Assiut University web-site: www.aun.edu.eg

EVALUATION OF MILK FRAUD FROM DIFFERENT SOURCES IN BEHEIRA GOVERNORATE

HANAA, F. SALAMA [1]; FAYZA, A. EL-TEDAWY [2] and FATMA, F. MOHAMED [3]

[1] Senior Researcher of Food Hygiene Unit, Damanhur Provisional Lab, Animal Health Research Institute (AHRI), Agriculture Research Center

[2] Chef Researcher of Biochemistry Unit, Damanhur Provisional Lab, Animal Health Research Institute (AHRI), Agriculture Research Center

[3] Senior Researcher of Chemistry, Shebin El-Kom Provincial Lab, Animal Health Research Institute (AHRI), Agriculture Research Center

Received: 25 October 2022; **Accepted:** 15 December 2022

ABSTRACT

Background and objective: Milk adulteration is an illegal treatment for milk in Egypt. Therefore, the goal of the current investigation was to identify and evaluate the potential milk adulterations that suppliers from different sources in El-Beheira governorate added to cow's and buffalo's milks. **Materials and methods:** A total of 180 samples of cow's and buffalo's milks (90 each) from different sources in El-Beheira governorate (farmer's houses, supermarkets and collecting milk centers, each 30 for each species) were collected randomly. The physicochemical analysis was performed, in addition to, detection of common commercial additives as well as antibiotics' residues. **Results**: The physicochemical parameters of specific gravity and freezing point reflected the addition of water in the examined cow's and buffalo's milk samples; in addition to, the detection of adulterants, in which, the milk samples from supermarkets had higher antibiotics' residues in a percentage of 20%, and also sodium bicarbonate, melamine and formalin in incidences higher than those from farmers' houses and collecting milk centers. **Conclusion**: The examined cow's and buffalo's milk samples from different sources were adulterated especially from the supermarkets which had higher incidences of sodium bicarbonate, melamine and formalin in addition to antibiotics' residues. Hence, these adulterants were dangerous to consumers' health, and did not satisfy recommended standards.

Keywords: Milk adulteration, physicochemical analysis, formalin, antibiotics' residues.

INTRODUCTION

Milk is known to be a nutrient dense food due to its variety of nutritional components. It contains proteins, lipids, carbs, minerals, vitamins and other nutrients necessary for supporting life and preserving excellent health (Poonia *et al.*, 2017). The safety and authenticity of milk have come under increasing scrutiny and concern as a result of rising milk production. Unfortunately, milk has been interfered by adding synthetic nutrients to maximize profits (Singh and Gandhi, 2015). To increase milk's volume and, consequently, profit

Corresponding author: Hanaa, F. Salama
E-mail address: hsalama689@gmail.com
Present address: Senior Researcher of Food Hygiene Unit, Damanhur Provisional Lab, Animal Health Research Institute (AHRI), Agriculture Research Center

margin, dealers commit the malpractice of adulterating it by either adding inexpensive ingredients or removing valuable ones (Chauhan *et al.,* 2019).

One of the keyways to adulterate milk is by adding water, skimming (either partial or complete), both and additives (like preservatives). Milk adulteration is a multi-chain process that begins with the owner of the animal, the milkman, rural collection stations, and ultimately, massive processing facilities (Swar *et al.,* 2021). Overly documented milk adulterants include diluents (water and ice), thickeners (starch, glucose, urea, flour, salt and chlorine), preservatives (sodium carbonate, sodium bicarbonate and formalin), reconstituting agents (seed oils, cane sugar, animal fats and milk powder), and cosmetics (detergent/soap and bleaching powder) (Afzal *et al*., 2011; Kandpal *et al.,* 2012; Shaikh *et al.,* 2013; Singh and Gandhi, 2015; Chauhan *et al.,* 2019; Lahankar *et al.,* 2019). In addition to antibiotic residues, that resulted from the use of antibiotics in dairy animals (Chen *et al.,* 2019).

Various techniques for detecting milk adulterants, while quantitative detections are intricate and varied, qualitative detections of adulterants in milk can be accomplished with ease using chemical reactions. Quantitative detection methods vary depending on the type of adulterants found in milk. For instance, LC (liquid chromatography) and ELISA (enzyme-linked immunosorbent assay) are the most popular methods for spotting foreign proteins; PCR (polymerase chain reaction) and PAGE (polyacrylamide gel electrophoresis) are frequently used to spot adulterants in the milk of a certain species (Azad and Ahmed, 2016; Poonia *et al.,* 2017).

Milk adulteration is an issue that not only has a negative impact on the human health that comes at a high cost to society but also prevents milk's beneficial components from being properly utilized, which is crucial for healthy body development. In Egypt, the dairy value chain is deeply ingrained in rural communities and is essential to the rural economy, as well as to the livelihoods of farmers and micro and small-scale dairy producers. Therefore, it is necessary to frequently and randomly check markets for adulteration of milk, in which, this present study's objective was to identify the adulteration of cow's and buffalo's milks in farmer's houses, supermarkets and collecting milk centers in El-Beheira governorate, as well as, to assess the effectiveness of the controls put in place to stop adulteration.

MATERIALS AND METHODS

Collection of milk samples:
A total of 180 random samples of cow's and buffalo's milks (90 each) were collected from different sources in El-Beheira governorate, Egypt, including farmer's houses (30 cow's & 30 buffalo's milk samples), supermarkets (30 cow's & 30 buffalo's milk samples) and collecting milk centers in the form of bulk milk tank (BMT) (30 cow's & 30 buffalo's milk samples). The milk samples were collected in sterile jars and then transferred to the laboratory with a minimum of delay.

Preparation of the samples:
Each milk sample (500 ml) was thoroughly mixed before being divided into 3 sub-samples; the first was used for physicochemical analysis; the second for the detection of common commercial additives, while the third for the detection of antibiotics' residues.

Physicochemical analysis:
The physical and chemical characteristics of the milk samples were determined shortly after arrival to the laboratory. Fat%, protein%, specific gravity and freezing point were determined using an automatic milk analyzer (MilkoScan^{TM} FT1, FOSS Analytical A/S, Postbox 260, DK-3400 Hillerød, Denmark) in the Animal Health Research Institute, Food Hygiene Department, Shebin El-Kom branch, Egypt. MilkoScan FT1 is in compliance with AOAC

(Association of Analytical Chemists) and IDF (International Dairy Federation).

Detection of common commercial additives:
The examined additives included palm oil, urea, sodium bicarbonate, citric acid, melamine, formalin and starch.
The detection of palm oil was performed by a simple rapid technique by Ramani *et al.* (2019), in which 2 ml of milk fat/ghee was taken in a clean dry test tube and heated to 60-70°C for 10 min; then after1 ml of ferric chloride solution (0.008 M) was added in that test tube, and there after 0.3 ml potassium fericynide solution (0.03 M) was added and mixed for 30 seconds; thereafter the color change was observed; for pure ghee the color would be light green and for adulterated ghee the color varied from lime green to blue.

Urea, sodium bicarbonate, citric acid, melamine and formalin were determined by using an automatic milk analyzer (MilkoScan^{TM} FT1) according to manufacturer instructions (AOAC, 2013). Starch was detected according to the Indian standards (IS 1479 part I, 1961 reaffirmed 2003), in which about 5 ml of milk in a test tube was subjected to boiling condition and allowed to cool to room temperature, then after 1-2 drops of iodine solution was added; the development of blue color indicated the presence of starch which disappeared when sample was boiled and reappeared on cooling; the limit of detection of method was 0.02%.

Antibiotics' residues were detected by the using of Twin sensor BT kit (Delvotest ®SP NT, DSM Food Specialties B.V., P.O. Box 1, 2600 MA Delft, the Netherlands).

Statistical analysis:
The data collected on different parameters were analyzed statistically by using frequency, percentage, mean, and standard deviation. Differences were considered significant at $p<0.05$.

RESULTS

Physicochemical analysis:

Table 1: Mean values of some physicochemical parameters of the examined milk samples

Milk source	Milk species	Fat%	Protein%	Specific gravity	Freezing point
Farmers' houses	Cow's milk	3.26±0.29	3.07±0.46	1027.6±3.94	-0.496±0.03
	Buffalo's milk	6.61±0.68	3.57±0.37	1021.5±7.3	-0.548±0.05
Supermarkets	Cow's milk	3.45±0.26	3.18±0.24	1028.2±3.29	-0.462±0.18
	Buffalo's milk	7.07±1.95	3.96±0.47	1006.8±12.9	-0.525±0.02
Collecting milk centers (BMT)	Cow's milk	3.96±0.4	3.15±0.29	1025.2±8.06	0.0471±0.07
	Buffalo's milk	6.88±1.29	3.51±0.49	1010.6±7.86	0.561±0.04

Detection of common commercial additives:

Table 2: Detection results of palm oil and urea in the examined milk samples

Milk source	Milk species	Palm oil	Urea
		+ve/30 (%)	Mean±SD
Farmers' houses	Cow's milk	2/30 (6.67%)	22.83±11.09
	Buffalo's milk	6/30 (20.00%)	36.67±12.45
Supermarkets	Cow's milk	4/30 (13.33%)	18.03±8.83
	Buffalo's milk	16/30 (53.33%)	16.57±17.35
Collecting milk centers (BMT)	Cow's milk	2/30 (6.67%)	23.87±6.79
	Buffalo's milk	4/30 (13.33%)	35.87±4.35

Table 3: Incidence of some additives in the examined milk samples

Milk source	Milk species	Sodium bicarbonate	Citric acid	Melamine	Formalin
		+ve/30 (%)	+ve/30 (%)	+ve/30 (%)	+ve/30 (%)
Farmers' houses	Cow's milk	2/30 (6.67%)	0/30 (0.00%)	0/30 (0.00%)	2/30 (6.67%)
	Buffalo's milk	4/30 (13.33%)	2/30 (6.67%)	0/30 (0.00%)	2/30 (6.67%)
Supermarkets	Cow's milk	4/30 (13.33%)	0/30 (0.00%)	4/30 (13.33%)	8/30 (26.67%)
	Buffalo's milk	10/30 (33.33%)	2/30 (6.67%)	8/30 (26.67%)	20/30 (66.67%)
Collecting milk centers (BMT)	Cow's milk	0/30 (0.00%)	6/30 (20.00%)	0/30 (0.00%)	1/30 (3.33%)
	Buffalo's milk	0/30 (0.00%)	14/30 (46.67%)	0/30 (0.00%)	1/30 (3.33%)

Detection of antibiotics' residues:

Table 4: Incidence of antibiotics' residues in the examined milk samples

Milk source	Milk species	Antibiotics' residues
		+ve/30 (%)
Farmers' houses	Cow's milk	2/30 (6.67%)
	Buffalo's milk	4/30 (13.33%)
Supermarkets	Cow's milk	6/30 (20.00%)
	Buffalo's milk	6/30 (20.00%)
Collecting milk centers (BMT)	Cow's milk	0/30 (0.00%)
	Buffalo's milk	2/30 (6.67%)

DISCUSSION

Dairy products are a group of food that plays an important role in nutrition of the population. Upon somewhat expensive raw material, milk might be a desirable product for alterations by partially substituting it with other dairy and non-dairy substances (Giglioti *et al.*, 2022). Authorities are aware of the issue of dairy adulteration; thus, it is imperative to create efficient ways for identifying goods that have been faked or otherwise contaminated (Hanganu and Chira, 2021). To ensure that consumers receive milk of high quality and safety, the Egyptian Standards for Specification have established regulations governing the manufacturing of raw milk (Egyptian Standards, 2013; Ghaffar *et al.,* 2019).

In general, cow's milk samples were more likely to be tampered with by adding water than buffalo's milk since cow's milk has low-fat content and will show any partial loss of fat. Retailers are therefore pressured to add more water to cow's milk than to buffalo's milk (Mansour *et al.,* 2012). Regarding fat content, the current findings in Table 1 revealed that the examined buffalo's milk samples had an average fat level that was much greater than those of cow milk's with averages 3.45 and 7.08%, respectively. Similar to the obtained results, Abbas *et al.* (2017) revealed that the fat contents of cow's and buffalo's milk were 4.1 and 6.30%, respectively.

According to the data recorded in Table 1, the specific gravity of cow's and buffalo's milk from different sources ranged between 1.025 - 1.028 and 1006 - 1021, respectively, which were below the Egyptian standards, that stated the specific gravity of cow's milk is 1.028 -1.034 with an average of 1.032 and for buffalo's milk is 1.033 - 1.036 with an average of 1.034 (Egyptian Standards, 2013). These findings reflected that the examined cow's and buffalo's milk samples had been diluted with by the addition of water (Table 1). Moreover, the obtained results in Table 1 showed slight variation in freezing points which indicated adulteration by addition of water, in which, the addition of water was the main cause of lowering freezing points than the Egyptian standard (- 0.53 to -0.56) (Egyptian Standard, 2013).

Hence the food items like milk or milk products are always tense to be adulterated by unscrupulous traders for earning more money. Adulteration with cheaper quality of oils (vegetable oil) and fats were getting a serious concern for food law enforcement agencies. As crooks involved in the business of ghee production are using designed oils/fats with or such oil that has almost same fatty acids profile of milk fat or ghee. Therefore, the present study examined the adulteration by addition of palm oil as a foreign fat (Table 2), in which, the obtained results found the presence of palm oil in the examined cow's and buffalo's milk samples from the supermarkets (13.33 and 53.33%, respectively), followed by bulk milk tank of the collecting milk centers (6.67 and 13.33%, respectively). According to the results reported in Table 2, Parmar (2005) reported a similar outcome.

The obtained results for the urea content in the examined cow's and buffalo's milk samples were recorded in Table 2. The obtained results were agreed with Swathi and Kauser (2015) for raw cow's milk.

In order to extend the shelf life of milk, formalin is added to raw milk. This additive has the potential to pose significant health risks (Chanda *et al.,* 2012; Salih and Yang, 2017). The data in Table 3 cleared the adulteration by addition of formalin, particularly in the examined cow's and buffalo's milk samples from the supermarkets (26.67% and 66.67%, respectively). Additionally, the supermarkets' samples had higher levels of chemical residues including sodium bicarbonate and melamine (Table 3). These results are analogous to those of Chanda *et al.* (2012) who discovered 10% sodium bicarbonate and 20% formalin as additional preservatives in raw milk samples from Bangladesh.

For starch, all the examined milk samples were starch-free; this conclusion is consistent with those made by Islam *et al.* (2013); Mohammed and Zubeir, (2021), whom discovered that no milk had been contaminated with starch. In contrast, Bari *et al.* (2015) found starch in 66.67% of the milk samples were contaminated with starch.

When focusing the light towards the detection of antibiotics' residues in the examined milk samples, Table 4 provided the results showing more prevalent (20%) in the milk samples from the supermarkets. Abo El-Makarem *et al.* (2020) analyzed the oxytetracycline residues in market raw milk samples obtained from Alexandria governorate, Egypt.

Chemical residues in raw milk can also impact upon food safety (McCarthy *et al.,* 2018). Citric acid is a useful organic compound that is commonly used in food. It is used in milk-washing, which involves mixing whole milk with a spirit, separating the milk into curds and whey, and then removing the curds using a coffee filter or a centrifuge and detected in milk due to lack of resining of system. Melamine is also added to

milk to cheaply improve the protein content (Liu *et al.*, 2012).

Conclusion and Perspective

The examined cow's and buffalo's milk from different sources were adulterated especially from the supermarkets which had higher incidences of sodium bicarbonate, melamine and formalin in addition to antibiotics' residues. Therefore, the milk monitoring for detection of any adulterants must be done regularly.

Funding: This research did not receive any specific grant from funding agencies in the public, commercial, or not-for-profit sectors.

Conflicts of Interest Statement: The authors declare that they have no conflict of interest that affects this study.

Availability of data and material: NA

REFERENCES

Abbas, Hayam; Ali, Afaf; Kassem, Jihan and Zaky, Wafaa (2017): Evaluation of Different Types of Egyptian Milk from Biochemical Aspects. International Journal of Dairy Science, 12. 130-136. 10.3923/ijds.2017.130.136.

Abo El-Makarem, H.; Leboudy, Ahlam and Mahmoud, Nesma (2020): Oxytetracycline and β-lactam residues in raw milk of different species marketed in Alexandria city, Egypt. Alex. J. Vet. Sci. 65:60-65.

Afzal, A.; Mahmood, M.; Hussain, I. and Akhtar, M. (2011): Adulteration and microbiological quality of milk (a review). Pakistan Journal of Nutrition, 10(12): 1195-1202

AOAC (2013): Official methods of analysis. https://www.aoac.org/wp-content/uploads/2019/08/Method_Format.pdf

Azad, T. and Ahmed, S. (2016): Common milk adulteration and their detection techniques. Food Contamination 3, 22. https://doi.org/10.1186/s40550-016-0045-3

Bari, L.; Hoque, M.; Reza, S.; Hossain, M. and Islam, A. (2015): Adulteration of Raw Milk in Selected Regions of Tangail District of Bangladesh. Journal of Environmental Science & Natural Resources, 8:41-44. 10.3329/jesnr.v8i1.24662.

Chanda, T.; Debnath, G.K.; Hossain, M.E.; Islam, M.A. and Begum, M.K. (2012): Adulteration of raw milk in the rural areas of Barisal district of Bangladesh. Bangladesh Journal of Animal Science, 41(2): 112-115

Chauhan, S.L.; Priyanka, K.D.M.; Paul, B.R. and Maji, C. (2019): Adulteration of milk: A Review. International Journal of Chemical Studies, 7(1): 2055-2057.

Chen, J.; Ying, G.G. and Deng, W.J. (2019): Antibiotic Residues in Food: Extraction, Analysis, and Human Health Concerns, J. Agric. Food Chem. 67: 7569–7586.

Egyptian Standards (2013): Raw Milk Egyptian for Standardization. No. 154. Egyptian standards. Milk and milk products Part1: Raw milk (1-154) Arab Republic of Egypt: Egyptian Organization for Standardization and Quality Control; 2005

Ghaffar, E.; El-Neshawy, A.; Siliha, H. and El-Shorbagy, Gehan (2019): Quality and safety assessment of Egyptian marketed flavored UHT milk: A survey study for sanitary monitoring. Zagazig Journal of Agricultural

Research, 46:455-465. 10.21608/zjar. 2019.33400.

Giglioti, R.; Polli, H.; Azevedo, B.T.; Katiki, L.M. and Filho, A.E.V. (2022): Detection and quantification of adulteration in milk and dairy products: A novel and sensitive qPCR-based method, Food Chemistry: Molecular Sciences, Volume 4,100074, https://doi.org/10.1016/j.fochms.2022.100074

Hanganu, A.M. and Chira, N.A. (2021): When detection of dairy food fraud fails: An alternate approach through proton nuclear magnetic resonance spectroscopy. Journal of Dairy Science, 104 (8): 8454-8466.

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

Islam, M.A.; Rashid, M.H.; Kajal, M.F.I. and Alam, M.S. (2013): Quality of milk available at local markets of Muktagacha upazila in Mymensingh district. J. Bangladesh Agril. Univ. 11(1):119-124.

Kandpal, S.D.; Srivastava, A.K. and Negi, K.S. (2012): Estimation of quality of raw milk (open & branded) by milk adulteration testing kit Indian Journal of Community Health, 124 (3) 188-192.

Lahankar, S.M.; Chavan, U. and Gore, N. (2019): A thorugh review on milk adulteration, its effect on health and detection tests. World Journal of Pharmaceutical Research, 8(2): 636-646.

Liu, Y.; Todd, E.D.; Zhang, Q.; Shi, J.R. and Liu, X.J. (2012): Recent developments in the detection of melamine. J. Zhejiang Univ. Sci. B Biomed. Biotechnol. 13(7): 525-532

Mansour, A.I.A.; El-Loly, M.M. and Ahmed, R.O. (2012): A preliminary detection of physical and chemical properties, inhibitory substances and preservatives in raw milk. Internet Journal of Food Safety, 14:93-103.

McCarthy, W.P.; Danaher, M.; Gleeson, D.; O'Connor, C.; Mark, A.; Fenelon, M.A. and Tobin, J.T. (2018): Chorate and other oxychlorine contaminants within the dairy supply chain. Comprehensive Reviews in Food Science and Food Safety, 17:1561-1575.

Mohammed, A.A.A. and Zubier, I.E.M.E. (2021): Occurrence of adulterants and preservatives in milk Solid in Rural areas of Omdurman, Sudan. Veterinary Medicine and Public Health Journal, 2(3):64-72, 10.31559/VMPH2021.2.3.1

Parmar, S.K. (2005): Evaluation of quality of market ghee. M.V.Sc. Thesis, NDRI, Karnal, India.

Poonia A.; Jha, A.; Sharma, R.; Singh, H.B.; Rai, A.K. and Sharma, N. (2017): Detection of adulteration in milk: a review Int. J. Dairy Technol., 70, pp. 23-42, 10.1111/1471-0307.12274

Ramani, A.; Hazra, T.; Parmar, M.P.; Sindhav, R.G. and Ramani, V.M. (2019): A simple rapid technique for detection of palm oil in ghee. Indian J. Dairy Sci. 72(4): 441-444.

Salih, M. and Yang, S.Y. (2017): Common Milk Adulteration in Developing Countries Cases Study in China and Sudan: A Review. *Advances in Dairy Research, 5*, 1-4.

Shaikh, N.; Soomro, A.; Sheikh; S.; Khaskheli, M. and Marri, A. (2013): Detection of adulterants and their effect on the quality characteristics of market milk Pakistan Journal of Agricultural Engineering Veterinary Science, 29(2): 175-183.

Singh, P. and Gandhi, N. (2015): Milk preservatives and adulterants: processing, regulatory and safety

issues. Food Reviews International, 31(3), 236 - 261. doi.org/10.1080/ 87559129.2014.994818

Swar, S.O.; Abbas, R.Z.; Asrar, R.; Yousuf, S.; Mehmood, A.; Shehzad, B.; Farhan, H.R.; Aleem, M.T.; Marcelino, L.A. and Mohsin, M. (2021): Milk adulteration and emerging health issues in humans and animals (a review). Continental Vet. J. 1(1): 1-8.

Swathi, J.K. and Kauser, N. (2015): A study on adulteration of milk and milk products from local vendors. International Journal of Biomedical and Advance Research, 6(9): 678-681.

تقييم غش اللبن من مصادر مختلفة بمحافظة البحيرة

هناء فتحي سلامة ، فايزة عبد العزيز التداوي ، فاطمة فتحي محمد

E-mail: hsalama689@gmail.com Assiut University web-site: www.aun.edu.eg

الخلفية والأهداف: غش اللبن هو معاملة غير مصرح بها في مصر. لذلك، كان الهدف من الدراسة الحالية هو تحديد وتقييم غش اللبن البقري والجاموسي والإضافات المحتملة الوارد إضافتها من الموردين من مصادر مختلفة في محافظة البحيرة.

الأدوات والمنهج: تم جمع ١٨٠ عينة من اللبن البقري والجاموسي (٩٠ لكل منهما) من مصادر مختلفة في محافظة البحيرة (بيوت الفلاحين ومحلات السوبر ماركت ومراكز تجميع الألبان ، ٣٠ عينة من كل مصدر لكل نوع لبن بقري وجاموسي) وذلك بشكل عشوائي. وقد تم إجراء الفحص الفيزيائي الكيميائي، بالإضافة إلى الكشف عن المضافات التجارية الشائعة وكذلك بقايا المضادات الحيوية.

النتائج: لقد عكست المعاملات الفيزيائية الكيميائية للكثافة النوعية ونقطة التجمد عن إضافة الماء في عينات اللبن البقري والجاموسي التي تم فحصها. وذلك بالإضافة إلى الكشف عن الغش، حيث إحتوت عينات اللبن من محلات السوبر ماركت على نسبة عالية من متبقيات المضادات الحيوية بنسبة ٢٠٪، وكذلك بيكربونات الصوديوم والميلامين والفورمالين وذلك في عينات أكثر من العينات التي تم فحصها من بيوت الفلاحين ومراكز تجميع الألبان.

الخلاصة: إستخلصت الدراسة الحالية أن عينات اللبن البقري والجاموسي التي تم فحصها من مصادر مختلفة أنها مغشوشة وخاصة التي من محلات السوبر ماركت والتي إحتوت على نسبة عالية من بيكربونات الصوديوم والميلامين والفورمالين بالإضافة إلى بقايا المضادات الحيوية. ومن ثم، فإن هذه المواد تشكل خطراً على صحة المستهلك، ولا تستوفي المعايير الموصى بها.

الكلمات المفتاحية: غش اللبن ، الفحص الفيزيائي الكيميائي، الفورمالين، بقايا المضادات الحيوية.