



## DETECTION OF UREA AND MELAMINE AS ILLEGALLY ADDED SUBSTANCES IN RAW MILK MARKETED IN CAIRO GOVERNORATE.

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### ABSTRACT

125 raw milk samples randomly collected from urban and slums areas (63 and 62 of each, respectively). These samples collected from dairy shops, markets and supermarkets in Cairo Governorate. These samples were examined for the presence of urea by phenol red method and for presence of melamine by ELISA technique. The results indicated that all samples collected were free from adulteration by urea and melamine.

KEY WORDS: Urea; Melamine; ELISA; Adulteration; *Raw milk*.

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### 1. INTRODUCTION

**M**ilk is the normal mammary secretion of obtained by milking of dairy animals intended for consumption as liquid milk or for further processing [1]. Milk is one of the most precious natural food and has been a basic component of human diet [2]. Due to increasing demand of milk, milk adulteration is a common phenomenon especially in certain areas of the world. The adulteration is an act internationally debasing the quality of food offered for sale either by admixture or substitution of inferior substances or by the removal of some valuable ingredients [3]. Milk adulteration leads to economic losses, deterioration of the quality of end products and a risk to consumers safety [4]. Therefore, it is important task for the milk authorities to confirm the quality of raw milk supplied in dairy shops and markets. Urea and melamine are being examples of illegally added substances to the milk for contribute the nitrogen and thus increase milk SNF.

Standard tests such as the Kjeldhal methods and Dumas tests estimate the

protein levels by measuring the nitrogen content, so values obtained can be increased by adding nitrogen rich compounds such as urea and melamine [5]. Urea is a normal constituent of milk and amounts to about 55% of the total non-protein nitrogen in milk [6]. The average values of urea in cow and buffalo milk have been estimated to be 40 and 17.5 mg/ 100ml, respectively [7]. When water was added in milk its foamy appearance diminishes, artificially detergents and urea are added for whitening milk and giving it a genuine look. Only few grams of urea enough to bring milk its original state [8]. In some countries Urea and sodium bicarbonate, sucrose, vegetable oils, detergent and water are used for making the synthetic milk. Adulteration of milk with synthetic milk up to 5% goes undetected, since there are no perceptible changes in taste, color and consistency of milk [9].

Melamine is a white powder with a high melting of 354 °C, it is slightly soluble in cold water at 20 °C and it is sparingly soluble on polar organic solvents (e.g. 95% ethanol) and essentially insoluble in non

polar solvents such as benzene and hexane. Melamine produced in high volumes mainly for the synthesis of melamine formaldehyde resins used in manufacture of laminates and plastic [14]. Melamine has no nutritional value but because it is high nitrogen content (66%) its addition to food makes it appear to have more protein than it actually does [5]. The incidence of melamine contamination was appearing from 2007 to 2009 especially in china, which led to human and animal illness and fatality [15].

The present study was performed to throw out a light on the incidence of urea and melamine as illegally added substances in raw milk marketed in dairy shops, markets and supermarkets in Cairo Governorate and mention the control measures adapted to prevent this.

## 2. MATERIAL AND METHODS

### 2.1. Collection of samples

125 random samples of raw milk were collected from different places in Cairo Governorate; these places were divided into two parts slums areas and urban areas (from dairy shops and supermarkets). Each sample was kept in ice tank with thermometer to maintain at 4 °C until reach to the lab to be analysed.

### 2.2. Preparation of sample

Each sample was wormed in a water bath at 38°C, thoroughly mixed, homogenized, and then cooled to 20°C. [16]

### 2.3. Detection of heat treated milk

Each sample of raw milk was subjected to Storch test to prove that the milk samples were raw (Not heat treat) [17].

### 2.4. Detection of externally added urea

Five ml of milk sample were placed in test tubes, and then the pH was adjusted to 7.0 in each tube, then 2 drop of phenol red solution in water (0.01g/100ml) and 1.0 ml of crude urease enzyme extract were added. The color change from orange to pink

within 2 minutes was noted in comparison with a blank tube without enzyme. A pink color compared with the yellowish –orange in the blank tube indicated the presence of added urea in the milk [9].

### 2.5. Detection of melamine

The AgraQuant Melamine Sensitive Assay Kit was used according to the manufactures instructions, Romers Lab, Singapore: The raw milk sample was prepared by centrifugation at speed 1500 rpm at 10 °C for 10 min, then 2 ml of milk serum was mixed with 8 ml of assay diluents. 150 µl of melamine standard or milk sample was added to the antibody- coated well. 50 µl of horseradish peroxidase conjugated melamine was added to each well and mixed by carefully pipetting it up and down three times. The plate was incubated for 30 min. at room temperature. After incubation, the liquid contents of microwell strips were discarded. The plate was washed with distilled water to remove unbound reagents. The washing step was repeated 4 times with 300 µl of distilled water. 100 µl horseradish peroxide substrate was added to each well, subsequently, the plates were incubated at room temperature for 20 min. the enzyme reaction was stopped by adding 100 µl of stop solution into each well. The optical density was read at 450 nm with ELISA reader. Final concentration of melamine were calculated based on the OD<sub>450</sub> values using the Romer log- logit spread sheet and considering the dilution factor of 5 [18].

## 3. RESULTS

125 raw milk samples from the urban and slums areas were collected from dairy shops and supermarkets in Cairo Governorate to detect the incidence of urea and melamine as illegally added substances.

The results showed that all raw milk samples were free from urea adulteration. For detection of melamine, the results showed that all milk samples were free from melamine adulteration

## 4. DISCUSSION

For the detection of urea, the procedure used was recommended to detect added urea that is higher than the normal level of the endogenously present urea (approximately 40mg/100ml). Therefore the test can detect only the urea added externally. These results were similar to the results demonstrated by Abdallah *et al.*, [10] who analyzed 30 raw milk samples collected from different localities in Damietta Governorate, they found that all samples were free from added urea. Also Wadekar *et al.*, [11] who examined 120 milk samples and found that none of the milk samples were found to be adulterated with urea; El-Mossalami *et al.*, [12] who examined 30 raw milk samples sold in Giza Governorate and the authors found that all samples were free from externally added urea. The results were lower than the results demonstrated by Farez *et al.*, [13] who examined 60 samples collected from different localities in Faisalabad city in Pakistan and they found that 63.87% of samples contain externally added urea. All samples were compatible with the Egyptian Organization for Standardization and Quality, EOS [19] that stated raw milk sample must be free from any added substances.

From the public health point of view, urea, added as adulterated item has a carcinogenic effect as suggested by a recent report of Indian Council of Medical Research (ICMR) [21]. Also Urea adulterated milk is very harmful to the girls as it hasten up the process of puberty [22]. Moreover, it is reported that excess of urea in milk on boiling decomposes to carbonic acid, acetic acid and ammonia. The ammonia containing calculi might be formed by the partial fermentation of urea in the bladder. In addition, it may convert into biuret which cause fall in blood pressure and produces strong irritation in the urinary tract [23]. These results were similar to the results of Suhiami *et al.*, [20] who examined 320 raw milk samples collected from different localities in Malaysia and they found that all samples

free from melamine addition. All raw milk samples were compatible with the Egyptian Organization for Standardization and Quality, EOS [19]. A safety limit of melamine ingestion has been officially set at 2.5 ppm for adult food and at 1 ppm for infant formula [24]. Illegal adulteration of food with melamine has resulted in illness and deaths of human infants, primarily as a result of kidney damage caused by crystals and stones in urinary tract [14]. Melamine is rapidly adsorbed from the gastrointestinal tract and rapidly extracted from the body [14]. Melamine toxicity is related to the urinary system in humans and, a constituent effect observed with melamine in experimental animals is bladder stones, carcinogenic effect observed with melamine are considered secondary to irritation caused by stones [15]. If melamine concentration in urine is sufficient to form crystals, it causes proximal tubular damage in the kidney. [14]. Humans may be more susceptible to corecipation of melamine with uric acid in the urine than most mammals [14].

### Conclusion and recommendation

All examined samples were free from addition of urea and melamine. In order to protect Egyptian consumer from the hazard effects of such chemicals the following recommendation should be applied; Periodical inspection of the substances and dairy products imported from outsides by inspectors of ministry of agriculture. Periodical inspection of markets by inspectors of ministry of health should be in forced control and to minimize risks of adulteration. Education programs should be imposed for producers and handlers to avoid addition of chemicals to raw milk and to improve the quality of raw milk and its product to ensure maximum safety to the consumers. Consumers are advice to avoid consuming raw milk. Intersectoral cooperation with different ministries and nongovernmental organization should be increased. Hazard analysis and critical

control point ( HACCP) system must be applied in dairy field.

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## الكشف عن وجود اليوريا والميلامين كمواد مضافة غير مسموح بها في اللبن الخام المتداول في محافظة القاهرة

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ب قسم الرقابة الصحية على الاغذية -كلية الطب البيطري - جامعة بنها.

### الملخص العربي

يعتبر اللبن المادة الوحيدة التي تستخدم كمصدر رئيسي للغذاء بحالتها الطبيعية. ولا يمكن استبدالها بماده اخري واللبن سائل حيوي معقد متكامل يمد الانسان بمعظم احتياجاته من الطاقة والبروتين والكربوهيدرات والفيتامينات والاملاح المعدنية ولا يمكن تخليقه. كما يعد اللبن من اهم الاغذية التي يحتاج اليها الانسان في جميع اطوار حياته. ونظرا لغياب الرقابة الصحية السليمة على مصادر تداول الالبان قد يتعرض للعش عن طريق اضافة بعض المواد التي قد تؤثر سلبا على الصحة العامة للإنسان. لذلك اجريت هذه الدراسة للكشف عن وجود الميلامين واليوريا كمواد مضافة غير مسموحة في اللبن الخام المتداول في محافظة القاهرة. وقد اشتملت الدراسة على فحص 125 عينة تم تجميعها من المناطق الحضرية (63 عينة) والمناطق العشوائية (62 عينة) وتم تجميع العينات من السوبر ماركت. وقد اظهرت النتائج على خلو العينات من وجود اليوريا والميلامين كمواد مضافة للبن.

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