. Worldwide animal milk has been used as major part of human diet since ages. Commercially there are two types of milk i.e. (a) ultra-high treated or processed milk brands and (b) fresh or raw milk.

The ultra-high treated milk brands include several popular brands which are being used widely. Synthetic milk or processed milk is refined milk which is refined by adding or mixing glucose, urea, different milk powder, and water and hydrogen also treated with ultra-high temperature and secured in box having four layers. The word synthetic is used with such kind of milk because of the alteration of the natural composition by adding different chemical components [1].

Increased milk demand and consumption increases the use of processed market milk to fulfil the requirement. When processed milk was first introduced in Pakistan in 1981, the idea was not welcomed. All leading processed milk brands in Pakistan are UHT (Ultra Heat Treated). UHT involves heating milk at a temperature of 135 degrees Celsius (which is above boiling point) for a few seconds and then cooling it down due to which it loses its nutritional value, taste, and smell [2]. However, Tetra Pak packaging has been found to release chemicals into milk such as urea. As per time, due to feasible availability, the growth trend of major processed milk is increasing in Pakistan Market. There are other risk factors associated with processed milk, which was further proved by Hanna Castro, Marjo Ruusunen, Miia Lindstrom who added a research article in the international Journal of Food Microbiology regarding the occurrence and growth of *Listeria monocytogens* in processed milk [3].

Due to limited awareness on this topic and people's false perception on the purity and wholeness of the processed milk people are unaware of the fact that natural or fresh milk is healthier to consume, as due to the addition of the glucose, urea, hydrogen and other synthetic chemical compounds, and the treatment with ultra-high temperature makes it unhealthy as the ultra-high temperature treatment makes the naturally occurring milk protein (casein and whey) denatured and the addition of the synthetic milk products make it unsafe for the consumption. Observational studies have suggested an association between high intakes of preformed vitamin A (more than 1,500 mcg daily—only slightly higher than the RDA), reduced bone mineral density, and increased fracture risk [4].

The country with the highest milk consumption in the world is Finland which consumes 361.19 kg per year while in Pakistan 828 million liters milk was produced in 2017 (82% cow milk, 14% buffalo milk, 2% goat milk, 1% sheep milk, and 0.3% camel milk), the production of milk in the urban and rural countries play an important role in the economy [5]. There are other factors that contribute great in the nutritional importance of milk such as presence of protein, lipid, and calcium ratio. In the handbook of dairy food and nutrition Gregor D. Miller, Judith K. Jarvis, and Lois J. McBean it is stated that Bovine milk protein is considered a high-quality, or complete protein, because it contains all 9 of the essential amino acids in proportions resembling amino acid requirements. Due to the high quality of bovine milk protein, it is regarded as a standard reference protein to evaluate the nutritive value of other food protein [6].

Milk is a good source of dietary calcium as it contains approximately 110 mg of calcium per 100 mL of milk [7]. Various adulterants of milk i.e. benzoic acid, urea, water, skimmed milk powder, sugar, detergent are detected in raw milk. Many studies have been carried out on milk adulteration and detection techniques [8]. Milk adulteration is common in developing countries and that has direct influence on physico-chemical properties of milk [9]. The current study is planned to analyze and compare different samples of buffalo milk being sold in mega city of Karachi, Pakistan and tetra pack milk available in this city.

Following nine biochemical tests are done to ensure the safety of milk.

Catalase Test

The catalase test has been utilized for a long time as a proportion of the leucocyte content of milk. This test depends on the decay of hydrogen peroxide by the chemical catalase which is available in milk. Milk regularlycontains some catalase, be that as it may, udder diseases increment the catalase action of milk. Catalase test is done to check the bacterial load in milk (except for lactobacillus), in which Hydrogen Peroxide is used to check the presence of catalase positive bacteria, if the catalase positive bacteria are present, bubbles are seen [10]. Chronic Granulomatous Disease (CGD) is the most

common disease which are generally caused by catalase positive bacteria.

Urea Determination

Urea, otherwise called carbamide, is a natural compound with substance recipe CO (NH 2) 2. This amide has two amino groups (-NH2) joined by a carbonyl group (-C(=O)-). Urea is a chemical compound which is naturally present in natural milk and is safe to consume but it is claimed that sometimes urea is artificially added in the milk to increase its shelf life, give consistency to milk and provide whiteness. [11]. To detect the presence of urea a biochemical test is done through Dimethyl Amino Benzaldehyde (DMAB), which is prepared in the ethyl alcohol and concentrated HCL, when the DMAB is added in the milk sample and if the milk color turns yellow it indicates the presence of the urea. [12]. There are many health hazards risk associated with the urea such as Ulcers, Indigestion, Early onset of diabetes mellitus II, Premature hair greying and Kidney dysfunctions.

Acidity

The genuine sharpness of milk is because of the presence of lactic acid bacteria which synthesizes lactic acid by utilizing lactose sugar of milk. The acidity test is done to check the load of lactic acid bacteria(LAB) in milk. Lactic acid bacteria are naturally present in the milk and is safe to consume, but as the milk gets old, the count of lactic acid bacteria increases in milk. The high load of LAB in milk indicates that milk sample is old and is not preferred for consumption. To detect the high titre of LAB in the milk sample, a biochemical test is done based on the concept of acid base titration in which phenolphthalein (as an indicator) and NaOH is used to neutralize the lactic acid which on neutralization turns light pink in color. The amount of NaOH used for neutralization is calculated using a mathematical formula. The value should be less than or equals to 0.14. [13]

Detection of Salt

Salts comprise a little amount of milk (8-9 g/L) [15]. The biochemical test for the detection of salt (specifically chlorides e.g. Calcium, Magnesium, Potassium Chloride) in the milk can be done by using potassium dichromate and silver nitrate, if the color of the milk turns yellow it indicates the presence of chloride salt in milk and in case of absence of salt, milk changes its color to chocolate [14]. It causes Clogging of the blood arteries which can cause stroke or a heart attack, Risks associated with the prolong chloride ions (Cl^-) consumption is high blood pressure, Effect on retina, and it also regulates the blood pH.

Skimmed Milk Powder Test

The arrangement of non-fat solids of skim milk is: 52.15% lactose, 38.71% protein (31.18% casein, 7.53% whey protein), 1.08% fat, and 8.06% debris [15]. Adding skimmed milk powder can change the taste of the milk, it can't always be lactose free as this can cause a great trouble for the people who are lactose intolerant. A method has been developed by for the detection of the skimmed milk powder in the milk by adding concentrated Nitric Acid (HNO_3) in the milk. Skim milk have low saturated fats which according to the studies are responsible to Increase bad cholesterol in your body and High risk of heart disease.

Detection of Formalin

Formalin is the preservative which is added to the milk in order to increase its shelf life, among all the preservatives which are used in the food formalin is considered the most dangerous preservative, as formalin is used in the preservation of the human body or it

parts. The method of the detection of the formalin has been developed which involves the use of concentrated sulphuric acid. Concentrated sulphuric acid is added to the milk sample, and if the formalin is present a purple/violet band is observed confirming the presence of formalin, the more the sulphuric acid is added for observing the purple band the higher amount of formalin is present [16]. Formalin is carcinogenic and the prolong use of formalin cause cancer.

Sugar Detection

The most basic sugar is glycerinaldehyde with a chain length of 3 carbons. Sugar is added as fake sugar in milk, to fabricate the starch content and thickness of the milk, table sugar is added as a contamination which furthermore extends the thickness of milk. There are three kinds of sugar included milk tests and are aldose, sucrose and fructose. Milk sugars are not identical to added sugars that give just calories and no enhancements. Generally, sugar is mixed in the milk to increase the solids not fat content of milk to increase the lactometer reading of milk, which was already diluted with water [17]. The biochemical test for the detection of sugar is adding concentrated sulfuric acid over milk with small amount of resorcinol and boiling in water bath, presence of red color indicates sugar as an additive. [18].

Starch

Another compound that is used as an adulterant in milk is starch. It is added to enhance the density in milk. Addition of starch prevents the detection of water in milk. Starch is used to increase solid-not-fat (SNF) and if high amounts of starch are added to milk, this can cause diarrhea due to the effects of undigested starch in colon. Its accumulation in the body may prove very fatal for diabetic patients [19]. Apart from the starch, wheat flour, arrowroot, rice flours are also added. Starch, cereal flours or arrowroot are added to make up the density of milk to prevent detection of added water. It is detected by starch- iodide test [20]. Biochemical test for the detection of starch is done using iodine, if the color changes to blue it determines the presence of starch in milk or the milk is adulterated with starch. [21]. The gathering of starch might demonstrate lethal for diabetic patients. Add a drop of color iodine or iodine arrangement. In the event that blue variety structures, it implies the milk is contaminated.

Pulverized Soap

Pulverized soap is a sodium salt or potassium salt of long chain unsaturated fats having purifying activity in water. They enhance the cosmetic nature of milk. Detergents cause gastro – intestinal complications [22]. Soaps are also added for adulteration and contamination of milk; used for the emulsification of remotely added vegetable fat. Biochemical test for the detection of pulverized soap is done using phenolphthalein (as an indicator) and sodium hydroxide following acid base titration, if the color changes to pink it determines the presence of pulverized soap [23]. Milk is adulterated with pulverized soap causing Food poisoning and Gastrointestinal infections.

MATERIALS AND METHODS

Fresh animal milk (buffalo milk) samples were collected from randomly selected areas in Karachi city. Animal milk includes 25 buffalo milk samples (S1 – S25). The collection points of animal milk include diary shops, diary markets and local shops. The sampling areas were Nazimabad, Dhoraji, Bahadurabad, Sharfabad, Shah Faisal, Burns Road, Landhi, Gulistan – e – Johar, Al – Hassan square, P.E.C.H.S,

Malir Cantt, Gulshan – e – Iqbal, Drig Road, and Clifton. Processed milk samples (tetra packs) include 6 Brands (B1 – B6). The collection point of packaged milks were local markets and supermarkets. About 31 milk samples of 250 ml each were collected in total. Milk samples were stored in sterile containers. Biochemical analysis of milk samples was done by performing different tests includes Chloride test (Silver Nitrate test), Sugar test (Seliwanoff's test), Urea test (DMAB determination method), Formalin test, Acidity % (Lactic acid %), Catalase test, Skim milk detection, Pulverized soap detection and Starch test.

Catalase test was done using the common method. small amount of milk sample was added on the surface of glass slide and few drops of hydrogen peroxide was added onto the milk to check the presence of catalase in it. For the positive control, staphylococcus aureus (which is a catalase positive bacteria) was added, if the milk gives off bubbles that means the test is positive and if the milk shows no activity that means milk is negative for catalase. Presence of urea was tested using DMAB (Dimethyl amino benzyl). 0.8% solution of DMAB was made in 95% ethyl alcohol. 2 ml of milk and 2 ml of DMAB solution was added in the test tube. For the positive control, few drops of urea was added in the milk. If the color of the milk changes to distinctive yellow color that shows that milk is adulterated with urea and if the test is negative, there will be no change in the color of milk [24]. Acidity % or the lactic acid % can be calculated using a formula acidity % = No. of ml of N/10 NaOH × 0.009 × 100 / weight of milk in gm. For this, phenolphthalein indicator was prepared by dissolving 4 gm of NaOH in 1 liter of distilled water. For making 250 ml of solution, dissolve 1 gm of NaOH in 250 ml of distilled water. To make N/10 NaOH, Dissolve 4 gm of NaOH in 1 liter of distilled water. For making 250 ml of solution, dissolve 1 gm of NaOH in 250 ml of distilled water. To calculate the value of acidity %, 3 ml of milk is added in a test tube and diluted with equal amount of water. To confirm the presence of acid, 2 – 3 drops of phenolphthalein indicator was added. NaOH is added from the burette until the appearance of light pink color that remains for about 10 – 20 sec. For the positive control, milk was stored for more than 10 days at – 20ºC and then defrost to check the acidity %. The value for acidity % must be less than or equals to 0.14%.[25]

Adulteration of milk with salt was tested using silver nitrate method. 0.8% of silver nitrate solution was prepared by dissolving 0.8gm of silver nitrate in 80 ml of distilled water and volume makeup till 100 ml and 1% of potassium dichromate solution was prepared by dissolving 1 gm of potassium dichromate in 80 ml of distilled water and volume makeup till 100 ml. 1 ml of milk and 5 ml of silver nitrate was added in a test tube and 2 – 3 drops of potassium dichromate was added as an indicator. The appearance of light-yellow color indicates the presence of salt. Other wise the milk shows chocolate or brick red color.[26] Sodium chloride, potassium chloride, magnesium chloride and calcium chloride were used as a positive control. On adding these salts, milk turns into light-yellow in color hence, this test is specified for the detection of chlorides only. To check the presence of skim milk in the milk, 5 ml of milk was added in the test tube and 2 – 3 drops of nitric acid was added. For the positive control, pure form of casein and market available skim milk were used. The color after the addition of pure casein protein and market available skim milk was orange and light yellow respectively. If the milk gives dark yellow color, that means milk is not adulterated with skim milk powder. Pulverized soap is the finely

grinded form of soap which is added for the purpose of providing specific texture to it. to detect the presence of pulverized soap, take 3 ml of milk in a test tube and add equal amount of hot water. 2-3 drops of phenolphthalein indicator are added. [27] For the positive control, milk was adulterated with fine soap particles and small amount of NaOH was also added as NaOH is the primary constituent of a soap. If no change in color is observed in milk and milk remains white in color that means, milk is not adulterated with soap. To detect the presence of artificial sugar in milk, saliwanoff's test is done. 0.5 gm of resorcinol is added in 5 ml of milk in a test tube along with 1 ml of conc, HCl. Then the test tube was placed in a water bath for around 5 minutes. For the positive control, fructose (honey), sucrose and aldose (dextrose) were added, and the appearance of color was deep cherry red, cherry red and faint pink red color respectively. In the absence of artificial sugar, the tubes remain unchanged and the white color of milk does not change.

Formalin is added in milk to increase the shelf life. In order to detect the presence of formalin in milk, 2 ml of milk is diluted with equal amount of water and few traces of ferric chloride (10%) was added in it. After that, sulphuric acid is added on the walls of the test tube. The appearance of violet/ purple ring at the junction gives the presence of formalin in it. the positive control was made by adding 1 ml of 10 % of formalin in milk. If the milk is not negative for the formalin, there will be no ring formed at the junction of sulphuric acid and milk. Starch is added to increase the consistency of milk, to detect the presence of starch, 3 ml of milk was added in a test tube and then boiled. Allow the milk to cool down at room temperature. Few drops of 1% iodine were added. The positive control for the starch test was made by adding small amount of starch in milk. Appearance of purple color indicates the presence of starch otherwise milk remains unchanged [30].

RESULTS

We carried out biochemical analysis of 25 fresh milk samples and 6 UHT milk samples sold in open market in different districts of Karachi division during May-July 2022.

Urea and formalin: Among raw milk and processed milk, there was maximum detection of urea and formalin in processed milk. Urea was detected in 5 out of 25 samples in raw milk and the intensity of distinctive yellow color was low, whereas 4 out of 6 samples of processed milk adulterated with urea with high intensity of distinguishable yellow color. Formalin was detected in 16 out of 25 samples in raw milk with low intensity bands whereas all 6 processed milk samples were adulterated with formalin with thick bands and high intensity.

Among pulverized soap, Starch, and Catalase, no adulterants were found either in raw milk or in processed milk .

All 25 raw milk samples and 6 processed milk samples were adulterated with salt(s). However, it should be noted that the protocol used was specific to chlorides.

There was no detection of skimmed milk in raw milk samples, whereas all processed milk samples were adulterated with skimmed milk.

There was no detection of artificial sugar in raw milk samples whereas 4 out of 6 samples were adulterated with Aldose sugar (artificial sweetener) in processed milk samples.

Value of acidity (in %) should be 0.14%. 5 out of 25 raw milk samples had acidity

more than 0.14% whereas processed milk samples had acidity more than 0.14%.

All milk samples including fresh milk and packaged milk were catalase negative.

All the fresh milk samples were free from added sugar. But when the brand/packaged milk were tested, 2 out of 6 samples tested negative and the rest showed positive towards added sugar. The types of sugars found in processed milk were aldose, sucrose, and fructose.

Titrate-able acidity or acidity percentage or lactic acid (%) has the normal value of < 0.14%. Higher levels of acidity than normal range indicates the poor quality of milk. When the milk is stored for a longer period, the count of lactic acid bacteria is increased that shows that the milk has been stored for a longer time. Among 25 fresh milk samples tested, only 5 milk samples showed value near or slightly high than the normal. On the other hand, all branded milk had acidity percentage higher than the limit which indicated that packaged milk samples have been stored for a longer period of time.

Starch test was performed to check the presence of added synthetic materials to increase the consistency of milk. The starch test was throughout negative in all the milk samples, either fresh milk or packaged milk.

Pulverized soap is a type of soap which is very finely grinded. These are sometimes added to milk to increase the foggy texture of milk. The presence of pulverized soap was negative in all the milk samples including fresh milk and packaged milk.

Skim milk is the type of milk which is in powder form and is added in milk to increase the consistency and to increase the sweetness in milk. Among all samples tested only processed milk samples were found adulterated with skim milk. The results were compared from two different positive controls. The one positive control contains purified form of casein protein, and the other contains the market available skim milk powder.

Formalin test was done to check the presence of preservative (formalin) in milk. Formalin is one of the most toxic and harmful chemicals for human health. Formalin is added to milk to increase the shelf life of the milk. When milk samples were tested, 16 out of 25 fresh milk showed presence of formalin but in very low quantity. Whereas 6 out of 6 processed samples tested positive for formalin and the appearance of dark color showed the heavy adulteration in milk.

While summarizing all the results, it can be concluded that fresh milk samples were performed ‘better’ in 8 out of 9 tests and packaged milk appeared ‘unhealthy’ in 6 out of 9 tests. The present study showed that the fresh milk being sold in open market in Karachi is ‘comparably’ safer to consume compared packaged milk.

CONCLUSION:

As the results shows, raw milk samples which are available in Karachi city were tested negative or have low detection in 8 out of 9 biochemical test and processed milk samples tested positive in 6 out of 9 biochemical tests performed. This suggest that raw milk samples are ‘healthier’ to consume in daily routine whereas processed milk samples are ‘hazardous’ in many ways and can bring different diseases along with better taste.

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