Development of Effective Methods for the Determination of Different Types of Adulteration in Milk and Milk Products

Project Report

Submitted in partial fulfilment of the requirements for the degree of

Master of Science in Biotechnology (Hons.)

Submitted By

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DECLARATION

I, Priya Verma, hereby declare that the project report entitled "Development of effective methods to determine different types of adulteration in milk and milk products" submitted for the partial fulfilment of the degree M.Sc. Biotechnology(Hons.) is record of work carried out by me under the supervision of "Dr Mohammad Murtaza Mehdi", Assistant Professor, Lovely Professional University, Phagwara, Punjab.

I further declare that the material taken from other sources have been duly acknowledged in this report.

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CERTIFICATE

This is to certify that PriyaVerma (Reg. No. 11611229)have completed theproject, entitled "**Development of effective methods for the determination of different types of adulteration in milk and milk products"**under my guidance and supervision. To the best of my knowledge, the present work is theresult of their original investigation and study.

No part of the report has ever been submitted for any other degree at any University.The report is fit for submission and the partial fulfilment of the conditions for the award of Master of Science in Biotechnology (Hons.)

Date: **Supervisor Signature:**

ACKNOWLEDGEMENT

I would never have been able to complete my writing without the guidance of my teacher, my friends and support from my family. I am heartily thankful to my supervisor Dr. Mohammad Murtaza Mehdi, Assistant Professor, Department of Biochemistry for encouragement and support me to understand this project. It is with my deepest gratitude and warmest affection that I dedicated this work to my sir, who has been a constant source of knowledge and inspiration and whose innovation and valuable ideas helped me to complete my work.

CONTENTS

INTRODUCTION

Milk is the principal nourishment of all new born children. So it assumes a fundamental part in our eating routine for the duration of the life. Milk has a high nutritive esteem and contains all the fundamental segments of our eating regimen like proteins, fats, vitamins and minerals. It is the main single finish sustenance for a well being and the development of individuals in nature. In India, dairy animals and wild ox are most essential for business creation of milk and milk items. This is the reason, why cow is considered as a hallowed creature and even loved and worshipped in India.

Individuals of all age bunches consume milk. In created nations, the utilization of milk and milk items is higher when contrasted with the restricted utilization in creating nations. This request is becoming higher in created nations because of the expanding population, ascend in pay, urbanization and the adjustment in dietary propensities. As indicated by the Food and Agriculture Organization of the United Nations, the utilization of milk and milk items is by more than 6 billion individuals around the world. The lion's share of these individuals have a place with creating nations. In India, around half of the milk is expended on-ranches.

Milk and milk items are accessible on shops and dairies. There is countless number of items that are delivered from milk and these change up our dinners. Cheddar, curd, spread, cream, khoa, rabri, frozen yogurt and so on are produced using milk. It is utilized at both family and mechanical levels. Ensure that the essence of milk is held amid the creation and capacity.

Milk is accessible in both new and handled structures. The previous being sold as fluid milk, drawn from the udder of the creature or after the gathering and sanitization to make it alright for human utilization.

A few people deliberately add particular substances to the milk to decrease its virtue which is named as milk defilement. At the point when the shoppers need to get the greatest amount at a cost as low as could reasonably be expected, the merchants must address the issues of the purchasers. Merchants at that point offer the milk of second rate quality and subsequently they debase the milk. An adulterant is any substance which is utilized to decrease the nature of the item and to receive most extreme advantage in return.

Debasement might be deliberate or inadvertent. Accidental defilement is for the most part because of obliviousness, carelessness or absence of legitimate offices. Accidental sullying may likewise happen because of small scale living beings. Some bundling perils are likewise there. The source may likewise be an infected creature. On the off chance that the creature is

infected, milk is additionally not beneficial. It additionally relies upon their encourage. Presently, milk is first prepared in enterprises. There it originates from various sources and afterward blended. Individuals intentionally add adulterants to get most extreme advantage. They don't consider the unsafe impacts that these substances can cause. Such individuals have lost their good and otherworldly esteems. There must be strict laws to rebuff these individuals.

Lamentably, a portion of adulterants show serious wellbeing sway, here and there over the long haul. Melamine intake at levels over as far as possible can actuate renal disappointment and demise in new born children. The two peroxides also, cleansers in drain might cause gastro-intestinal difficulties, that can prompt gastritis and aggravation of digestive tract. Over the top starch in the drain can cause looseness of the bowels because of the impacts of undigested starch in colon, be that as it may, amassed starch in body demonstrate exceptionally deadly for the patients of diabetes (Singuluri and Sukumaran, 2014). Milk containing urea can overload the kidneys because they have to sieve through excess urea from the body (Kandpal, Srivastava, and Negi, 2012). Likewise, carbonate and bicarbonates may result interruption in hormone flagging to facilitate improvement and propagation.

SCOPE OF THE STUDY

There are various methods to check different types of adulteration in milk. Some of these include some biochemical tests which are done in laboratory. Different kinds of kits are also available. But as per the commercial level, these tests should be economic because in industries they collect milk from different sources. So there should be development of such methods which are not costly and can be done at the spot and are effective with the same extent. There should be still progress to determine different cost effective methods for checking milk adulteration.

OBJECTIVE OF THE STUDY

The present study has been designed with the following objectives:-

1. To determine the different kinds of adulteration in milk and milk products.
2. To develop some cost effective methods to determine adulteration of milk and milk products.
3. Quantitative analysis of the adulterants in milk.
4. Development of economic adulteration detection kit which can be used at commercial level.

REVIEW OF LITERATURE

Luis A. Dias (2009) built an electronic tongue having 36 cross-sensibility sensors which permit an effective acknowledgment of the five fundamental taste measures. He further tested its taste acknowledgment ability to detect presence of goat's milk in cow milk, that is an important issue with the dairy business. This technique was a contrasting option to the traditional ones used to recognise goat milk adulterated in cow milk. This method was less time consuming, simple and efficient. This e-tongue gadget recorded distinctive flag profiles that together with straight discriminant investigation permitted the usage of an innovative model which could determine crude skim drain gatherings with 97% sensibility and 97% specificity.

Kandpal et al (2012) conducted an investigation at Department of Community Medicine at HIHT, Dehradun. Each sample of the raw milk was provided by the merchants in 5km range of HIHT. Samples of drain were also taken from hostels. They estimated that just 12% samples were having a particular gravity that was 26 and more, which has been considered as undiluted milk. 80% samples were having a particular gravity under 26 which demonstrates that drain is diluted with water. The discoveries of this investigation features the need to execute enhanced cleanliness hones and to apply successful checking all through the generation to conveyance chain.

Ian P. Hurley (2006) build up an examine fit for distinguishing defilement of bison, sheep and delicate goat milk cheese with cow milk that is from less expensive means. Indirect ELISA had a poorer affectability with cheese as comparison to milk. He produced a sandwich ELISA using a similar monoclonal counter acting agent in mix with a polyclonal goat hostile to cow like IgG immune response. Recognition restrains in drain were 0.001% bovines' milk defilement of sheep or wild ox milk and 0.01% cow's milk contaminated of goat milk.The measure was reproducible with both intra and between examine coefficient of variety <10%

Musara et al (2013) investigated to assess osmometry as an instrument in quality investigation of drain. The osmolality of crude drain, cleaned drain, skimmed UHT i.e. ultra-high temperature treated drain, sanitized drain, institutionalized UHT drain and matured drain (Lactococcus lactis culture) was resolved by the aim of solidification osmometry. The connection between osmolality also, pH of matured drain was additionally explored amid unconstrained maturation of UHT drain at 37 °C for 48 h. Normal osmolality esteems (mean ± SD) were crude drain 290.2±7.98, disinfected drain 290.2±5.84, skimmed UHT drain 290.8±3.31, purified drain 283.6±2.28, institutionalized UHT drain 281±4.59 and matured

drain 466.0±17.30 mOsmoles kg−1. For fresh milk tests, 88%showed typical osmolality, 8 % were hypo-osmotic and 4 % hyper-osmotic. Aging examinations uncovered a high negative connection amongst osmolality and pH, with a relationship coefficient of −97.49 %. Hypo-osmotic drain demonstrates blending of drain with water along the generation chain. Hyper-osmotic drain shows maturation of drain at high surrounding temperatures or with delayed capacity. It might likewise uncover defilement of new drain with a solvent substance. Osmolality was most elevated for aged drain inferable from generation of lactic corrosive amid aging. It was affirmed by high negative relationship amongst osmolality and pH of drain in aging ponders. Thus the osmolality of matured milk might be utilized as a list of the degree of maturation.

Barui et al (2011) described a strategy to determine nearness of anionic cleanser in drain utilizing azure A dye. Their strategy needed brief blending of drain, azure A dye and EDTA arrangements, trailed by chloroform expansion and resulting centrifugation. The blue shading in the chloroform stage was measured at 635 nm. The cut off points of location (LOD) and utmost of measurement (LOQ) for sodium dodecylbenzene sulfonate in drain were 10 and 20 mg/ L, separately and for a lab review anionic cleanser, labolene, 50 and 100 mg/ L, separately. Recuperation of cleanser in spiked milk tests was quantitative. The nearness of different added substances, i.e., NaOH, NaHCO3, KNO3 and NaCl at levels of 250, 750, 500 and 500 mg/ L milk, individually, did not meddle with estimation of cleanser. The technique was too material to drain tests put away within the sight for formalin and sodium azide for 39 days at 4 ^{0}C.

Priyanka et al (2012) The target of their research was to appraise sialic acid in drain and drain items by fluorimetry examination which do not need an earlier hydrolysis step accordingly the estimation time diminished. In milk, the recuperation of included sialic acid was found to be 91·6 to 95·8%. Sialic acid in milk was observed to be subject to dairy cattle breed and was in the scope of 1·68– 3·93 g/kg (dry issue premise). The test was additionally reached out for identifying milk adulteration with sweet whey which depends on the location of glycomacropeptide (GMP) bound sialic corrosive in defiled drain. The C-terminal piece of κ-casein is GMP that is discharged into the whey amid cheese making. To recognition corruption, particular precipitation of GMP was finished utilizing trichloroacetic acid(TCA). TCA focus in milk was first raised to 5% to encourage drain proteins, particularly κ-casein, taken after by raising the TCA focus to 14% to accelerate out GMP. In the accelerates GMP bound sialic acid was evaluated utilizing fluorimetric strategy and the fluorescence force was

found to be specifically corresponding to the level of sweet whey in defiled drain tests. The strategy was found to distinguish the nearness of 5% sweet whey in drain.

Awan A et al (2014) The point of their examination was to dissect and analyze the synthetic creation of 8 tetra packs drain tests Dairy Umang (S7), Haleeb (S2), Everyday (S4), Drain Pack (S5), Nurpur (S8), Dairy Queen (S6), Olpers (S1), Good drain (S3) accessible in neighborhood advertises and recognized the nearness of different synthetic adulterants in drain tests in Southern Punjab (Pakistan). Thickness, pH, add up to solids, lactometer perusing, particular gravity and fat substance were broke down to decide concoction piece of drain tests. Their outcomes uncovered that every contemplated parameters had factually non critical contrasts ($P>0.05$) but add up to fat in drain tests which was fundamentally unique ($P=0.03$) among the 8 examined drain tests. Nearness of various compound adulterants, formalin, salicylic corrosive, unadulterated sweetener, starch, benzoic acid, glucose, skim milk powder, ammonium sulfate, salt, alkality, pounded cleanser, cleansers, borax and boric acid were likewise identified for drain tests followed by standard techniques. Outcomes demonstrated that natural sweetener, benzoic acid, alkalinity, formalin and glucose were available in every specimens whereas salt test was sure just for Olper drain. Remained examined adulterants had not been identified in eight drain tests in investigation.

Jiali et al. (2011) revealed assurance of microbes checks in new drain continuously utilizing piezoelectric transducer.The identification framework comprises of cell for recognition, oscillator, recurrence counter and PC where self created programming is introduced to catch the transducer reaction with the difference in culture media amid microscopic organisms development. The transducer could obtain adequate information quickly and empowered constant checking of microscopic organisms development.

Bishop et al. (1984) depicted an impedimetric strategy to decide the time span of usability of sanitized entire drain. They considered 100 examples of sanitized entire drain furthermore, contemplated diverse parameters, for example, organoleptic assessment, standard plate check (SPC), psychrotrophic microscopic organisms check (PBC), altered psychrotrophic microscopic organisms tally (mPBC), Moseley test (MSPC), and impedance recognition time (IDT) at 18 and 21 degrees centigrade. The relationship between the timeframe of realistic usability and the immediate check strategies are not satisfactory to make any forecast. Moseley test (MSPC) aside from impedance strategy has huge connections to timeframe of realistic usability. What's more, it has been guaranteed that impedance strategy had was good

indicator of time span of usability, it is less work concentrated, and requires just 1– 2 days, instead of 7– 9 days to finish.

Monso et al (2002) An interlaboratory think about, with the investment of 8 research centers, was directed to assess a sodium dodecyl sulfate - slim gel electrophoresis strategy for assurance of defilement of drain powder with soy and pea proteins. Adjustment benchmarks (0– 8%, w/w, soy and pea protein altogether protein) and tainted skim drain powders (0– 5%, w/w, soy and pea proteins in all out protein) were delivered. Vegetal proteins were resolved after evacuation of drain proteins by pretreatment of the examples with tetraborate– EDTA cradle, pH 8.3. Repeatability standard deviations went from 9 to 15% and reproducibility standard deviations gone from 25 to 30% in the specimens containing 5% vegetal protein in complete protein.

After olive oil, milk powder ranks second in the agenda of food items that are in the zone of threat for adulteration (Moore et al. 2012). Financially pushed methods for aduleration include summation of whey, vegetable protein and water (Fischer, Schilter, Tritscher, and Stadler, 2011). Such corruptions don't represent any extreme wellbeing hazard. Be that as it may, a few adulterants are too unsafe to ever be ignored. A portion of the real adulterants in drain having genuine unfriendly wellbeing impact are salicylic acid, formalin, boric acid, melamine, ammonium sulphate, urea, caustic soda detergents, sugar, benzoic acid and hydrogen peroxide. Point of solidification, SNF (solid not fat), protein content and percentage of fat are the general parameters which are taken into account to access milk quality. To elevate such kind of parameters, people add adulterants in milk and in this manner expanding the drain quality in untrustworthy way. To expand the SNF value of the milk, substances which are added are pure sweetener, normal salts, starch and urea. Urea, being a characteristic constituent of crude milk, has a greatest point of confinement forced by FSSAI (Food Safety and Standards Authority of India) Act 2006 and PFA (Prevention of Food Adulteration) Rules 1955 which is to be 70 mg/100 ml. To increment the non- protein nitrogen content in the milk, urea is added commercially (Sharma et al. 2012). When someone intentionally add water to milk to gain profit, ammonium sulphate is added alongwith water so that lactometer reading can be maintained by balancing the milk density. To lift up the content of protein, melamine is added (Liu et al. 2012). To increment the time span of usability of the drain, additives such as benzoic acid, formalin and salicylic corrosive are used (Singh and Gandhi, 2015). As drain fat is extremely costly, a few makers of drain and dairy items evacuate drain fat for extra monetary profit and remunerate it by including

non-drain fat, for example, vegetable oil. As foamy arrangement is a coveted quality of milk, cleansers are added for emulsifying and smash up the oil in water. (Singuluri and Sukumaran, 2014).

EQUIPMENTS, MATERIALS AND EXPERIMENTAL SETUP

I. Equipments

- Water bath
- Spectrophotometer
- Weighing Balance
- Refrigerator
- Autoclave

II. Materials:-

Chemicals Used

- Resorcinol
- Conc. HCl
- Iodine
- Potassium iodide
- Ethanol
- 10% Sodium hydroxide
- Sodium carbonate
- Urea
- p-Dimethyl amino benzaldehyde
- Anhydrous potassium dihydrogen orthophosphate
- Anhydrous dipotassium monohydrogen orthophosphate
- Trichloroacetic acid
- 2% Sodium hypochlorite
- 5% phenol solution
- Silver nitrate
- Potassium chromate
- Formalin
- Starch

- Glucose
- Sucrose
- Conc. Sulphuric acid
- Zinc chloride
- Cellulose
- Ammonium sulphate
- Barium chloride
- Ammonium molybdate
- Acetic acid
- Sodium tungstate
- Copper acetate
- Sodium chloride
- Sodium carbonate
- Diphenylamine
- Ethanol
- Methylene blue dye
- Hydrogen peroxide
- Boric acid
- Salicylic acid

Glasswares Used

- Conical flasks
- Culture test tubes
- Beakers
- Measuring cylinder
- Funnel
- Glass pipette

Miscellaneous

- Microcentrifuge tubes
- Microtips
- Pipette
- Tips
- Cotton
- Foil paper
- Test tube stand
- Whatman filter paper

III. Experimental Setup

Spectrophotometer was utilized for the quantitative approximation of various adulterants in milk sample. Absorbance was measured at 420nm. A spectrophotometer works on the basic principle that each compound assimilates or transmits light finished at a specific scope of wavelength. It works by passing a light shaft through a specimen to quantify the light force of an example.

REASEARCH METHODOLOGY

The present investigation entitled “Development of effective methods for the determination of different types of adulteration in milk and milk products” is conducted in Project Lab 28-301, School of Bioengineering and Biosciences at Lovely Professional University, Phagwara. A summary of the methodology adopted in this work is presented in this section under the following headings:-

PREPARATION OF MILK SAMPLE

Tests are gotten following couple of days of drawl and contain additive (0.4% formalin). Warm the example to 37-40°C by exchanging it to the measuring utencil and keeping it in a water shower kept up at 40 - 45°C. Blend gradually for legitimate homogenisation. Blend test altogether by emptying over into the jug, blending to oust any lingering fat adhering to the sides and pour it back in the measuring utencil. Amid blending don't shake the jug overwhelmingly. Enable the specimen to come to room temperature (26-28°C) and pull back promptly for investigation. In the event that little coagulations or bumps are seen in the specimen which can't be scattered, a couple of drops of alcohol alkali might be utilized amid homogenisation. On the off chance that even after homogenisation the specimen indicates bumps or clumps or beads of oil are unmistakable suggestive of turning sour/part of drain, the example ought to be regarded unfit for examination and rejected.

QUALITATIVE ANALYSIS OF DIFFERENT ADULTERANTS

The milk samples which were taken do not show any adulteration. All the tests were found negative. So, milk was intentionally debased to check the efficiency of the following tests.

DETECTION OF STARCH

Reagent used: Iodine Solution

Steps involved: Took 3ml of milk sample in a test tube. Then it was brought to boiling conditions in a water bath and then cooled to room temperature. About 1 or 2 drops were added to the test tube. Appearance of blue colour indicated the presence of starch.

DETECTION OF FORMALIN

Reagent used: Conc. Sulphuric acid

Steps involved: Took 5ml of milk sample in a test tube. 5ml concentrated sulphuric acid was added from walls of test tube without any shaking. Formation of a blue ring showed the presence of formalin in the milk sample.

DETECTION OF SUGAR

This method is modified Seliwanoff's Method.

Reagents used: Resorcinol solution: 0.025g resorcinol was weighed in 2ml of distilled water. 2ml of conc. HCl was added to it and final volume was made upto 5 ml using distilled water.

Steps involved: 1ml of milk sample was taken in a test tube and 1 ml of resorcinol solution was added to it and mixed properly. The test tube was placed into a boiling water bath for around 5 minutes. The presence of red colour showed that the milk sample had added sugar. Milk having no added sugar remains as such i.e. white in colour.

DETECTION OF DETERGENTS

Reagents used: Bromocresol purple solution

Steps involved: Took 3 ml of drainsample in a test tube and 0.1 ml of bromocresol purple solution was added. Appearance of violet colour indicated the presence of detergent in milk. Pure drain sample showed a faint violet colour.

DETECTION OF UREA

Reagents used: p-dimethyl amino benzaldehyde solution: 0.32g DMAB was taken and 2ml conc. Hydrochloric acid was added to it. Then finally 20ml ethanol was added.

Steps involved:1ml of milk sample was taken and it was mixed with 1.6% DMABsolution. Appearance of distinct yellow colour showed the presence of added urea in milk. As milk naturally contains urea. So, the normal milk had a slight yellow colour.

DETECTION OF AMMONIUM COMPOUNDS

Reagents used:

(A) 2% sodium hydroxide: 0.1 g NaOH dissolved in 5ml of distilled water.

(B) 2% sodium hypochlorite: 0.1g sodium hypochlorite dissolved in 5ml of distilled water.

(C) 5% phenol solution: 0.1ml phenol in 2ml of distilled water.

Steps involved: 1ml of milk sample was taken and 0.5ml of 2% sodium hydroxide was added to it. Then 0.5 ml 2% sodium hypochlorite and 0.5ml 5% phenol solution was added to it. It was heated in a boiling water bath for around 30 seconds. Deep blue colour showed that ammonium compound was present. The pure milk showed pink colour as an end point.

DETECTION OF SULPHATES

Reagents used:5%Barium chloride solution: 0.25g barium chloride wan added to 5ml of distilled water.

24% trichloroacetic acid: Took 2.4g TCA and dissolved it in 10ml distilled water.

Steps involved: I took 5ml of milk sample and 5ml TCA solution was added to it. Milk coagulates and the coagulated milk was filtered out through whatman filter paper. The filtrate was taken and few drops of barium chloride solution was added to it. Milky white precipitates were observed and this showed the presence of sulphates like ammonium sulphate in milk.

DETECTION OF SODIUM CHLORIDE

Reagents used: 0.1N aq. Silver nitrate solution and 10% potassium chromate

Steps involved: 5ml of milk sample was taken and 1ml of 10% silver nitrate solution was added to it. 0.5ml of 10% potassium chromate solution was added to it. Appearance of yellow colour indicated presence of chloride.

DETECTION OF NITRATES

Reagents used: Diphenylamine(2%): Took 0.04g diphenylamine and dissolves in 2ml of sulphuric acid.

Steps involved: 2ml of milk sample was taken. The test tube was rinsed with milk and the milk was drained from the tube. Along the walls of the test tube, a few drops of diphenylamine solution were added. Blue colour was noticed which indicates the presence of nitrates in the milk.

DETECTION OF CELLULOSE

Reagents used: Iodine- zinc chloride solution

Steps involved: 10ml of milk sample was taken in a 100ml beaker. 50ml hot water was added to it and mixed for 3minutes. Mixture was poured on a nylon cloth and the residue was washed again with 50ml hot water. Residue was scrapped with help of a spatula. It was divided into two parts. One part was stained with Iodine-Zinc chloride reagent and second with iodine solution. Blue colour in iodine zinc chloride reagent and absence of blue colour in iodine solution depicted presence of cellulose.

DETECTION OF HYDROGEN PEROXIDE

Reagents used:- (A) Potassium Iodide solution: 0.7g potassium iodide in 10ml water was taken. (B) Dilute HCl :- 20ml of water was added to 10ml conc. HCl. (C) Starch solution:- 0.1g starch in 10ml water was taken.

Steps involved:- 1ml of milk sample taken in a test tube. 1ml of potassium iodide starch solution was added to it and mixed well. Blue colour appearance indicated the presence of hydrogen peroxide and pure milk remained white.

DETECTION OF PULVERIZED SOAP

Reagent used: Phenolphthalein indicator

Steps involved: I have taken 10 ml drain sample in a test tube and diluted with same quantity of hot water i.e 10ml.I have added few drops of phenolphthalein indicator to it. Development of pink colour indicated that the milk was adulterated with soap.

DETECTION OF SKIM MILK POWDER

Reagent used: Nitric acid

Steps involved:I have taken5ml milk sample in a test tube and few drops of nitric acid were added to it drop by drop. Appearance of orange colour indicated that the milk was adulterated with skim milk powder. Pure drain sample showed yellow colour.

DETECTION OF BENZOIC ACID AND SALICYLIC ACID

Reagents used: concentrated sulphuric acid, 0.5% ferric chloride solution

Steps involved: I have taken 5ml drain sample in a test tube and I also added1ml of conc. Sulphuric acid to it. 0.5% ferric chloride solution was added drop by drop. Appearance of buff colour indicated presence of benzoic acid and appearance of violet colour indicated salicylic acid.

QUANTITATIVE ANALYSIS

Quantitative Analysis of Urea in Milk

Reagents prepared:

A. DMAB i.e. p-Dimethyl amino benzaldehyde solution: I have taken 0.32 g of p-Dimethyl amino benzaldehyde and 2ml of conc. Hydrochloric acid was added to it. Then 20 ml of ethanol was added to it.

B. Preparation of phosphate buffer with pH 7.0: I have taken 3.403 g quantity of anhydrous potassium dihydrogen orthophosphate in a 100ml dH2O. Also 4.355 g quantity of anhydrous dipotassium monohydrogen orthophosphatedisjointedly in dH20 (100 ml). These two soliutions were combined and the dilution was made upto one litre.

C. 24% of Trichloroacetic acid: The quantity of TCA taken was 2.4 g in 10ml dH2O.

D. Diluting Reagent: To prepare diluting reagent same volumes of phosphate buffer and 24% TCA were combined.

E. The standard solutions for urea with different concentrations were prepared.

Steps to proceed:

Firstly, preparation of the standard curves of urea takes place and the graph plotted should be a straight line. This graph is used to estimate urea content in the defiled milk samples. Secondly, estimation of urea is done in milk illustrations. The blank which is considered is diluting reagent. The O.D. is taken at 420nm.

Detection and Estimation of Added Glucose in Milk

Milk is already having an accepted amount of glucose which is 10 mg/100 ml.

Reagents used:

A. Modified Barford's reagent: Firstly 1.2 g of copper acetate is taken and dissolved into 25ml boiling water. Then 2ml of acetic acid of 8.5% concentration is taken and cooled to room temperature. This is kept in a dark bottle.

B. Phosphomolybdic acid: To prepare this, 0.5 g of sodium tungstate and 3.5 g of ammonium molybdate are taken and 10% of sodium hydroxide solution is added. Water

is added to it and heat up to eliminate ammonia. It is cooled and diluted with water. 85% of concentrated H3PO4 is added.

C. Acetate buffer: Same volumes of 1N acetic acid and1 N Sodium acetate with pH 4.75 are taken.

Steps to proceed:

Equal amount of acetate buffer and milk are taken in a test tube. Then it i filtered. The filtrate is taken and around 2 ml of water and modified barford's reagents are added to it. Test is placed for 5 minutes in a pre heated water bath. Afterwards, it is cooled and app. 2ml phosphomolybdic acid is added. The contents are mixed thoroughly. If deep blue colour appears that demonstrates the glucose. With the help of a filter paper, the solution is filtered and again the filtrate is taken. Then by using standard curve, glucose estimation can be done.

Quantitative analysis of Cane Sugar in Milk

This is done with the help of Lane- Eynon method. To curdle the milk, two chemicals are used:

- Zinc acetate
- Potassium ferrocyanide

Reagents used: Conc. Hydrochloric acid, Fehling solution, stock and standard solution of invert sugar

Quantitative Analysis of Starch in Milk

In this, alcohol is used to curdle milk. This step makes the milk sample free from lactose. Wash the precipitates with alcohol. Lane Eynon method is used to estimate reducing sugar content.

Reagents used:

A. Sodium carbonate

B. 98% ethanol

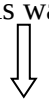
C. 10% sodium hydroxide.

Steps to proceed:

25ml of milk sample is taken in a beaker.

Milk is curdled with 20ml ethyl alcohol.

Precipitate is washed with 50% ethyl alcohol



Precipitate is transferred into a flask and water and conc. Hydrochloric acid are added.

For 2 hours place it in a boiling water bath.

After this, it is cooled with sodium carbonate and 10% sodium, hydroxide.

By Lane Eynon method, reducing sugar can be estimated.

EXPECTED OUTCOME

The samples from nearby sources were collected. They were generally domestic and commercially available. These were collected from Ludhiana and their qualitative analysis was done. The main focus was to detect the presence of any adulterant by using certain biochemical tests. The quantitative estimation of various adulterants in milk can also be determined. Here in this study, the quantitative analysis of urea has been done and the sensitivity of the method is also checked. The technique used mostly here was spectrophotometry. The methods that can be used to detect adulteration are ELISA (Enzyme linked immunosorbent assay) based methods, methods based on chromatography like HPLC (High performance liquid chromatography), liquid chromatography- tandem mass spectrometry and near infrared spectroscopy. Biuret method and lowry method are also there. Pesticides can also be a source of adulteration. Milk can also be naturally contaminated from udder.

PROPOSED WORK PLAN WITH TIMELINES

Workplan	**Timeline**
Visit to various industries	February to march 2017
Discussion with specific industry	March
Review of literature	April to May
Training at Verka, Ludhiana	June to July
Review of methodology	August to September
Detection of adulterants with biochemical methods	October to November
Collection of sample from various sources and performance	January to April

RESULTS

I have taken milk samples which were domestic and industrial (milk sample of Verka) and carried out various tests to determine whether any adulterant is present in that samples or not. Every adulterant has a specific biochemical test for its detection. The samples which I had collected were not giving a desired colour with the reagents used for that specific chemicals used as adulterants. It showed that they were showing negative tests and samples were not adulterated with any substance or chemical. Then to check the efficiency of these biochemical tests chemicals used as adulterants were added intentionally to the milk samples and different tests were done separately for that particular chemicals. They showed the positive results and colour which depicts the positive tests as described above the given table.

ADULTERANT	RESULT
Starch	Blue colour observed
Formalin	Blue ring formed
Sugar	Red colour appeared
Detergent	Violet colour appeared
Urea	Yellow colour appeared
Ammonium compound	Deep blue colour observed
Sulphates	Milky white precipitates were formed
Nitrates	Blue colour indicates positive test
Hydrogen peroxide	Blue colour observed
Pulverized soap	Pink colour appeared
Skim milk powder	Orange colour observed
Benzoic acid	Buff colour indicates positive test
Salicylic acid	Violet colour appeared

DISCUSSION

Contamination of drain is considered to decrease the idea of drain. From the regular deplete from dairy animals and wild bulls, cream, margarine, and fats are disengaged from cream separator machine. To make this drain again thick, urea, cleanser, burning Soda, starch oil, glucose, skimmed powder, cleanser, white paint, hydrogen peroxide et cetera are mixed to design manufactured deplete. It is exceptionally hurtful. Adulterants are for the most part added to grow the time allotment of reasonable ease of use of deplete. At the point, when purchasers buy drain, they have the benefit to acknowledge that it will be unadulterated. Regardless, shockingly, this isn't by and large the case. It is to be seen that most Indians have surrendered to drink deplete debilitated with water which not simply decreases the nutritious estimation of the refreshment yet furthermore acts peril to prosperity. Produced drain is set up by mixingglucose/sugar, urea, caustic soda, starch/blotching paper, refined vegetable oil, unassuming cooking oil, white paint and typical chemical or chemical. Drain is most by and large debilitated with water. This declines its dietary regard, and additionally cause additional medicinal issues.

Draining machine intended for quick and effective evacuation of drain. In any case, the steady vacuum was left on the nipple end of a expanded period, blood and lymph will amass toward finish of the nipple, making injury the nipple. This would resemble connecting a vacuum hose to the finish of ones finger. The region presented to the hose will be turn red with amassed blood. Erroneous vacuum or throb settings or worn nipple container liners all could upgrade part of the draining machine in adding to intramammary contamination.

By and large corrosives like Benzoic acid and Salicylic acid, are utilized like an additive of drain. Along these lines expands the timeframe of realistic usability of drain. Cleanser is added to drain to build the frothing of milk and in this way to have thick milk. Expansion of these chemicals result many medical issue particularly identified with stomach and kidneys.

Formalin which is a additive and can safeguard drain for drawn out stretch of time. Because of its high poisonous quality, it is considered to cause liver and kidney harm.

Ammonium Sulphate is added to the drain as it builds the lactometer perusing by keeping up the thickness of drain.

Urea can prompt heaving, sickness and gastritis. Urea may especially destructive for the kidneys, and scathing pop can be hazardous for individuals experiencing hypertension and heart illnesses.

Formalin may make serious harm to the body like liver harm. Wellbeing effect of drinking drain defiled with those chemicals is more regrettable to kids. Acidic pop damages the mucosa of nourishment pipe, particularly in children. The substance which contains sodium, can go about as moderate toxin for them agony from hypertension and heart afflictions.

SUMMARY

Defiled drain is a sweet dangerous substance which however does not kill immediately but instead it bit by bit make the body a nutritive place for infirmities. It shows dangerous for pregnant women and patients encountering conditions of heart torment and hypertension. It is enormously unsafe for little children. Drain adulterants have dangerous prosperity impacts. The chemical in drain can cause sustenance hurting and other gastrointestinal burdens. Its high essential level can in like manner hurt body tissue and pulverize proteins. Other designed parts can cause blocks, heart issues, development or notwithstanding passing. While the fast effect of drinking drain corrupted with urea, acidic pop and formalin is gastroenteritis, the whole deal impacts are altogether more certifiable.

CONCLUSIONS

Albeit monetary profit is thought to be one of the real explanations behind drain defilement, deficient supply for the expanding populace everywhere throughout the world has cleared the ground for this also. This issue is more intense in the creating and immature nations because of absence of sufficient checking and law implementation. Existing normal recognition procedures are most certainly not continuously advantageous and open in these nations making it hard to address the differing methods for deceitful debasement in drain. This calls for consolidated endeavors from mainstream researchers and the administrative specialists through the advancement, execution and dispersal of better methods for the recognition of drain defilement. Also, mindfulness and access to data can assume indispensable part in these districts to defeat this issue. Some of these simple location techniques at the purchaser level.

Adulteration in milk has been a reason for worry for both the Government and the Dairy Industry. The Indian Council of Medical Research has announced that "milk adulterants have perilous wellbeing impacts. The cleanser in milk can cause nourishment harming and other gastrointestinal intricacies. Its high basic level can likewise harm body tissue and obliterate proteins. Other manufactured parts can cause disabilities, heart issues, malignancy or even passing. While the quick impact of drinking milk defiled with urea, burning pop and formalin is gastroenteritis, the long haul impacts are much more genuine."

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