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A STUDY ON DETECTION OF ADULTERATION IN MILK AND SOME MILK PRODUCTS

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Received: 25 June 2024; **Accepted:** 18 August 2024

ABSTRACT

One hundred and fifty cows' milk and some milk product samples were collected at retail outlets and from street vendors in Sohag province (Egypt) on a random basis including raw milk, UHT milk, thick cream, farmers' butter, imported butter (30 samples each) for detection of various adulterants. Specific gravity added water%, fat% and S.N.F% were determined using an automatic milk analyzer to detect adulteration with the water addition and partial skimming. Additionally, samples were subjected to some chemical analyses to detect different adulterants. The obtained results revealed that the most common methods of adulteration were the addition of water at various percentages, partial skimming or both; and adding preservatives to the examined raw milk samples. Notably, UHT milk and cream were free from any kind of adulteration except the addition of gelation (thickner) which was present in 63.3% of the examined thick cream samples. Additionally, the mean values of iodine number for the farmer's butter and imported butter were 36.34 ± 6.13 and 41.10 ± 7.27, respectively. Interestingly, all the examined samples of farmers and imported butter had no evidence of starch and cotton seed oil. On the other hand, sesame oil was detected in 36.67 % of the examined samples of the imported butter. In conclusion, the qualitative analysis that was conducted demonstrated that some of the milk and milk products purchased did not meet the legal standard. Overall, the obtained findings indicated that some of the milk and milk product samples were adulterated with some adulterants that did not meet the legal standard and may decrease the nutritive value of dairy products as well as cause public health hazards to the consumers.

Keywords: Adulteration, milk, Milk products, Automatic milk analyzer, Cream, Butter.

INTRODUCTION

Milk is the best food for both infants and adults because of its high nutritional value as it is an excellent source of protein, fat, lactose, vitamins, and minerals. It is used for the processing of many types of dairy products, like cheese, butter and cream (Dennis and Robert, 2021). Cream and butter are rich in easily digested fat, as well as triglycerides, cholesterol, saturated and unsaturated fatty acids (Jensen, 2002).

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Food adulteration is a serious problem worldwide, particularly in developing nations, due to inadequate monitoring and policies in the food system. Of particular note, adulteration can be easily performed in milk and milk products. Some milk adulterants can pose health concerns and even fatal diseases (Azad and Ahmed, 2016).

Milk and its products can be adulterated in different ways to increase volume, and viscosity, enhance their physical appearance and extend the shelf life. Furthermore, adulterants mostly found in milk involve the addition of food additives like vegetable protein, cheaper fat, starch, glucose, whey, salt, and watering. These kinds of adulterants are referred to as economically motivated adulteration, and do not induce any severe health risks (Singh and Gandhi, 2015; Tomaszewska-Gras, 2016). However, milk adulterants can result from the addition of harmful chemical compounds have serious adverse effects on consumer health such as urea, formalin, detergents, boric acid, salicylic acid, hydrogen peroxide, and melamine (Salih and Yang, 2017). The ingestion of adulterated milk and milk products has several severe health impacts. For instance, detergents and peroxides in milk can lead to gastrointestinal problems such as gastritis and colitis (Singuluri and Sukumaran, 2014). While addition of excess starch to dairy products leads to severe diarrhea. However, kidney failure can be produced due to urea in milk (Kandpal *et al.*, 2012). Moreover, consuming carbonates and bicarbonates adulterated milk can interfere with development and reproduction (Bhamare *et al.,* 2016).

The detection techniques for milk adulteration should be both specific and rapid. There are two methods used to identify the adulterants in milk and its products called qualitative and quantitative methods. Qualitative detection depends on color detection through chemical reactions. Quantitative detection techniques that is more complicated include liquid chromatography, ELISA, PCR and Polyacrylamide Gel Electrophoresis (Garcia *et al.*, 2012). Due to the probable serious risks of milk adulterants on consumers' health, the present study was designed to examine milk and some milk products for detection of different adulterants.

MATERIALS AND METHODS

Samples collection and preparation:

One hundred and fifty milk and milk product samples were randomly collected from collection points, food stores and street vendors in Sohag City. Samples include raw cows' milk, UHT milk, thick cream, farmers butter and imported butter, 30 samples each. They were collected in sterile, clean, and dry containers, and then sent to the laboratory just after collection. Milk samples (500 ml) were mixed thoroughly for proper homogenization and then divided into 3 parts for physicochemical analysis, detection of heat treatment and detection of preservatives or common commercial additives. While 250 g of cream samples was warmed (30- 40 °C) in water bath and cooled to room temperature with interval shaking. For butter, 250 g was mixed thoroughly until become soft and divided into two parts. The initial one was used for iodine number measurement, whereas the latter was melted in a water bath at less than 50°C for the examination of other parameters (IS 1479 part II, 1961 reaffirmed 2003).

Physical and chemical examination of milk samples:

Determination of added water percentage, specific gravity, fat percentage, and non-fat milk solids percentage, was carried out at the Department of Dairy Science, Faculty of Agriculture, Sohag University, utilizing an automatic milk analyzer (Milk Analyzer Lactoscan MCC, Milkotronic LTD) (Draaiyer *et al.*, 2009). While, the detection of heat treatment in milk samples was done using Storch′s test (Lampert, 1975).

Detection of preservatives in milk and cream samples:

a. Formalin and Hydrogen peroxide detection (Kamthania *et al.,* 2014).

b. Salicylic acid and Boric and borax detection (Arvind Singh *et al.*, 2012).
c. Carbonate & bicarbonate detection (Parikh, 1945).

Detection of additives in milk and cream samples:

a. Detection of Starch, Ammonium Sulphate, Detergent and Urea (Reddy *et al.* , 2017).
b. Gelatin detection in cream (AOAC, 2000c).

For butter samples:

a. Iodine number determination (AOAC, 2000a).
b. Sesame oil detection (Baudouin's Test) (Recio and Olieman, 1996).
c. Cotton seed oil detection (halphen's test) (AOAC, 2000b).
d. Starch and cereal flour detection (Kumar *et al.*, 1998).

RESULTS

Table 1: Added water percentages in the examined raw and UHT milk samples.

Samples	positive samples		Added water %	
	No./30	%	Minimum	Maximum
Raw milk	20	66.7	2.1	61.2
UHT milk	17	56.7	3.5	21.0

Table 2: Specific gravity of the examined raw and UHT milk samples.

Samples	Examined samples	Specific gravity		
		Minimum	Maximum	Average
Raw milk	30	1.021	1.035	1.029
UHT milk	30	1.023	1.033	1.031

Table 3: Frequency distribution of examined raw and UHT milk samples depending on their specific gravity.

Specific gravity	Raw milk		UHT milk	
	No./30	%	No./30	%
1.020-	5	16.7	3	10
1.024-	9	30	10	33.3
1.028-	10	33.3	11	36.7
1.032-	4	13.3	6	20
1.035-	2	6.7	0	0

Table 4: Fat % of the examined raw and UHT milk samples.

Samples	Examined samples	Fat%		
		Minimum	Maximum	Average
Raw milk	30	2.0	4.7	3.2
UHT milk	30	2.7	3.5	3.4

Table 5: Frequency distribution of the examined raw and UHT milk samples depending on their fat content.

Fat %	Raw milk		UHT milk	
	No./30	%	No./30	%
2.0-	10	33.3	8	26.7
3.0-	14	46.7	22	73.3
4.0-	6	20	0	0

Table 6: Non-fat milk solids percentages of the examined raw and UHT milk samples.

Samples	Examined samples	Non-fat milk solids (S.N.F %)		
		Minimum	Maximum	Average
Raw milk	30	4.7	9.1	6.9
UHT milk	30	6.3	8.8	6.8

Table 7: Frequency distribution of the examined raw and UHT milk samples depending on their non-fat milk solids %.

Non-fat milk solids (S.N.F %)	Raw milk		UHT milk	
	No./30	%	No./30	%
4.25-	2	6.7	0	0
5.25-	3	10	0	0
6.25-	6	20	12	40
7.25-	9	30	5	16.7
8.25-	10	33.3	13	43.3

Table 8. Milk samples with legal and illegal values compared to Egyptian standards.

Source of milk	Added water%		Specific gravity		Fat%		S.N.F.%	
	L.	I.L.	L.	I.L	L.	I.L	L.	I.L
Raw milk	33.3	66.7	46.6	53.4	66.7	33.3	33.3	66.7
UHT milk	43.3	56.7	56.7	43.3	73.3	26.7	43.3	56.7

L.: Legal I.L.: Illegal

Egyptian standards values: (Specific gravity 1.028: 1.034) (Fat % 3%) (S.N.F. 8.25%)

Table 9: Preservatives and commercial additives detected in the examined samples of milk and milk products.

Items	Raw milk		UHT milk		Cream	
	+ve/30	%	+ve/30	%	+ve/30	%
a. preservatives:						
1- Formalin	9	30	0	0	0	0
2- Salicylic acid	0	0	0	0	0	0
3- Hydrogen peroxide	1	3.3	0	0	0	0
4- Boric acid & Borax	7	23.3	0	0	0	0
5- Carbonate & bicarbonate	2	6.7	0	0	0	0
b. Commercial additives:						
1- Starch	0	0	0	0	0	0
2- Urea	0	0	0	0	0	0
3- Ammonium sulfate	0	0	0	0	0	0
4- Detergent	0	0	0	0	0	0
5- Gelatin	0	0	0	0	19	63.3
c. Heat treatment (Storch's test)	10	33.3	30	100	-	-

Table 10: Statistical analysis results of the tested butter samples depending on their iodine number.

Samples	No. of the exam. samples	Min.	Max.	**Mean ± Sd**
Farmers butter	30	25.58	43.51	36.34 ± 6.13
Imported butter	30	30.48	51.88	41.10 ± 7.27

Table 11. Frequency distribution of Iodine values in the tested butter samples.

Iodine value	Farmers butter (n=30)		Imported butter (n= 30)	
	No. of samples	%	No. of samples	%
<26	2	6.67	0	0
26-43	24	80	17	56.67
>43	4	13.33	13	43.33

Iodine Value limits range 26.4-43.1 in E.O.S.Q.C. for natural butter (No. 154-5/2005).

Table 12: Statistical analysis results of the tested butter samples depending on the presence of cotton and sesame seed oils and starch.

Samples	Cotton seed oil		Sesame oil		Starch	
	+ve/30	%	+ve/30	%	+ve/30	%
Farmers butter	0	0	0	0	0	0
Imported butter	0	0	11	36.67	0	0

DISCUSSION

The current study confirmed that raw milk, UHT milk, cream and butter could be adulterated in different ways such as addition of water, partial skimming and preservatives. Herein, 10 (33.3%) raw milk samples were heat treated, whereas all UHT milk samples were positive for heat treatment using Storch's test. On other hand, EL-Bessary (2006), El- shameya (2014), Amin (2016), and Shinawy *et al.* (2018) failed to detect heat-treated milk in the examined samples of raw milk. Although heat treatment is not implicated in health hazards to the consumer, it is considered a typical type of milk adulteration as it covers the unhygienic conditions under which milk is produced.

Adulteration of milk with addition of water is more obvious in raw milk than in UHT milk, as presented in Table 1. 66.7% and 56.7% of the examined raw and UHT milk samples, respectively, were adulterated with the addition of water, these findings were in line with those stated by Chanda *et al.* (2012), Shaikh *et al.* (2013) and Swathi and Kauser (2015). On the other hand, Adam (2009) and Debnath *et al.* (2014) detected lower results in raw milk and UHT milk. It is worth noting that adulteration of milk by adding water leads to a reduction in the milk's nutritive value and is considered as a source of harmful substances such as microorganisms.

The specific gravity of the raw milk samples ranged from 1.021 to 1.035 with a mean value of 1.029, while the UHT milk samples had a specific gravity of 1.023 to 1.033 and a mean value of 1.031 (Tables 2 & 3). Moreover, the highest frequency distribution of the specific gravity was found in the range of 1.028 – 1.031 for both raw and UHT milk samples (Table 3). Additionally, the current findings were in line with those revealed by Mansour *et al.* (2012) and Amin (2016), whereas higher findings were found by Wafy (2019), but lower findings were stated by Fahmid *et al.* (2016) in raw milk. Also, our findings were in accordance with those obtained by Wafy (2019), and lower findings were found by Awan *et al.* (2014) in UHT milk.

According to the Egyptian standards (Egyptian Standards, 2010), 46.6% and 56.7% of raw and UHT milk samples were found to be within the legal limit (1.028–1.034), and the remaining samples were found lower than the reported standards, that may be as a result of the adulteration caused by adding water. In contrast, 6.7% of raw milk samples exceeded the standard values; this could be because of adulteration of milk with partial skimming.

As shown in Tables 4 and 5, the average values of fat % in raw and UHT milk were 3.2 and 3.4, respectively. The highest frequency distribution of fat% was recorded with a range of 3.0 – 3.9 in both kinds of milk. On the other hand, in comparison to the legal (Egyptian Standards, 2010) range value of fat% (not less than 3%), 66.7% and 73.3% of raw and UHT milk samples, respectively, coincided with the Egyptian standards (Table 8). While, 33.3% and 26.7% of the examined samples of raw and UHT milk, respectively were found lower than the reported standards. The lower fat % may be because of adulteration of milk by the addition of water, partial skimming, or both of them. Furthermore, failure in stripping after milking and reduced forage consumption may be another cause of low fat in milk (Nickerson, 1995). In addition, the given results in raw milk were in line with the result revealed by Mansour *et al.* (2012), Amin (2016) and Shinawy *et al.* (2018). Whereas, lower findings were found by Fahmid *et al.* (2016) and a higher fat% was determined by Debnath *et al.* (2014). On the other hand, the findings of UHT milk agreed with those recorded by Bendale *et al.* (2015) and Shinawy *et al.* (2018).

The current study showed that S.N.F. % in the examined samples of raw milk ranged from 4.7% to 9.1% with a mean value of 6.9% (Table 6). Consonant results were found by Shinawy *et al.* (2018) and Wafy (2019), whereas lower findings were determined by Faraz *et al.* (2013). Moreover, for UHT milk, it had an average of 6.8% and a high-frequency distribution (43.3%) was recorded with the range of 8.25 to 9.24% (Tables 6 &7). Also, higher findings were obtained by Bendale *et al.* (2015) and Wafy (2019). Notably, 33.3% and 43.3% of raw and UHT milk samples, respectively were within the normal range value of S.N.F. % (not less than 8.25%) according to the Egyptian Standards (2010). S.N.F. % decreases mainly as a result of the addition of water; consequently, the decrease in such parameters is an indicator of adulteration by adding water (Harding, 1995).

Regarding preservatives, it is obvious from Table 9 that all the examined samples of UHT milk and cream had no evidence of all the examined types of preservatives. While raw milk samples were adulterated with formalin, hydrogen peroxide, boric acid, borax, and carbonate & bicarbonate at various percentages, however, salicylic acid was not detected. Past studies performed in Sohag and Assiut cities detected preservatives in raw milk, but in different percentages (Shaker *et al.,* 2015, Amin, 2016 and Wafy 2019). No doubt, some preservatives have severe health impacts on our health in the long run. For instance, the ingestion of hydrogen peroxides and carbonates in milk will lead to gastrointestinal complications involving diarrhea, gastric ulcers, and colon ulcers (Ayub *et al.,* 2007), and could also disturb the antioxidants in the body so cause impairment of the natural immunity, therefore, increasing aging. In addition, formalin is implicated in liver damage as well as increasing the total oxidant capacity and destroying the lung tissues (Aydin *et al.,* 2015). The obtained result in the current study was in line with those reported by Barham *et al.* (2014). In contrast, Wahba and Korashy (2006), Amin (2016) and Shinawy *et al.* (2018) could not detect salicylic acid in raw milk samples.

Results shown in Table 9 demonstrated that all of the examined milk samples were found free from commercial additives, including starch, urea, ammonium sulfate, and detergents, except gelatin, which was detected in 19 (63.33%) of the tested cream samples. Gelatin is a type of thickners that increases the firmness of cream and reflects its richness with fat (Stokes, 1897). The gained findings are in consistent with those previously reported by Debnath *et al.* (2014), Uddin *et al.* (2016) and Shinawy *et al.* (2018).

Regarding butter samples, the mean values of iodine number in the examined samples of farmers and imported butter were 36.34 ± 6.13 and 41.10 ± 7.27, respectively (Table 10). As a rule, the iodine value measures the level of unsaturation of fat (Knothe, 2002). Ibrahim (2006) and; EL-Mossalami and Abdel-Hakem (2014) determined higher iodine values in farmers butter and imported butter, respectively. While lower findings were recorded by Sagdic *et al.* (2004) and Park *et al.* (2007). The IV was within the normal range in 24 (80%) and 17 (56.67%) of the farmers butter and imported butter samples, respectively, however 4 (13.33%) and 13 (43.33%) of the same samples were found above the normal limit (26-43) (Table 11). El-mossalami and Abdel-Hakem (2014) noticed that 12% and 16% of farmers and imported butter samples, respectively, showed higher iodine values than the maximum value, and they concluded that these can be adulterated by vegetable oils. Strikingly, cotton seed oil and starch were absent from all of the examined samples of butter. In contrast, 11 (36.67%) samples of the examined imported butter were tested positive for sesame oil (Table 12). A similar result was recorded by Shinawy *et al.* (2018), who found sesame oil in 56.67% and 36.67% of examined samples of imported and farmers butter, respectively. While Rao *et al.* (2004) could not find sesame oil in the tested samples; but starch was determined by Swathi and Kauser (2015) in the examined butter samples.

CONCLUSION

Adulteration of milk and milk products is a worldwide concern, particularly in developing countries. The current study could detect different kinds of adulterants in milk and some milk products. Consequently, adulterated dairy products could be implicated in various health hazards for consumers. Hence, more efforts are required from the authorities through putting strict restrictions to control and reduce the risks of milk and milk products adulteration. Besides, better and more sensitive techniques for the detection of adulteration in milk should be applied.

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دراسة حول اكتشاف الغش في الالبان وبعض منتجات الالبان

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تم تجميع ١٥٠ عينة من اللبن وبعض منتجات اللبن المباعة في مدينة سوهاج بمصرعشوائيًا من محلات الألبان وباعة الشوارع (٣٠ عينة من كل من اللبن الخام، واللبن المعقم، والقشدة السميكة، والزبد الفلاحي، والزبد المستورد) للكشف عن مختلف المواد المختلطة. تم استخدام محلل الحليب التلقائي لتحديد الكثافة النوعية، ونسبة الماء المضاف، ونسبة الدهون، ونسبة المواد الصلبة غير الدهنية للكشف عن الغش عن طريق إضافة الماء ونزع الدهن. تظهر النتائج أن أكثر أنواع الغش البارزة هي إضافة الماء بنسب مختلفة، ونزع الدهن أو كلاهما، بالإضافة إلى إضافة مواد حافظة إلى العينات المفحوصة من اللبن الخام. اللبن المعقم والقشدة يبدوان خاليين من المواد الحافظة والاضافات التجارية باستثناء الجيلاتين الذي وجد بنسبة ٦٣,٣٪ في عينات القشدة . كانت قيمة الرقم اليودي المتوسط لعينات زبد الفلاحين والزبد المستورد هي ٣٦,٣٤ ± ٦,١٣ و ٤١,١٠ ± ٧,٢٧ على التوالي. جميع عينات زبد الفلاحين والزبد المستورد المفحوصة كانت خالية من زيت بذور القطن والنشا، بينما كان زيت السمسم موجودًا في ٣٦,٦٧٪ من عينات الزبد المستورد المفحوصة. في الختام، أثبتت هذه الدراسة النوعية التي تم إجراؤها أن بعض عينات اللبن ومنتجاته المجمعة من السوق لا تتوافق مع المعايير القانونية وتم غشها مما يقلل قيمتها الغذائية وقد تسبب خطرًا على الصحة العامة.