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Research/Technical Note

Laboratory Manual for Common Milk Quality Testing

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Abstract

Milk quality assessment is critical to ensure safety and prevent adulteration in dairy supply chains. This study outlines rapid, cost-effective methods for evaluating milk quality at collection points, including sensory inspection, heat-induced solidification or clot on boiling (C.O.B), Alcohol Coagulation, Lacto scan, Milk Density, Freezing Point, Inhibitor, Phosphatase, and tests for adulterants like cane sugar, urea, formalin, starch, and soaps/detergents. Organoleptic tests rely on sensory evaluation to detect abnormal smell, taste, or appearance, indicating issues like bacterial contamination or acidification (pH < 6.4). The C.O.B. and Alcohol Coagulation tests identify high acidity (pH < 5.8) or protein instability, signaling unsuitable milk for processing. The Alcohol-Alizarin test provides dual insights into protein stability and pH via color changes. Lacto scan offers precise compositional analysis, measuring fat, solids-not-fat (SNF), proteins, and more. Density and freezing point tests detect water adulteration, while inhibitor and phosphatase tests ensure safety by identifying antibiotics or inadequate pasteurization. Adulterant-specific tests reveal cane sugar, urea, formalin, starch, or detergents, safeguarding consumer health. These methods, requiring minimal equipment, enable rapid quality control, ensuring only safe, high-quality milk enters the supply chain. Generally, enhancing test sensitivity to identify lower levels of adulterants and incorporating automated systems for real-time monitoring could be the main goals of future developments.

Keywords

Adulteration, Coagulation, Milk Quality, Organoleptic, Safety

1. Introduction

Safeguarding dairy standards and reliability is essential within the production sector to protect users and sustain confidence in items. Since milk is highly perishable, it is vulnerable to spoilage and adulteration, which can reduce its nutritional value and safety. This laboratory manual offers a comprehensive guide to quick, reliable, and cost-effective methods for evaluating milk quality at various points in the supply chain, especially at collection sites. The tests described including organoleptic assessment, heat solidification (C.O.B), Alcohol Coagulation, Alizarin test, Acidity, Lacto scan, Milk Density, Freezing Point, Inhibitor, Phosphatase, and tests for detecting adulterants like cane sugar, urea, formalin, starch, and soaps or detergents are designed to identify spoilage, contamination, or fraudulent practices. These methods, based on established standards [3, 6], require minimal equipment and are suitable for both field and lab use. By using these tests, dairy professionals can ensure adherence to quality standards, detect issues such as bacterial contamination, high acidity, or adulteration, and protect public health. This manual is a practical resource for milk graders, technicians, and quality control staff to maintain the integrity of the dairy supply chain.

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2. Milk Quality Testing

Milk quality testing is essential to ensure the safety, nutritional integrity, and authenticity of milk in the dairy supply chain. It serves as a practical guide for laboratory and field applications. The tests are designed to detect spoilage, adulteration, and ensure compliance with safety standards, as referenced in established protocols [3, 6].

2.1. Organoleptic Tests

Sensory analysis allows quick identification of inferior dairy at receiving areas. An evaluator must be capable of distinguishing appearances, odors, and flavors accurately [10].

Materials

No dedicated instruments or reagents are necessary [5].

Procedure

1. Unseal a milk holder.
2. Detect the aroma instantly.
3. Assess the visual traits.
4. If uncertain about standards from aroma or visuals, test the flavor.
5. Review the holder's cover and inside for cleanliness.

Comments

Irregular odors or flavors may arise from:

a. Environmental scents (feed or body-related).
b. Animal health concerns, such as delayed milking or natural tartness.
c. Bacterial byproducts.
d. Artificial colorants in the product.
e. Heightened tartness (pH < 6.4).

Precaution

The milk sample should be spat into the designated container rather than swallowed. Milk might contain sensitizing agents for the evaluator [12].

2.2. Clot on Boiling Test (COB) or Heating Coagulation Test

The heating coagulation check is a simple, fast way to spot dairy that is too sour (pH under 5.8) or unusually high in initial milk proteins [10]. If milk is negative for the C.O.B. test, it may contain acids, acid-producing bacteria, or an abnormally high protein content, as seen in colostrum [5]. Such milk forms curds or clots during heat treatment in milk processing, making it unsuitable for further use and requiring rejection [12].

Materials

1. Testing container
2. Dairy sample
3. Heating device or alcohol lamp
4. Ignition source

Procedure

1. Collect 5 ml dairy sample.
2. Secure the container with a holder.
3. Heat the dairy in the container over a flame for 5 minutes.

Result

Formation of solids or coagulation shows the sample does not pass the heating check.

Comments: This approach might not identify quality if:

a. Newly extracted dairy has bacterial presence.
b. Sourness level is below 0.20-0.26% lactic content.
c. Dairy is mixed with non-sour-producing microbes.

2.3. Alcohol Coagulation Test or Ethanol Stability Test

The ethanol stability check is straightforward and quick. It relies on the diminished stability of dairy proteins when sourness rises or other acidic conditions exist. Protein presence grows with higher sourness or in initial secretions. Heightened salt levels, like in salty dairy, could fail the evaluation [12].

Materials

1. Testing container
2. Dairy sample
3. Reagent in a sealed bottle (86% ethanol mix)

Preparation of alcohol solution

An ethanol mix is created by combining 68 cc of 96% (pure) ethanol and 28 cc of purified water.

Procedure

1. Collect a 2 ml sample in a testing container.
2. Introduce 2 ml of 86% ethanol mix.
3. Allow the blend to rest at 25-37 ℃ for 5 minutes.

Result

Solid formation, solids, or settling of the dairy sample, even minor clumps, indicates failure for the check.

2.4. Lactoscan (Milko-Scan) Test

Dairy from all mammal types is a balanced nourishment for offspring. Its elements are assessed manually through color-based techniques, but currently, Lactoscan or similar devices offer fast evaluations of dairy makeup, including lipids, warmth, pH, non-lipid solids, sugars, total proteins, liquid content, minerals, conductivity, and thickness. Samples can be gathered from new dairy, tank dairy, processed, or bagged dairy.

Materials

1. Milk analyzer with acidity sensor and sampling tube.
2. Milk specimen.
3. Cleaning the container with purified liquid.
4. Container of 500 ml size.
5. Glass 250 ml size.

Procedure

1. Adjust the dairy heat to room level.
2. Place 20 mL of dairy in a container and immerse the acidity sensor in it.

3. Introduce 20 mL of dairy into the glass holder and supply the specimen via the intake tube.
4. Change on the device.
5. In 5 minutes, it will absorb the specimen and show the outcome on the display role.

2.5. Alcohol-Alizarin Test

This evaluation is similar to an ethanol check. Alizarin acts as an acidity marker. Ethanol-alizarin (A-A) check is more detailed. It will indicate the coagulation as well as the intensity of acidity. The Alcohol Alizarin (A–A) Test, also known as the alizarol test, enhances the classic alcohol stability assay by integrating the pH indicator alizarin. In this test, equal volumes of milk and a saturated alizarin–alcohol solution (typically 70% ethanol) are mixed. Alcohol destabilizes milk proteins, causing coagulation if acidity or other destabilizing factors are present, while alizarin provides a color change that reflects the milk's pH, such as red-brown for normal, yellow-brown in acidic samples, or lilac/violet for alkaline conditions. As a result, this test offers a rapid, dual-purpose check of both protein stability and acidity, making it a practical and widely used tool for ensuring milk quality, especially for heat processing applications [8].

Materials

1. Ethanol-Alizarin mix
2. Testing container
3. Milk specimen

Preparation of the solution

Ethanol mix is made by blending 61 mL of 96% (pure) ethanol and 39 mL of purified liquid.

Procedure

1. Collect 2 mL of the dairy sample in a testing container.
2. Introduce 2 ml of ethanol-alizarin mix.
3. Allow the blend at 25 ℃ for 5 minutes.

Result

Solidification, solids, or settling of the dairy along with its shade.

2.6. Acidity Test

Microbes that normally develop in raw dairy convert sugar to lactic acid, increasing its sour level. The quantity of sour content is measured, and from this amount, the percentage of sour content is determined. Raw milk naturally contains bacteria that ferment lactose, producing lactic acid as the main by-product. It measures the degree of this fermentation by titration [2, 3, 7].

Materials

1. Small tapered container (100 ml size)
2. Scaled dropper (10 ml size)
3. Scaled dropper (1 ml size)
4. Stirring tool for mixing product
5. Phenol color indicator mix (0.5% in 50% ethanol)
6. N sodium alkali solution

Procedure

1. Collect 9 mL of product in the container.
2. Transfer phenol color indicator (1 ml) to the product in the container.
3. Add alkali solution (0.1 N) with steady stirring from the dropper until a faint rose tone appears.
4. Note the volume of alkali used and divide by 10 to get tart percentage.

Comments

The new product has "innate tartness" due to natural resistance to pH shifts. The natural tartness of the product is 0.16 – 0.18%. Values above this suggest bacterial breakdown of sugar.

2.7. Measurement of Milk Density Test

Milk's density is known as its specific gravity. It can be ascertained using a lactometer. In order to increase the volume of milk and obtain a higher price for the same pure milk, middlemen add water to it. It is an attempt to mislead the purchasers. The fact that middlemen add water to milk is well known. As a result, buyers make sure that water is added to milk [1-3].

Materials

1. Density meter
2. Product sample 500 mL

Procedure

1. Blend the product gently and pour into a measuring cup (500 mL capacity).
2. Insert the density meter slowly into the product sample.
3. Read and note the final density reading (℄) just above the surface.
4. If the temperature of the milk is different from the calibration temperature, apply the correction factor.

2.8. Freezing Point Determination

This assessment consistently shows values compared to all other measurable traits. Minor dilution with fluid causes a noticeable shift in its chill point from normal. Values of -0.54 ℃ or above indicate accurate and sensitive to added fluid in the product. It is thus employed to track dilution with fluid [3, 4, 13].

Material

1. Milk sample
2. Freezing point apparatus or a small test tube and ice-salt bath
3. Thermometer (accurate to 0.01 °C if possible)
4. Stirring rod
5. Clamp and stand (optional)

Procedure

1. Pour a small amount of milk into the test tube.
2. Placed the tube in the freezing point apparatus or an ice-salt bath.
3. Gently stir the milk continuously to ensure even cooling.

4. Monitor the temperature carefully with the thermometer.
5. Record the temperature at which the first ice crystals appear; this is the freezing point.
6. Repeat the measurement 2–3 times for accuracy and take the average.

Result

The chill point of the product is generally around -0.55 ℃.

2.9. Inhibitor Test

Product or milk is sometimes treated with antimicrobials or cleaners during long-distance transport. Lactic acid-producing bacteria are inhibited in their growth by these antibacterial agents. Additionally, it poses a risk to end users' health. After a few hours, the suspected product sample is evaluated following a fermentation test using a starter organism. The measurable tartness values obtained are contrasted with those of a sample that was similarly handled but lacked any restrictive agents [8].

Materials

1. Test vessels
2. Starter organism
3. One ml dropper
4. Water immersion
5. Material for tartness measurement

Procedure

1. Fill three test vessels with 10 ml sample for tested, and three vessels are filled with standard product.
2. All vessels are warmed to 90 ℃ by placing them in a water immersion for 5 minutes.
3. After cooling to 30 ℃ temperature for the starter organism, add one ml of starter organism to each test vessel, and incubate for 3 hours.
4. After each hour, one test vessel from each of the "tested" and the "reference" vessels is removed and processed for estimation of its tart content.

Results

If acid generation in the suspected sample is the same as the standard, the suspected sample does not contain any inhibitory substances. If the acid production in the suspect sample is less than in the standard product sample, then the suspected sample contains any antibacterial or other inhibitory substances.

2.10. Phosphatase Test

Standard product is vulnerable to infection and comes from pathogenic sources. It may harbor harmful bacteria if animal is suffering from ailments. Some of the disease-causing bacteria are zoonotic (Mycobacterium tuberculosis, Brucella abortus, Q fever bacteria etc.) and may cause infection in product users. Product also contains heat-sensitive phosphate that is normal enzyme present in somatic cells of product. The enzyme is heat labile and is destroyed at the pasteurization temperature. Pasteurized product is therefore considered as safe for human users [13, 14]. Inactivation of the enzyme in the pasteurized product milk is indication that milk is properly pasteurized and all the pathogenic bacteria are destroyed. If the product is positive for the enzyme, it means the pasteurization process was inadequate and the product may not be safe for human use and will have a short shelf life.

Materials

1. Test vessels
2. Droppers (one and 10 mL capacity)
3. Volumetric container (100 mL and 500 mL capacity)
4. Buffer solution is prepared by mixing 0.75g anhydrous sodium carbonate and 1.75g sodium bicarbonate in 500 ml of distilled water
5. Buffer-substrate solution is prepared by placing 0.15 g of di-sodium para nitrophenyl phosphate (the substrate) into a clean 100 mL measuring cylinder.
6. All glassware must be rinsed, cleaned, demineralized in dichromate solution, and boiled in water for 30 min. Store this buffer and substrate solution in a refrigerator and protect from light. It should not be used after one week.

Procedure

1. Pipette 5 ml buffer-substrate solution into a test vessel, cover with a suitable stopper, and warm in the water bath at 37 ℃.
2. Add to the test vessel 1ml of the sample and mix well, and place in a water bath at 37 ℃.
3. Prepare a blank sample from the heated sample type that has undergone boiling for 2 ℃.
4. Incubate both the test samples and the blank sample at 37 ℃ for 2 hours. After incubation, remove the tubes and mix them thoroughly.
5. Place one sample against the blank in a Lovibond comparator "All purposes using "A.P.T.W" disc and shake until the color of the test sample is matched and read the disc number.

3. Milk Adulteration Test

3.1. Cane Sugar

One of the common adulteration practices in milk is the addition of sugar (sucrose). It is added artificially to increase SNF content, thereby raising the lactometer reading, which would otherwise fall below the normal range if the milk had been diluted with water [1, 6]. In the laboratory, the presence of added cane sugar is detected using the Resorcinol Test (Seliwanoff's test principle). In this test, sucrose in milk undergoes hydrolysis under acidic conditions to yield glucose and fructose. The fructose then reacts with resorcinol in hydrochloric acid, producing a red-colored complex, and accepts the occurrence of added sugar [3, 6].

Materials

1. Test chemical
2. Test vessels
3. Pasteur dropper
4. Product sample

Composition of the reagent

The ratio of resorcinol and HCl is (1:1.5). Acid solution should be prepared in a fume chamber because fumes of the concentrated acid are toxic to lab staff. Add the acid to the water along the sides of the container.

Procedure

1. Take two test vessels and label one as "test" and the other as "control".
2. Transfer one ml product sample to each of the vessels.
3. Add 1.0 ml of the reagent in the vessel marked as "test" from the side of the test vessel slowly, and 1.0 ml of distilled water in the vessel marked as "control".
4. Mix the contents of each vessel.

Results

The appearance of deep red color shows the presence of sucrose, or cane sugar.

3.2. Urea Test

Urea is often added during the preparation of a synthetic product to increase the SNF value [11]. The turmeric paper method is used to test for urea in a product. This test applies to formaldehyde-treated products and can detect urea. Concentrations greater than 0.1% thus distinguish it from the naturally occurring urea in the product, which is usually below 0.1% [1, 6].

Preparation of Turmeric Paper

About 50 g of coarsely powdered turmeric root is boiled for 30 minutes, and the extract is strained. Filter paper is dipped in this extract and dried. The resulting turmeric paper turns brown when exposed to alkalis, and also reacts with boric acid or borates [3, 6].

Materials

1. Test reagent
2. Test vessels
3. Pasteur dropper
4. Product sample

Procedure

1. Take two test vessels as "test" and "control".
2. Transfer one ml product sample to each of the vessels.
3. Add 1.0 ml of the reagent to the vessel as "test" and 1.0 ml of distilled water to the vessel as "control".
4. Mix the contents of each vessel.

Results

A different creamy color is detected in milk comprising urea. However, the standard milk may display a minor creamy color due to the occurrence of urea.

3.3. Formalin Test

Formaldehyde is generated in rural and urban regions of the nation and is transported twice a day to big cities. In the summer months, the high ambient temperature accelerates microbial growth, leading to rapid spoilage. To prevent this, transporters may add ice blocks to cool the milk, but in some cases, they also use formalin (aqueous formaldehyde solution) as a preservative [1, 3]. While formalin effectively delays spoilage by inhibiting microbial activity and enzymatic action, it is highly toxic and carcinogenic. Consumption of milk adulterated with formalin can cause gastrointestinal irritation, kidney damage, respiratory distress, and increased cancer risk [6, 9]. Therefore, the addition of formalin to milk is strictly prohibited.

Materials

1. Test reagent
2. Test vessels
3. Pasteur dropper
4. Milk sample

Procedure

1. Take two test vessels and label one as "test" and the other as "control".
2. Transfer one ml milk sample to each of the vessels.
3. Add 1.0 mL of the reagent in the tube marked as "test" from the side of the test tube slowly, and 1.0 mL of distilled water in the tube marked as "control".
4. Mix the contents of each tube.

Results

Formaldehyde is present in milk when a purple ring forms at the junction.

3.4. Starch Test

Addition of cereal flours and others are commonly added to increase the density of milk (Lactometer reading), which reduces if it is adulterated with water. Uncooked starch may be a health hazard for consumers, but it is deceiving/cheating the end consumers. The presence of starch or cereal flours is detected in the lab.

Materials

1. Test reagent
2. Test vessels
3. Pasteur dropper
4. Milk sample

Procedure

1. Take two test vessels and label one as "test" and the other as "control".
2. Transfer one ml milk sample to each of the tubes.
3. Add 0.5 ml of the reagent to the tube marked as "test" and 0.5 ml of distilled water to the tube marked as "control".
4. Mix the contents of each tube.

Results

Occurrence of starch is shown by the presence of a navy color, which disappears after the sample is bubbled and returns on chilling.

3.5. Soaps/Detergents Test

Milk producers remove the fat and add water to the skimmed milk before transporting it from rural to urban areas. Because of this, the milk is thin and doesn't have any foam, so milk producers use detergents to increase the amount of milk foam that is produced.

Materials

1. Test reagent

2. Test tubes
3. Pasteur pipette
4. Milk sample

Procedure

1. Take two test tubes and label one as "test" and the other as "control".
2. Transfer one ml milk sample to each of the tubes.
3. Add 0.5 ml of the reagent to the tube marked as "test" and 1 ml of distilled water to the tube marked as "control".
4. Mix the contents of each tube.

Results

The appearance of a rosy or red color.

4. Conclusion

The described milk quality tests provide a robust framework for ensuring dairy safety and integrity. Organoleptic, C.O.B., Alcohol Coagulation, and Alcohol-Alizarin tests offer quick, low-cost screening for spoilage and protein instability, critical at collection points. Lacto scan delivers precise compositional data, enhancing quality assurance. Density and freezing point tests effectively detect water adulteration, while inhibitor and phosphatase tests confirm the absence of harmful substances and proper pasteurization, respectively. Adulterant detection tests for cane sugar, urea, formalin, starch, and detergents protect consumers from fraudulent practices and health risks, such as formalin's carcinogenic effects. These methods, grounded in established protocols, are practical for widespread adoption due to their simplicity and minimal resource requirements. Implementing these tests ensures only safe, unadulterated milk reaches consumers, supporting public health and trust in dairy systems. Future improvements could focus on enhancing test sensitivity to detect lower adulterant levels and integrating automated systems for real-time monitoring.

Abbreviations

COB	Clot on Boiling
HCL	Hydrochloric Acid
SNF	Non-Fat Solids

Author Contributions

Muluken Getachew: Conceptualization, Data curation, Formal Analysis, Funding acquisition, Investigation, Methodology, Project administration, Resources, Software, Supervision, Validation, Visualization, Writing – original draft, Writing – review & editing

Mekasha Seid: Writing – review & editing, Conceptualization, Data curation, Formal Analysis, Funding acquisition, Investigation, Methodology

Conflicts of Interest

The authors declare no conflicts of interest.

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