DEPARTMENT OF NUTRITION AND DIETETICS

COMPARATIVE QUALITATIVE DETECTION OF ADULTERANTS IN SELECTED COMMERCIAL BRANDS OF POWDERED MILKS SOLD WITHIN ILORIN METROPOLIS

By

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ND/23/NAD/PT/0008

BEING A PROJECT SUBMITTED TO
THE DEPARTMENT OF NUTRITION AND DIETETICS, INSTITUTE OF APPLIED SCIENCES, KWARA STATE POLYTECHNIC ILORIN, KWARA STATE
IN PARTIAL FULFILLMENT OF THE REQUIREMENT FOR THE AWARD OF NATIONAL DIPLOMA (ND) IN NUTRITION AND DIETETICS, KWARA STATE POLYTECHNIC ILORIN, KWARA STATE

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2024/2025 SESSION

CERTIFICATION

This is to certify that this project work presented by **Oguntayo Sherifah Omowunmi** with Matriculation Number **ND/23/NAD/PT/0008** has been read, approved and submitted to the Department of Nutrition and Dietetics, Institute of Applied Sciences, Kwara State Polytechnic, Ilorin.

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DEDICATION

I dedicate this to Almighty Allah for given the knowledge throughout the journey.

To my incredible brother, for your unwavering support, encouragement, and the silent strength you have always shown me. Your belief in me has been a steady force through every challenge.

And to my darling husband, thank you for walking beside me, holding me up when I faltered, and never letting me give up on myself. Your love, patience, and constant presence have been my anchor.

This work is a reflection of your sacrifices, prayers, and love. I dedicate it to you all, with all my heart.

ACKNOWLEDGMENT

I would like to express my deepest gratitude to Almighty Allah for granting me the strength, knowledge, and perseverance to complete this project successfully.

My heartfelt appreciation goes to my dear parents and my husband for their unconditional love, endless prayers, and constant encouragement throughout this journey. Your support has been my greatest motivation.

I am also grateful to my supervisor/lecturer, Mr Adeyemo .O. Elijah, for his valuable guidance, feedback, and patience during the course of this work.

To my friends, coursemate, and everyone who supported me in one way or another thank you for being part of this journey.

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Abstract

Five commonly sold commercial brands of powdered milk were analyzed to detect the presence of adulterants in these products. Fifteen 15 different adulterants were tested for in each commercial product sample. The results revealed that all the commercial powdered milk samples analyzed for contained at least one adulterant (several adulterants were detected in each sample). This outcome calls for increased and more aggressive monitoring and standardization of these group of products.

Keywords: Powdered milk, adulterants, nutrition, health

CHAPTER ONE

1.0 INTRODUCTION

Food safety and quality are rising concern all occur over the word particularly when it comes to human health in this regard, many countries have been running quality control programs for all food ingredient including animal sources foods[1]. Food safety is a scientific discipline dealing with handling preparation, and storage of food in the manner that prevent food bone illness, this require a number of routine activities that should be followed to prevent occurrence of potentially severe health hazard. Hazard is a biological, chemical, or physical agent that is contributing likely to cause a great deal of or injury in the obscene of its control wide-angle of food bone can be caused by routine activities like keeping personal hygiene, proper processing of the food, heat treatment at higher temperature, adequate cooking before consumption and not subjecting the food to temperature where bacteria can grow[2].

1. M. G. El-Ziney, and A. I. Al-Turki. Microbiological quality and safety assessment of camel milk (Camelus dromedaries) in Saudi Arabia. Appl. Ecol. Environ. Res. 2007, 5(2), 115-122. Penkala Bt., Budapest, Hungary.
2. M. Addis, and D. Sisay. "A Review on Major Food Borne Bacterial Illnesses. J. Trop. Dis. 2015, 3:176.

Milk is highly nutritional food and ideal for microbial growth the fresh milk deteriorate easily to become unsuitable for processing and human consumption[3]. Milk obtain from healthy animal udders is free from pathogenic bacteria but some of the animal in field condition may be suffering from subclinical mastitis and are excreting causative agent in milk, such milk contaminate the bulk milk moreover fresh milk may get microbial contamination from utensils animals skin, environment, or water may be used for cleaning etc[4]. Foodborne diseases are common wide speed global problem several out-break have been reported as a result of consuming contaminated milk that may look, taste and smell perfectly normal but are in fact contaminated with large number of harmful bacteria[5]. Milk contaminated by high level of spoilage bacteria usually become unsuitable for further processing since it does not meet the consumer expectation in term of heath (nutritional value) safety

3. FAO. "The lacto-peroxidase system of milk preservation". Regional Lacto-peroxidase Work-shop in West Africa; Burkina Faso: 2001, 17-19.
4. FAO. Milk Hygiene, In Milking, Milk Production Hygiene and Udder Health, FAO Animal Production and Health Papers-78, FAO Corporate Document Repository 2008, 1-7 pp.
5. Centers for Disease Control and Prevention, CDC. 2009. Surveillance for foodborne disease outbreak. United States. 2006. Morbidity Mortality Weekly Rep. 58: 609-615.

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(hygiene quality) and satisfaction (sensory attributes)[6]. Salmonella are considered among the most important enteric food bone pathogen whose presence in the food constitute a serious health hazard contamination of raw milk with salmonella spp is mostly due to infected person of the udder are rare and seldom contribute as one of the major reason for contamination of milk with both spoilage and pathogenic bacteria[7] yersina enterocolitica is the most prevalent yersina species connected to disease in human. The organism has received considering attention as a causative agent of human gastroenteritis[8].

Milk (Lac) which was used by human in the early of the seventh millennium BC[9,10] is a nutritious white world food secreted by the

6. E. Nanu, et al. "Quality assurance and public health safety of raw milk at the production point". American Journal of Food Technology. 2007, 2:145-152,

7. Mubarack, H. M.: Dhanabalan, R., Balachander. S.2010."Microbial quality of raw milk samples collected from different village of combatore district,Tamilhadu, South India," Indian J. Sci. Technol. 3 (1) 61-63.

8. Robins-Browne, R.M. 2001. Yersinia enterocolitica in food microiology fundamentals and Fronties, pp. 215-245. Edited by M.P.Doyle, L.R.Beuchat and T.J. Montiville .Washington, DC :American Society for Microbiology.

9. Evershed RP, Payne S, Sherratt AG, Copley MS, Coolidge J, Urem-Kotsu D, et al. Earliest date for milk use in the near east and southeastern Europe linked to cattle herding. Nature. 2008;455:528–31.

10. Dudd SN, Evershed RP. Direct demonstration of milk as an element of archaeological economies. Science. 1998;282:1478–81.

mammary glands of mammals, cow milk consumption varies around the world, with an average of 10-212 kg per person per year[11]. Milk contain 18 of 22 essential nutritients[12] include a various of bioactive peptide and fatty acid such as casein, when protein milk polar (MPL), α linolenic acid (ALA) (16:0) lactose and other minor constituents (i.e. calcium, phosphorus, magnesium and vitamin D) which have an important impact on human metabolism and heath[13,14] evidence showed that milk has wide range of physiological functionality including anti-carcinogenic[15], anti-inflammatory[16], anti-oxidative[17], anti-dipogenic[18], antihypertensive[19],

11. World Health Organization. Global and regional food consumption pat-terns and trends. 2018. http://www.who.int/nutri tion/topic s/3_ foodc onsum ption /en/index 4.html.
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13. Bougle D, Bouhallab S. Dietary bioactive peptides: human studies Crit Rev Food Sci Nutr. 2017,57.335-43.
14. Fulgoni VL, Keast DR, Bailey RL, Dwyer J. Foods, fortificants, and supplements, where do Americans get their nutrients? J Nutr 2011141(10) 1847-54
15. P. W. Parodi. “Cows’ milk fat components as potential anticarcinogenic agents”. J Nutr. 1997, 127, 1055–60.
16. M. S. Da Silva and I. Rudkowska. “Dairy nutrients and their effect on inflammatory profile in molecular studies”. Mol Nutr Food Res. 2015, 59, 1249–63.
17. S. Sultan, et al. “Therapeutic potential of dairy bioactive peptides: a contemporary perspective”. Crit Rev Food Sci Nutr. 2018, 58, 105–15.
18. M. Milard, et al. “Milk polar lipids in a high-fat diet can prevent body weight gain: modulated abundance of gut bacteria in relation with fecal loss of specific fatty acids”. Mol Nutr Food Res. 2019, 63, e1801078.
19. J. He, et al. “Effect of dietary protein supplementation on blood pressure: a randomized, controlled trial”. Circulation. 2011, 124, 589–95.

anti-hyperglycoma[20], and antiosteoporosis[21]. Milk has not been only the primary source of nutrition for any new born mammals species, but also an excellent source of the nutrients for children growth and most adult which has been recommended by the great amount of dietary guidelines all over the word[22,23]. Word wide consumption of milk from livestock product were expected to project up to 89.5kg per year/ person in 2030 from only 78.1kg per year in between the year 1997-1999[24]. It is well documented and known that milk process valuable nutrients and contribute to our body requirement such as calcium, magnesium, selenium, riboflavin, vitamin B_{12} and vitamin B_5[24]. Breast milk has been recommended (WHO) for growing young children[24]. For full term new born baby the consumption of human milk of up to six month and above

20. S. O'Connor, et al. "Increased dairy product intake alters serum metabolite profles in subjects at risk of developing type 2 diabetes". Mol Nutr Food Res. 2019, 63, e1900126.
21. J. Cadogan, et al. "Milk intake and bone mineral acquisition in adolescent girls: randomised, controlled intervention trial". BMJ. 1997, 315:1255–60.
22. World Health Organisation European Region. Food based dietary guidelines in the WHO European Region. Copenhagen: WHO, Europe; 2003.
23. Food and Agricultural Organization, World Heath Organisation. Prepara-tion and use of food-based dietary guidelines. Report of a joint FAO/ WHO consultation. Nicosia, Cyprus: WHO; 1996.
24. FAO. Milk and dairy products in human nutnition Milk and Dairy Products in Human nutrition, 2013.

has led to fewer cases of diarrhea, vomiting and infection, apart from that it also help to reduce the predisposition of obesity and non-communicable diseases such as diabetes and cardiovascular disease in the adult[25] particularly for pre term babies Breast milk is the best option to support the survival of pre term babies (very low birth weight babies) benefit from the intake of human milk because it promotes good neuro development outcome protection against infection, low rate of metabolic syndrome in the long term and during adolescent years associated with low blood pressure and lower risk of insulin resistance milk glycan component in the breast milk has pre biotic effect that help with prevention of disease such as necrotising enterocolthis a common and devastating disease of protein infact[26].

25.Lonnerdal B. Infant formula and infant nutrition:Bioactive protein of human milk and implications for composition of infant formullas.American Journal of clinical nutrition. 2014;99(3).

26.Pacheco AR, Barile D, underwood MA,muis DA. The impact of the milk glycobiome on the neonate gut micro biota. Annual review of animal biosciences.2015;419-45.

1.1 PROBLEM STATEMENT

Milk is a popularly consumed dairy product. It is in fact the most consumed and industrialized animal product. However, its consumption may pose certain health concerns if its quality and composition are not properly monitored.

The analysis of milk may be compared among different brands as a measure of the variations in their composition and quality.

This study is designed to qualitatively assess the major adulterants in milk as a result of sampling from the source,presentation of the raw samples and processing as well as industrial production patterns of each manufacturer. Some adulterants are intentionally added while some are introduced during processing.

1.2 AIMS AND OBJECTIVE OF THE STUDY

Aims

The study is aimed at qualitatively detection of a number of non-milk-components which may be intentionally or unintentionally added to milk products being sold for public consumption and may have effect on

peoples' nutrition and health. On the basis of the adulterant, this study also aimed at comparing the quality of the selected commercial brands of powder milk sold within Ilorin metropolis.

Specific objective

i. To collect commercial powdered milk samples readily available on sale within Ilorin metropolis.

ii. To identify common adulterants presents in commercial brands of evaporated milk available in Ilorin.

iii. To compare the level of adulteration across different selected brands using qualitative methods.

iv. To assess the possible health risks associated with the detected adulterants in these milk samples.

v. To compare the quality of the milk samples based on the absence of harmful adulterants.

1.3 SCOPE OF THE STUDY

The study encompasses such areas as collecting commercial powder milk samples readily available to the public within Ilorin metropolis.

Simple preparation of the samples(Dissolution) where necessary.This is to prevent any major change in the product before analysis.The eventual detection of the selected adulterants using simple chemical tests.

1.4 JUSTIFICATION OF THE STUDY

This study will be an eye opener to the quality of commercial products readily consumed by the public, the respective nutritional concerns it may cause as a result of unhealthy adulterants.

There is the need to provide such details of adulterants to assist relevant monitoring agencies and reducing these adulterants in products made available to the public. As nutritionists, it calls for knowledge of the unwanted and unexpected components to further effectively attend to patients with related health issues.

1.5 RELEVANCE OF THE STUDY

The study of the Comparative Qualitative Detection of Aldultrants in powdered milk sold within Ilorin metropolis relevant for promoting consumers' health and safety. By identifying the presence and type of adulterants in different brands, the study can help protect consumers from potentially harmful substance and ensure they are getting the quality of milk they expect.

1.5.1 Public health protection

Identifying Harmful adulterants: milk can be adulterated with various substance, some of which can be harmful to human health. For example formalin, urea, am detergents can be added to extend shelf life or increase volume. identifying these adulterants help prevent consumers from unknowingly ingesting harmful substance.

1.5.2 Promoting safe consumption

By identifying adulterated milk, the study can help guide consumers towards safer brands and products, promoting the consumption of milk that meet quality standards Quality control and Assurance: This study

will be relevant to the functions of appropriate governmental and non-governmental monitoring agencies on what component of milk products to enforce its removal during processing and production.

CHAPTER TWO

2.0 LITERATURE REVIEW

In most dairy industrialized countries, milk quality is defined by the level of somatic cells count (SCC) and the microbial load of milk in the pre-pasteurized bulk tank. These are the key components of international regulations put in place for milk quality[27].

2.1 QUALITY REGULATION

Governments, all over the world, have put in place various mechanisms for protecting their citizens from food borne illness to ensure the socio-economic development of their countries. Milk quality standards have been regulated by the respective food and drug administration in the countries[27].

27. Merwan Ahmedsham, Nezif Amza and Metekia Tamiru, "Review on milk and milk product safety, quality assurance and control". Livestock production. 9.4(2018):67-78.

2.2 MORTALITY

Milk consumption was not connected with total mortality[28], CVD mortality[29] or all cancer mortality[30], while it was associated with a elevated risk of mortality from coronary heart disease (CHD)[28] and prostate cancer[30].

2.3 CARDIOVASCULAR DISEASE

Although high verse low milk consumption was not related to the risk of CVD, CHD and stroke[31,32], dose response analysis manifested a 6%

28. M. Mazidi, DP. Mikhailidis, NG. Sattar, I. Graham, M. Banach, Lipid and Blood Pressure Meta-analysis Collaboration (LBPMC) Group, "Consumption of dairy products and its association with total and cause specific mortality". Clinical nutrition. 38.28(2019):33-45.
29. TA. O'Sullivan, K. Hafekost, F. Mitrou and D. Lawrence. "Food sources of saturated fat and the association with mortality". Amj Public Health. 103(2013):31-42.
30. W. Lu, H. Chen, Y. Niu, H. Wu, D. Xia and Y. Wu. "Dairy products intake and cancer mortality risk". Nutrition and metabolism. 15.91(2016).
31. J. Guo, A. Astrup, JA. Lovegrove, L. Gijsbers, DI. Givens and SS. Soedamah-Muthu. "Milk and dairy consumption and risk of cardiovascular diseases and all-causes mortality". European journal Epidemic. 32.2(2017):69-87.
32. P. Mullie, C. Pizot and P. Autier. "Daily milk consumption and all-cause mortality, coronary heart disease and stroke". BMC public health. 16.12(2016):36.

lower risk of CVD and a 4% lower risk of hypertension[33] with increment of 200mls milk consumption per day. However high-fat milk intake was connected with a 4% higher risk of stroke[34].

2.4 CANCER OUTCOME

High milk intake was consistently related to decreased risk of colorectal cancer (CRC)[35]. The highest milk intake was connected with a lower risk of both colon and rectal cancer, especially in colon cancer[36].

33. S. S. Soedamah-Muthu, EL. Ding, WK. Al-Delaimy, FB. Hu, MF. Engberink, WC. Willet and et al."Milk and dairy consumption and incidence of cardiovascular disease and all-cause mortality". Am J clinical nutrition. 93.1(2011):58-71.
34. S. S. Soedamah-Muthu, LDM. Verberne, EL. Ding, MF. Engberink and JM. Geleijnse. "Dairy consumption and incidence of hypertension". Hypertension. 60.113(2012):1-7.
35. J. de Goede, SS. Soedamah-Muthu, A. Pan, L. Gijsbers and JM. Geleijnse. "Dairy consumption and risk of stroke". J Am Heart Assoc.(2016).
36. L. Barrubés, N. Babio, N. Beverage-Tomás, N. Rosique-Esteban and J. Salas-Salvadó. "Association between dairy products consumption and colorectal cancer risk in adults". Advanced nutrition. 10.5(2019):190-211.

CHAPTER THREE

3.0 EXPERIMENTAL

3.1 REAGENTS AND APPARATUS

3.1.1 Reagents

Resorcinol Solution (0.5%), Iodine Solution, DMAB reagent (1.6%, w/v), Nessler's reagent (mercuric chloride, sodium hydroxide, potassium iodide, Barium chloride solution, Trichloroacetic acid (TCA), 24% (w/v, aq.), Silver nitrate ($AgNO_3$) solution: Potassium chromate (K_2CrO_4) solution, Diphenylamine (2%, w/v, in sulfuric acid), Dilute HCl solution, Starch solution, eosin indicator solution, 25g citric acid, Methylene blue dye solution, Turmeric Paper, ferric Chloride solution, methanol, Ethanol, Lead acetate, Cupric copper, Potassium hydroxide (KOH) solution, ferric chloride, sodium hydroxide (NaoH), distilled water, Mayer's reagent, Hagner's reagent, Wagner's reagent Dragendoff's reagent, acetic anhydride, hydrochloride, hydrochloric acid solution, ethanolic KOH, chloroform, pyridine, indicator solution (for Kjedahl titration), boric acid, nitropruside, Kjedhal tablet, potassium

hydroxide (KOH), pellet, concentrated tetraoxosulfate (vi) acid (H_2SO_4), acetone, hexane.

3.1.2 Apparatus and Equipment

Beaker, glass funnel, separating funnel, filter paper, stirring rod, test tubes, test tube rack, dropper, round bottom flask, measuring cylinder, volumetric flask, conical flask, spatula.

Equipment: weighing balance, water bath. Heating mantle.

3.2 COLLECTION OF SAMPLES

The selected samples of the various commercial brands (powdered/evaporated) milk were purchased from different shopping malls and retail sellers within Ilorin metropolis in sealed sachet and cans.

3.3 PREPARATION OF SAMPLES

Each milk sample was used as found in its package (with expiry date confirmed to be sure it was not an expired product). However, dilution

and/or dissolution were done where necessary as indicated in the procedure.

3.4 PREARATION OF REAGENTS

3.4.1 Preparation of Resorcinol Solution (0.5%)

0.5g of resorcinol was weighed into a beaker containing about 40 ml of distilled water. 35 ml of concentrated HCl (12 M) was added to it. The content was quantitatively transferred into and made up the volume to 100 ml volumetric flask using distilled water.

3.4.2 Preparation of Iodine Solution

2.6g of iodine crystals and 3g of potassium iodide crystals were dissolved in a sufficient quantity of water and made up to mark in a 200 ml standard flask.

3.3.2 Preparation of Iodine–Zinc chloride reagent

20g $ZnCl_2$ was dissolved in 8.5 ml water and when cooled, the iodine solution (3g potassium iodide and 1.5g iodine in 60 ml water) was introduced drop by drop until iodine begins to precipitate.

3.4.3 Preparation of DMAB reagent (1.6%, w/v)

p-Dimethylaminobenzaldehyde (DMAB) solution was prepared as follows; 1.6g DMAB was dissolved in 100ml ethanol and add 10 ml concentrate HCl. The reagent is stable for 1 month. Prepare new standard curve with each new batch of reagent.

3.4.4 Preparation of Nessler's reagent

The following chemicals were dissolved separately.

A. 8.0 g of mercuric chloride in 150 ml distilled water.

B. 60.0 g of sodium hydroxide in 150 ml distilled water.

C. 16.0 g of potassium iodide in 150 ml distilled water.

Reagent ‘A’ was added to reagent ‘B’ and mixed well. To this mixture, was added reagent ‘C’, mixed and diluted the contents to 500ml. This solution was left undisturbed and the clear upper layer of the solution was decanted and stored in a stoppered glass bottle.

3.4.5 Preparation of Barium chloride solution

Barium chloride ($BaCl_2.2H_2O$) 5% (w/v) aqueous solution: 5.0g of barium chloride crystals were dissolved in distilled water and made the final volume to 100 ml using appropriate standard flask.

3.4.6 Preparation of TCA solution

Trichloroacetic acid (TCA), 24% (w/v, aq.): 24g of TCA were dissolved into distilled water and make the final volume to 100 ml to obtain 24% TCA.

3.4.8 Preparation of Silver nitrate ($AgNO_3$) solution:

0.1M, aqueous solution: 16.99g of silver nitrate crystals were weighed and dissolved in distilled water, then made up to 1L with more distilled water in an appropriate volumetric flask.

3.4.9 Preparation of Potassium chromate (K_2CrO_4) solution

Potassium chromate (K_2CrO_4) solution: 10% (w/v) aqueous. 10g of the chromate salt was dissolved in 100ml of solution.

3.4.10 Preparation of Diphenylamine

Diphenylamine (2%, w/v, in sulfuric acid): 2g of diphenylamine were weighed and dissolved in sulfuric acid to obtain final volume of 100 ml.

3.4.11 Preparation of Potassium Iodide solution

Prepared fresh by dissolving 7g of potassium iodide in 100ml of water.

3.4.12 Preparation of Dilute HCl solution

To 200ml of water, 100 ml of Concentrated Hydrochloric acid (sp. gr. 1.16). was added.

3.4.13 Preparation of Starch solution

1g of soluble starch was dissolved in 10ml water. 90ml of boiling distilled water added to prepare the solution. It was cooled before using.

3.4.14 Preparation of eosin indicator solution

A stock solution was prepared by dissolving 0.05g eosin in 100 ml acetone. 10 ml of stock solution was shaken with 90 ml of tetrachloroethane and 1g citric acid and filter before use.

3.4.15 Preparation of Buffer solution

25g citric acid was dissolved in 100ml water and was adjusted to pH 3.5 with 50% Sodium Hydroxide solution (approximately 15 ml required).

3.4.16 Preparation of Methylene blue dye solution

12.5mg of Methylene blue dye was dissolved in 100ml of distilled water and the solution was protect against direct sunlight.

3.4.17 Preparation of Potassium iodide-starch solution

20g of potassium iodide was weighed and dissolved it in distilled water to obtain a 100 ml solution. Starch solution was prepared by weighing 1g starch powder and dissolved it in distilled water by heating and make up the volume to 100 ml.

Potassium iodide–starch reagent was finally prepared by mixing equal volumes of 20% potassium iodide solution and 1% starch solution.

3.4.18 Preparation of Turmeric Paper

1.5 to 2.0 g of turmeric powder was weighed into 250ml Erlenmeyer flask and 100ml 80% (v/v) ethanol was added, shaken for 5minutes and filtered. The filtrate was transferred into a flat bottom dish. A Whatman No 1 filter paper was soaked in the clear filtrate. The paper was then removed and hanged to dry in air. After 1 h, the paper impregnated with turmeric was cut into 6 x 1cm strips and stored in tightly stoppered bottle protected from light.

3.4.19 Preparation of 0.5% (v/v) neutral Ferric Chloride solution

0.5g of $FeCl_3$ was weighed into a beaker, 150ml of distilled water added to dissolve it with stirring. 5ml of dilute sodium

3.4 ANALYSIS OF SAMPLES

3.4.1 Detection of Cane Sugar in Milk

Sucrose is absent in milk and its presence in milk indicate adulteration. Presence of sucrose in milk can be determined by the Modified Seliwanoff'smethod.

1ml of milk sample was placed in a test tube. 1ml of Resorcinol Solution was added and mixed. The tube was placed in boiling water bath for 5 min. On withdrawing the tube and subsequent careful observation of the colour, an appearance of deep red colour indicates presence of sucrose, or a ketose sugar. In pure unprocessed milk samples no such red color is developed and sample remains white in nature. The limit of detection of method is 0.1% according to Bureau of Indian Standards (2003)[37].

37. IS 1479 (Part I) 1961 (Reaffirmed 2003) Methods of test for Dairy Industry – Rapid Examination of Milk.Bureau of Indian Standards, New Delhi).

3.4.2 Detection of Starch in Milk

5ml of milk sample was measured into a test tube and brought to boiling condition and allowed the test tube to cool to room temperature. 1-2 drops of iodine solution was added to the content of the test tube. Development of blue colour indicates presence of starch which disappears when sample was boiled and reappeared on cooling. The limit of detection of method is 0.02%[37].

3.4.3 Detection of Cellulose in Milk

10g of milk weighed into a 100ml beaker and 50ml of hot water was added and stirred thoroughly for about 2 min. The mixture was filtered and washed the residue with 50ml of hot water twice. The residue was scraped with a spatula and placed it in a spotting plate. A part of residue was stained with Iodine-Zinc chloride reagent and another part with iodine solution. Development of blue colour in Iodine-Zinc chloride reagent and absence of blue colour in Iodine solution confirms presence of cellulose[38].

38. Manual Methods of Analysis for Adulterants & Contaminants in Foods. I.C.M.R 1990 page 27).

3.4.4 Detection of Added Urea in Milk

This method is based on the principle that urea forms a yellow complex with DMAB in a low acidic solution at room temperature. 1 ml of milk with was mixed 1ml of 1.6% DMAB reagent. Distinct yellow colour is observed in milk containing added urea. The control (normal milk) shows a slight yellow colour due to presence of natural urea. The limit of detection of method is 0.2%.

3.4.5 Detection of Ammonium Compounds in Milk

5ml of milk sample was taken into a test tube. 1ml of Nessler's reagent was added. The content of the tube was mixed thoroughly. The color was observed and noted.

The control milk sample gives slight grayish colour. At low concentration of ammonium compounds, brownish shade appears which is distinguishable at 0.15% followed by yellowish colour and then orange colour development at higher concentration. The limit of

detection of method is 0.15% based on works of Guleria (1998)[39] and Naik (2012)[40].

3.4.6 Detection of Sulphates in Milk

The presence of sulfate salts, which may be added to milk to raise its SNF level in milk, can be detected by using barium chloride. SNF (Solid-Not-Fat; all the solid components of milk other than the milk fat and water)

10ml of milk sample (or 5g dissolved in 5ml of distilled water) was taken into a 50ml stoppered glass container 10ml of TCA solution was added. The coagulated milk was filtered through Whatman filter paper Grade 42. 5ml of clear filtrate was taken and to it was added few drops of barium chloride solution. Observation of any visible precipitates in the tube as formation of milky-white precipitates indicated the presence of

39. Guleria, V. (1998). Detection of added ammonium salts in milk with and without the addition of formalin. M. Sc. Thesis. NDRI, Karnal, India; Sharma, R.; Rajput, Y.S. and Naik,
40. N.L. (2012). Detection of adulterants in milk – a laboratory manual. NDRI Publication No. 88/2012, NDRI, Karnal, page 49-51).

added sulfates like ammonium sulfate, sodium sulfate, zinc sulfate and magnesium sulfate etc. to milk. The limit of detection of method is 0.05%[41].

3.4.7 Detection of Sodium chloride in Milk

To 5.0 ml of milk sample was added 1.0ml of 0.1M silver nitrate solution. The content was thoroughly mixed and added was 0.5ml of 10% potassium chromate solution followed by colour observation. Appearance of chocolate brown precipitate indicated the absence of dissolved chloride in milk and appearance of yellow colour indicated presence of dissolved chloride. The limit of detection of method is 0.02%.

3.4.8 Detection of Nitrates (Pond Water) in Milk

Pond water is heavier than the tap water; some unscrupulous persons for adulteration in milk usually prefer it. However, it can be easily

41. Sharma, R.; Rajput, Y.S. and Naik, N. L. (2012). Detection of adulterants in milk–a laboratory manual. NDRI Publication No. 88/2012, NDRI, Karnal, page 20-21).
42. Pearson's Composition and Analysis of Foods, 9th edn,1991 – Modified Mohr method, page 14).

detected by the following method. This method actually detects nitrates present in the pond water. In the pond water nitrates may come from fertilizers used in the fields.

2ml of milk sample (5g of powdered milk dissolved in 5ml of distilled water) in a test tube. The tube was rinsed with the milk and drain out from the test tube. Two-to-three drops of the Diphenylamine reagent were added along the side of the test tube. The development of a deep blue colour was formed in presence of nitrate in the milk sample. Pure milk sample will not develop any colour[43].

3.4.9 Detection of Hypochlorites and Chloramines in Milk

A. To 5ml of milk sample in a test tube 1.5ml of Potassium Iodide solution was added, mixed thoroughly and was observed for colour.

B. If unaltered, 4ml of dilute HCl was added, mixed thoroughly with a glass rod flattened at one end and the colour of curd was noted.

43. Sharma, R.; Rajput, Y.S. and Naik, N.L. "Detection of adulterants in milk– a laboratory manual". NDRI Publication No. 88/2012, NDRI, Karnal, 2012, page 47-48).

C. Subsequently, the tube was placed in a water bath previously heated to 85°C and was allowed to remain for 10 minutes. The curd would rise to the surface. The tube was cooled rapidly by placing in cold water. The colour of the curd and the liquid was noted.

D. 0.5.to 1.0ml of starch solution was added to the liquid below curd and the colour noted[44].

Interpretation

The proportion of available chlorine may be ascertained from Table 3.1 below

44. IS 1479 (Part II) 1961 (Reaffirmed 1997) Methods of test for Dairy Industry– Chemical Analysis of Milk. Bureau of Indian Standards, New Delhi; A.O.A.C 17th edn, 2000 Official Method 922.08 Hypochlorites and Chloramines in milk).

Table.3.1: Reactions with various tests to detect residual chlorine in milk.

Test No*	Concentration of available chlorine					
	1:1000	1:2000	1:5000	1:10000	1:25000	1:50000
A	Yellowish brown	Deep yellow	Pale yellow	-	-	-
B	Yellowish brown	Deep yellow	Light yellow	-	-	-
C	Yellowish brown	Deep yellow	Yellow	Yellow	Pale yellow	Yellowish
D	Blue purple	Blue purple	Blue, dark purple	Dark purple	Red purple	Pale red-purple

*** Indicates the step number followed in the above procedure.**

Note: The method is not reliable in the presence of more than 2.5 ppm of copper.

3.4.10 Detection of in Quaternary Ammonium Compounds Milk

Quaternary ammonium compounds (QAC) may be present in milk due to some residual detergent solution remaining after bottle washing. The following method detects about 5 mg / Kg in milk and is included in B.S 1741: Part II[45].

To a centrifuge tube 1ml milk was placed, 5 ml water, 1 ml indicator solution and 0.2 ml buffer and shake hard for 10 seconds, centrifuged for 5 minutes at 3200rpm. If QAC is present the bottom layer assumes a red or pink colour. Samples containing about 1 mg /kg of QAC show a faint pink colour. If the colour is deep pink or red, the amount of QAC can be approximately determined by titration with a standard anionic detergent solution[45].

3.4.11 Detection of in Anion Detergent Milk

Alkyl benzene sulphonic acid (ABS) or anionic detergent may be present in milk due to intentional addition of detergent in milk or due to insufficient rinsing of dairy equipment. The following method is based on the ionic interaction between the anionic detergent and cationic dye. Anionic detergents have a property to form a complex with cationic dyes.

45. B.S. 1741: Part II, Pearson's Composition and Analysis of Foods 9th edn 991, page 548).

The solubility of dye and dye-detergent complex differs significantly as dye-detergent complex is relatively less polar in comparison to dye

alone. Formation of dye-detergent complex between cationic dye and anionic detergents and subsequently its extraction into the hydrophobic solvent layer (lower) is the principle behind the method. The method is performed by addition of methylene blue dye solution and chloroform to milk, mixing of the content followed by centrifugation. This results in distribution of dye colour in upper layer and lower layers. Relative intensity of the colour is noticed in these layers. Appearance of relatively deeper blue colour in lower layer indicates the presence of detergent in milk. The developed test is sensitive to detect anionic detergent up to 0.0125% (12.5 mg/100 ml).

Procedure: 1ml of milk sample was pipetted into a 15 ml test tube. 1ml of dye solution was added followed by addition of 2 ml chloroform. The contents were vortex for about 15 sec and centrifuge at about 1100 rpm for 3 min. Note the intensity of blue color in lower and upper layer. Relatively, more intense blue color in lower layer indicates presence of detergent in milk. Relatively more intense blue color in upper layer

indicates absence of detergent in milk. The method can detect presence of 0.15% level of laboratory grade detergent in milk[46].

3.4.12 Detection of Formalin in Milk

2ml of milk sample in a test tube and add 2ml of 90 percent H_2SO_4containing traces of ferric chloride from the side of the test tube slowly. Formation of purple ring at the junction indicates formaldehyde is present in milk.

If sucrose is present, distil the milk sample (25 ml) and then carry out the test on the distillate by taking 2-3 ml of distillate and adding 2 ml of formaldehyde free milk. The violet coloration does not appear usually when relatively large quantities of formaldehyde are present.

Precaution: If H_2SO_4 is added from the top and not from the side of the test tube, it may burn the milk solids and affect the end result.

46. Rajput, Y.S.; Sharma, R. and Kaur, S. (2006) A kit for detection of detergent in milk. Indian Patent Office file no. 1970/Del/2006.
47. Pearson's Composition and Analysis of foods, 9th edition, 1991 page 90; IS 1479 (Part II) 1961 (Reaffirmed 1997) Methods of test for Dairy Industry – Chemical Analysis of Milk. Bureau of Indian Standards, New Delhi).

3.4.13 Detection of Hydrogen Peroxide in Milk

1ml of milk sample was placed in a clean test tube. 1ml of the potassium iodide-starch reagent was added and mixed well. The color of the solution in the tube was observed. Blue color will developed in the presence of H_2O_2, whereas pure milk sample remain white in color[43].

3.4.13 Detection of Presence of Boric acid and Borates in Milk

20ml of milk sample was measured into a porcelain dish and 1.4 ml of conc. hydrochloric acid added. The content was mixed thoroughly. A strip of turmeric paper was dipped in the acidified milk. Appearance of characteristic red colour on the turmeric paper indicates the presence of boric acid or borax ($Na_2B_4O_7.10H_2O$). The red colour changes to dark blue green on adding ammonium hydroxide, but reappears on re-acidification with hydrochloric acid[44].

3.4.15 Detection of Presence of Salicylic acid in Milk

50ml of milk sample was placed in a separating funnel. 5ml of dilute HCl (1ml of conc acid : 3ml of distilled water) was added, stoppered and

mixed. The content was then extracted with 50ml ether. If mixture emulsifies, 10-15ml petroleum ether was added and shaken. If this treatment fails to break emulsion centrifuge or let stand until considerable portion of aqueous layer separates. The latter was drained, shaken vigorously and again allowed to separate. The ether layer was washed with two 5ml portions of water, evaporate ether in a porcelain dish and add 1 drop of 0.5 % (v/v) neutral Ferric Chloride solution. A violet colour indicates presence of Salicylic acid[48].

48. A.O.A.C 17th, edn Official method 975. 30 Salicylic acid in Food and Beverages/ IS 1479 (Part II) 1961 (Reaffirmed 1997) Methods of test Dairy Industry – Chemical Analysis of Milk. Bureau of Indian Standards, New Delhi).

CHAPTER FOUR

4.0 RESULTS AND DISCUSSION

4.1 RESULTS

- The table 4.1 below presents the result of the analysis on the samples used.

Table 4.1: Results of detection of adulterants in powdered milk samples

Adulterants	Three crown milk	Cowbell milk	Peak milk	Hollandia
Suc	-	+	-	-
Ur	-	-	+	-
Stch	-	-	-	-
Celu	-	-	-	-
NH_4^+	++	++	++	-

Sulf	-	-	-	-
NaCl	+	+	+	-
NO_3^-	-	-	-	-
HOCl	-	-	-	-
Qtn	+	+	+	+
Amdet	-	+	++	-
For	+	+	+	+
H_2O_2	-	-	-	-
Bor/Bora	-	-	-	-
Sal	-	-	-	-

Key: Suc = sucrose; Ur = urea; Stch = starch; Celu = cellulose; NH_4^+ = Ammonium compounds; Sulf = sulphates; NaCl = sodium chloride; NO_3^- = nitrates; HOCl = hypochlorite; Qtn = quaternary ammonium

compounds; Amdet = ammonium detergents; For = formalin; H_2O_2 = peroxides; Bor/Bora = boric and borates; Sal = salicylic acid.

4.2 DISCUSSION

From the table of results above (Table 4.1), all the commercial powdered milk samples analyzed contain at least one adulterant. These adulterants may be conveniently viewed as belonging to two groups; those that may have toxic effects in the body once consumed and those that may later develop health issues. For example, sucrose, starch and sodium chloride belong to the latter group, while hypochlorite, ammonium detergent and formalin are seen as toxic to health.

From the results, *Cowbell milk* sample had the highest number (six) of adulterants, while the others showed the presence of five (5) adulterants.

This study did not intend to establish whether these adulterants were intention added to these samples. It is possible that some of these adulterants were introduced into the products in the course of production processes and operations. For instance, improper washing of the production line may introduce detergents into subsequent products.

However, one may be forced to believe that one or two of these adulterants may be added on purpose as a form of value addition to such product e.g. addition of sugar to powdered milk may increase its sweetness but introduce sucrose as an adulterant.

Of more concerns from the results of this study are the detection of quaternary ammonium compound, ammonium detergents and formalin. It is hoped that the manufacturer may have to reassess their production processes starting with the sourcing of their raw milk to be able to cub the introduction and contamination of these adulterants.

4.3 CONCLUSION

This work has brought to fore, the issue of adulteration of edible products be it of plant or animal. The study reveals the presence of one or more adulterants in all commercial evaporated milk samples analyzed. There is therefore the need for increased and more aggressive monitoring and standardization of these group of products.