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Detection of some chemical hazards in milk and some dairy products

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Chemical contaminants in milk comprise of hazardous chemicals that may be introduced during milking, dairy processing or packaging. They possess some hazards to humans who consume milk and other dairy products. Total of one hundred and fifty (150) samples were collected; thirty each of UHT milk, yogurt, soft, hard and processed cheese. The samples were analyzed to investigate the presence of some chemical hazards. Chemical analysis indicated that tetracyclines were present in samples of UHT milk with variable percentages, while aflatoxin M1 was detected in all of the examined UHT milk samples and cheese. Sorbic acid, benzoic acid, sulphur dioxide and nitrite were detected in some samples of cheese. The potential to cause toxicological harm to the consumers is common for all of the detected chemical contaminants.

Key words: Chemical hazards milk, dairy products, antibiotic, mycotoxin, preservatives.

INTRODUCTION

The presence of chemical contaminants in milk are very harmful for the consumers and it can be a matter of public health concern because milk and dairy products are widely consumed by humans throughout the world (Jahed, 2007). They are usually classified as naturally occurring chemicals and added chemicals.

Naturally occurring chemicals include toxins that are produced by some microorganisms (e.g. mycotoxins) which can enter through animal feed and deposited some residues in milk and dairy products.Aflatoxins are groups of toxic fungal metabolites produced by certain molds of the genus *Aspergillus* growing in number of raw-food commodities. Aflatoxin M1 (AFM1) may be found in the milk of animals that are fed with aflatoxin B1 (AFB1) containing feed (Wood, 1991; Cathey et al., 1994). These toxins are very stable and may pass through quite severe processes. For this reason they may constitute a problem in processed foods (Lawley et al., 2008).

International Agency for Research on Cancer (IARC) of WHO classified AFB1 as primary and AFM1 as secondary groups of carcinogenic compounds (Cathey et al., 1994; Dragacci et al., 1995). Chronic toxicity is probably

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more important from a food safety point of view; ingestion of low levels over a long period has been implicated in primary liver cancer, chronic hepatitis, jaundice, cirrhosis and impaired nutrient conversion (Lawley et al., 2008).

The added chemicals include chemicals that are intentionally added such as permitted food additives and non-intentionally added chemicals to a food such as cleaning and sanitizing chemicals. Food and Agricultural Organization (FAO, 1995) describes preservative as any substance which is added to food and enables the physical properties and chemical composition of food to remain unaffected by microbial or other spoilage, so that the milk retains its original wholesomeness and nutritional value. The most likely preservatives to be found in milk are formaldehyde, hydrogen peroxide and neutralizers such as sodium bicarbonate.

Chemical additives such as sodium carbonate and sodium bicarbonate added to milk as neutralizers to preserve it for longer time and to prevent curdling. The continuous use of such milk may cause health hazards to the society (Iswariah and Guruswami, 1973; Satoskar et al., 1999).

Benzoic acid, sorbic acid and their salts are commonly used as chemical preservatives in food products including cheese to prevent alteration and degradation by microorganisms during storage. However, excessive addition of these preservatives may be harmful to consumers, because of the tendency to induce allergic contact dermatitis and convulsion. Therefore, the development of convenient and inexpensive analysis methods of these preservatives is of great importance for food safety (Fang et al., 2008).

The widespread use of antimicrobials in dairy cattle management may result in the presence of their residues; such residues in animal products may be toxic to humans, or may cause serious reactions in sensitive individuals (Lawley et al., 2008) and presents technological difficulties in the milk processing industry (Heeschen and Blüthgen 1991), so many countries have enacted regulations that limit the level of chemical residues in milk and dairy products; milk chemical safety is important for public health (Tenant, 1997). Tetracyclines display a wide spectrum of antimicrobial action: it has a stronger action against gram-positive bacteria and weak action against gram-negative ones. They possess exercise against mycoplasmas, chlamydiae, rickettsias, spirochetes, actinomyces, and some protozoa (Sundin, 2003).

MATERIALS AND METHODS

Collection of samples

UHT milk, yogurt, hard, soft and processed cheese samples were randomly collected from dairy shops and supermarkets in Giza governorate. Collected samples were transferred to the laboratory in an insulated ice box with a minimum of delay to be immediately examined.

Chemical examination

Detection of Inhibitory substances in milk

Using general test (Wynther, 1927) 10 ml of milk sample were transferred aseptically into sterilized cotton pluged test tube. Ten ml of milk free from preservatives “control” were transferred to another test tube under the same condition. Two milliliters of litmus solution (10%) “as indicator” were added to both test tubes. Two test tubes were boiled in a water bath for 20 min to kill all lactic acid producing microorganisms then cooled at room temperature. 1 ml of diluted broth culture of lactic acid producing microorganisms (diluted sour milk) was added to both test tubes, then incubated at suitable temperature and kept under observation till coagulation of milk and color reduction of the litmus solution occurs. Result was recorded as positive if the difference in time was 2 h or more between the control and tested sample.

The positive samples were subjected to the following tests:

Detection of chemical preservatives

Detection of formalin: Hehner’s test (Ling, 1963)

Two milliliters of milk sample were mixed with 2 ml of distilled water in a test tube, and then sulphuric acid (90 % containing a trace of ferric chloride) was poured down the side of the tube. Development of violet ring at the junction between the two layers indicates the presence of formalin.

Detection of Hydrogen peroxide (Pien, 1953)

In a clean test tube, 2 ml of milk sample were added and 2 ml of HCL (1%) were added, thoroughly mixed, then 2 ml of potassium iodide (10%) were added. The tube was immersed in hot water (80-90°C) for 1 min after which the tube was quickly cooled in running water. 2 ml of starch solution (1%) were added as an indicator which develops blue color in the presence of hydrogen peroxide in the milk sample.

Detection of Alkalinizer (sodium bicarbonate): (Foley et al., 1974)

One milliliter of milk sample was pipetted into a test tube; the concentrated hydrochloric acid (0.5 ml) was added into the sample, the positive samples showed effervescence due to formation of carbon dioxide gas, when this gas was directed into lime water, it makes it turbid.

Quantitative analysis of tetracycline residues in milk

Analysis of tetracycline residues in milk was carried out using ELISA test kit (competitive enzyme immunoassay for the quantitative analysis of tetracycline in milk) (MaxSignal).

Preparation of samples

One and half milliliter of the cold milk samples (100C) were

centrifuged at 10000 rpm for 10 min and the upper fat layer was discarded. 50 µl of milk sample was taken then 9.95 ml of 10 mM PBS buffer (pH 7.4) was added. The tubes were vortexed for 30 s, and then 75 µl of the sample were used for the assay.

Result measurement by photometer was at wavelength of 450 nm. Standard curve was constructed by plotting the mean relative absorbance (%) obtained from each reference standard against its concentration in ng./ml.

$$\text{Relative absorbance (\%)} = \frac{\text{Absorbance standard (or sample)}}{\text{Absorbance zero standard}} \times 100$$

The mean relative absorbance values were used for each sample to determine the corresponding concentration of the tested drug in ng/ml from the standard curve.

Detection and determination of preservatives in cheese

Detection of benzoic acid

The test was done using a qualitative test (ferric chloride test) as described by AOAC (2000).

Determination of sorbic acid

Determination of sorbic acid was done using oxidation method as described by AOAC (2000).

Preparation of standard curve

Sorbic acid standard solution was pipetted (5, 10 and 15 ml) into separate 500 ml volumetric flask. Then each was diluted to 500 ml and mixed. Two millimeters of each solution (2 ml of H_2O for blank) were pipetted into separate 15 ml test tubes, then 1.0 ml of 0.15 H_2SO_4 and 1.0 ml of $K_2Cr_2O_7$ solution was added to each tube and heated in boiling water bath exactly for 5 min after that the tubes were immersed in ice bath and 2 ml of thiobarbituric acid solution was added. Then the tubes were replaced in boiling water bath and heated for additional 10 min, cooled, then the absorbance (A) of each solution was determined at 532 nm against blank, using matched 1 cm cells. The absorbance was plotted against µg sorbic acid.

Preparation of test portion

Test sample was cut into strips and passed through food chopper for grinding. Then 300-600 test sample were blended (< 150°C) in high speed blender till homogenous mixture was obtained. Then 1.5 - 2 g of prepared test portion were weighed into distill tube containing SiC chips then 10 ml of (1 M) H_2SO_4 and 10 g. $MgSO_4.7H_2O$ were added. Steam distillation was done. 100-125 ml. were collected in 250 ml volumetric flask within 45 min. The prepared sample was treated as in the case of standard curve preparation and then the absorbance was plotted against µg sorbic acid/ml.

Determination of nitrite using sulfanilic acid method (Edward, 1981)

Preparation of standard curve

Into 50 ml volumetric flasks, 0 (blank), 1.0 ml, 2.0 ml, and so on up to 10 ml. of the dilute standard nitrite solution was transferred then water was added to each flask up to 40 ml and mixed. Two milliliters of the solution (3) of Griess reagent were added to each flask and made to volume (50 ml) with distilled water hich was mixed thoroughly and allowed to stand at room temperature for 1 h.

Suitable portion of each solution was transferred to separate cuvet (Blank + 10 samples); the transmittance of the blank (without nitrite) was adjusted to 100% transmittance (zero optical density) at 520 nm, then the optical density of each standard solution was measured. The optical density readings were plotted on coordinate paper against the nitrite concentration.

Procedure

Five grams of prepared sample were weighed in volumetric flask (225 – 250 ml) and mixed with 100 ml of water, and then the flask was heated in water bath (80°C). At the end of heating, 5 ml of saturated mercuric chloride solution were pipetted into each sample and cooled, then made to volume of 225 or 250 ml with distilled water. The solution was filtered through 12.5 cm filter paper. Ten milliliters of filtered solution were transferred to 50 ml volumetric flask. Water was added up to 40 ml and mixed with 2 ml of Griess reagent and made to volume (225 or 250 ml) with water.

The color was allowed to be developed within 40 min to 1 h at room temperature. A suitable portion of the solution was transferred to the cuvet, and the optical density was determined at wave length of 520 nm and adjusted to zero optical density with blank which contained 2 ml of reagent in 50 ml water. The sodium nitrite content was obtained in ppm through the prepared plot.

Detection of Sulphur Dioxide in different types of cheese (AOAC, 2000)

The test portion was placed in distilling flask, and then diluted to 400 ml with H_2O, and then 90 ml of HCl was added to separator which was forced into flask with gentle pressure. The flask was heated until condensation was showed in the first U-tube then the separator was removed and the heat was turned off.

One drop of methyl red was added, and titrated with NaOH (0.1 M) till clear yellow color was obtained (0.1 M NaOH =3.203 mg SO_2.). Similarly, the second U-tube was titrated. After titration, gravimetric determination was made by adding four drops of 1 M HCl and excess of filtered 10% $BaCl_2$ solution, then stood overnight. The precipitate was washed by decantation 3 times with hot H_2O through weighed gooch, then washed with 20 ml alcohol and 20 ml ether, and then dried at 105 - 110°C (mg. $BaSO_4$ × 274.46/g. test portion = SO_2 µg. /g).

The blank was determined, both by titration and gravimetrically, and the results were corrected accordingly.

Quantitative analysis of aflatoxin M1 in milk and cheese

Using ELIZA Kit (RIDASCREEN® Aflatoxin M1 30/15) which is a competitive enzyme immunoassay for the quantitative analysis of aflatoxin M1 in milk and cheese (Riedel de-Haen, 1997).

RESULTS AND DISCUSSION

It is evident from the obtained results that the chemical preservatives are the most prevalent type of inhibitory substances. Some unscrupulous producers tend to add

Table 1. Incidence of inhibitory substances in examined samples of UHT milk

Test	No. of examined samples	Positive samples		Negative samples	
		No.	%	No.	%
Wynter blyth test	30	11	36.67	19	63.33
Hydrogen Peroxide residues test	30	6	20.00	24	80.00
Formalin residues test	30	0	0.00	30	100
Alkalinizer (Na_2CO_3)	30	2	6.67	28	93.33
Tetracycline residues	30	9	30.00	21	70.00

Table 2. Statistical analytical results of tetracycline residues in the examined samples of UHT milk.

Tetracycline residues concentration (ppb.)	No. of examined samples	Positive samples		Tetracycline residues		
		No.	%	Min.	Max.	Mean ± S.E.M.
	30	9	30	10.00	78.71	23.62 ±7.01

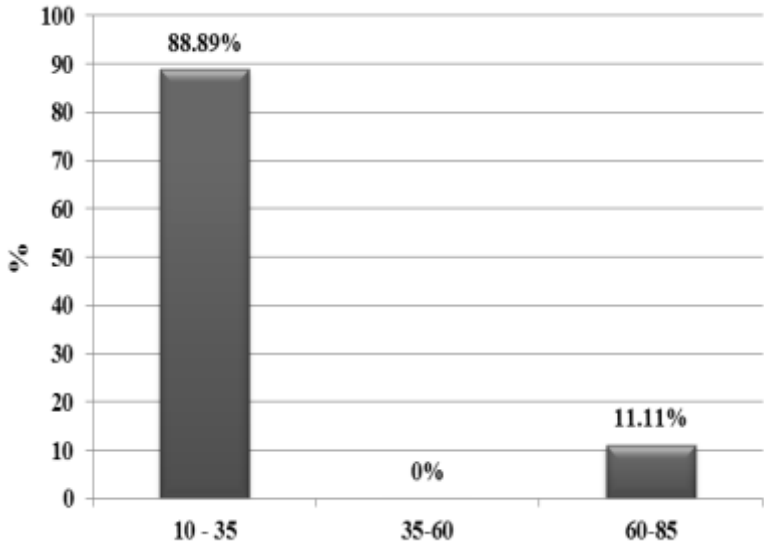

Figure 1. Frequency distribution of the examined samples of UHT milk based on their tetracycline residues concentration (ppb).

preservatives to milk in order to mask the neglected sanitary measures and to improve its keeping quality. Chemical preservation works either as direct microbial poisons or as acid neutralizer to prevent the microbial growth (Australian Academy of Science, 2004).

The results recorded in Table 1 show that 11 (36.67%) out of 30 examined UHT milk samples contained inhibitory substances; 6 samples (20%) contained hydrogen peroxide, 2 samples (6.67%) contained sodium bicarbonate (alkalinizer), while formalin was not detected in any of the examined samples. Tetracycline residues were detected in 9 samples (30%).

It is known that long continuous intake of sodium carbonate or bicarbonate and formalin might be harmful, as it causes damage to the gastrointestinal tract, primarily the stomach and lower oesophagus (Resmini et al., 1988). In addition, it may have a carcinogenic effect on human (Haddad and Winchester, 1990) and severe acidosis which result from rapid conversion of formaldehyde to formic acid (Mohanan et al., 2002).

Table 2 reveals that the mean value of tetracycline residues in the examined samples of UHT milk was 23.62 ± 7.01 with a minimum value of 10.00 and a maximum value of 78.71 ppb. The highest frequency distribution of tetracycline residues in the examined samples of UHT milk 8 (88.89 %) lies in the range of 10 to 35 ppb (Figure 1).

Table 3 represents the incidence of inhibitory

Table 3. Incidence of inhibitory substances in the examined samples of cheese.

Type of samples	No. of samples	Benzoic acid		Sorbic Acid		Nitrite		Sulphur Dioxide	
		No. of positive samples	%	No. of positive samples	%	No. of positive samples	%	No. of positive samples	%
Hard cheese	30	15	50.00	6	20.00	20	66.67	0	0.00
Soft cheese	30	5	16.67	13	43.33	17	56.67	3	10.00
Processed cheese	30	0	0.00	16	53.33	29	96.67	0	0.00

Table 4. Statistical analytical results of sorbic acid and nitrite in examined samples of cheese.

Types of samples	Sorbic acid (ppm.)			Nitrite (ppm.)		
	Min.	Max.	Mean ± S.E.M.	Min.	Max.	Mean ± S.E.M.
Hard cheese	9.00	13.30	2.12± 0.8	1.40	8.00	1.59 ± 0.31
Soft cheese	1.00	10.3	1.64 ± 0.5	0.40	3.20	0.67± 0.14
Processed cheese	2.00	14.00	1.71 ± 0.5	0.60	9.00	1.33 ± 0.027

Table 5. Statistical analytical results of aflatoxin M1 concentration (ppt.) in the examined samples.

Types of samples	No. of examined samples	Positive samples		Aflatoxin M1 conc. (ppt.)		
		No.	%	Min.	Max.	Mean±S.E.M.
Hard cheese	15	14	93.33	3.33	80.00	22.93 ± 6.31
Soft cheese	15	14	93.33	14.00	80.00	62.26 ± 7.03
UHT milk	15	15	100	7.5	80.00	53.30 ± 7.12

substances in cheese samples as benzoic acid was detected in 50% of hard cheese and 16.67% of soft cheese samples but it was not detected in the examined processed cheese samples. Sorbic acid was detected in 53.33% of processed, 43.33% of soft and 20% of hard cheese samples. The nitrite was detected in all examined cheese samples (100%) with highest incidence (96.67%) in the processed cheese samples, while sulphur dioxide was detected in examined soft cheese samples with incidence of 10%, it was not detected in the rest of the examined cheese samples.

Sorbic acid was detected in the examined hard, soft and processed cheese samples with mean values of 2.12± 0.8, 1.64 ± 0.5 and 1.71 ± 0.5, respectively; while nitrite content was detected with mean values of 1.59 ± 0.31, 0.67 ± 0.14 and 1.33 ± 0.027 ppm., respectively (Table 4).

Excessive addition of these preservatives may be harmful to consumers, because of the tendency to induce allergic contact dermatitis and convulsion (Fang et al., 2008). Sorbic acid is permitted in cheese, at levels below 3000 mg/kg. At these concentrations, sorbic acid acts as an efficient fungicide (Skovgaard, 1992) while Glass et al. (1998) mentioned that addition of sorbate with concentration of 0.1% can inhibit the growth of *Staphylococcus aureus* in processed cheese slices**.**

Nitrate is used in Europe to prevent late blowing of certain cheeses due to *Clostridium butyricum* and *C. tyrobutyricum* (Skovgaard, 1992; Glaesser, 1989).

The toxic effect of SO_2 in human is variable; some persons may tolerate up to 50 mg/kg body weight, while others feel headache, nausea and diarrhea at this concentration (Schroeter, 1966).

According to the obtained results in Table 5, it was cleared that aflatoxin M1was detected in all examined samples (100%) of UHT milk, (93.33%) of hard and soft cheese samples, with mean values of 53.30 ± 7.12, 22.93 ± 6.31and 62.26 ± 7.03, respectively.

Results represented in Figure 2 show the highest frequency distribution of AFM1concentration in the examined soft cheese and UHT milk samples (60 and 33.33%, respectively) lies in the range 40-80 while it lies in the range 0-10 in 40 % of examined hard cheese samples.

The higher concentration of aflatoxin M1 in cheese may be attributed to the association of aflatoxin M1 with casein fractions during manufacture (Egmond, 1989) while lower levels of aflatoxin M1 in some milk and milk

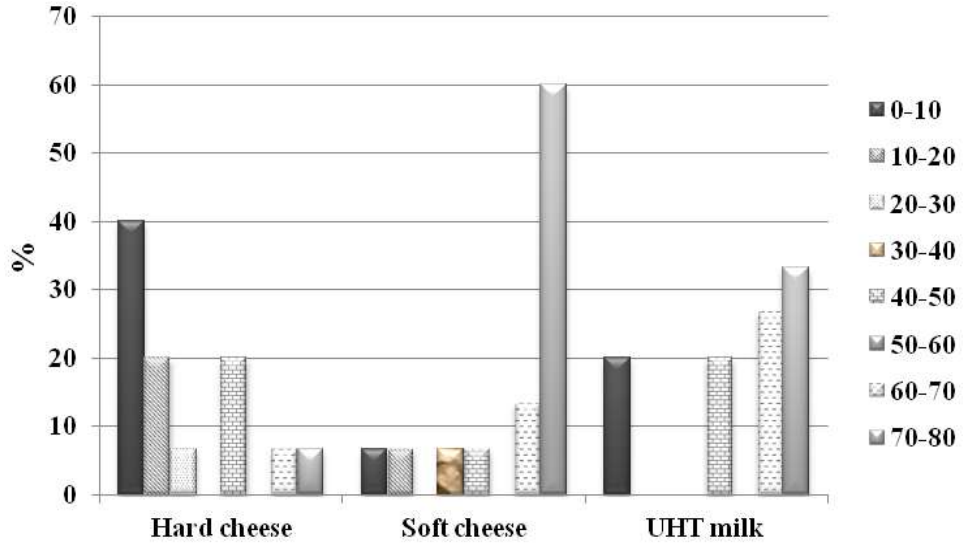

Figure 2. Frequency distribution of the examined samples based on their aflatoxin M1 concentration (ppt).

products may be attributed to greater amount of grass consumed by dairy cattle and lower amount of concentrates (Brown, 1982; Egmond, 1991; Dragacci and Fremy, 1996).

Conclusion

The results obtained from this work allow us to concluded that the majority of yogurt, hard, soft and processed cheese exposed for sale in Giza governorate are considered being hazardous to the consumers.

Tetracyclines were present in samples of UHT milk with variable percentages, while aflatoxin M1 was detected in all examined samples of UHT milk and cheese, sorbic acid, benzoic acid, sulphur dioxide and nitrite were detected in some samples of cheese. Therefore, in order to produce safe milk products, contamination should be avoided in order to reduce economic losses and expenses. To ensure safety in dairy industries the following suggestions should always be observed:

1) Strict hygienic measures should be adopted in dairy farms to ensure production of high quality milk.
2) Good sanitary conditions should be applied during production and processing of milk. Educational programs should be imposed to producers, processors and and handlers to improve the quality of the product and to ensure the maximum safety to the consumers.
3) Application of effective technological measures (pasteurization, sterilization, acidification in technological processes to prolong the product shelf life and decreases or eliminations of pathogens in milk and milk products.
4) Application of adequate control measures through periodical examination of market milk and milk products by specialists to ensure maximum safety to the consumers.
5) Good manufacturing practices should be maintained throughout and HACCP should be applied to ensure safety of the finished products.

Conflict of interests

The authors did not declare any conflict of interest.

REFERENCES

Association of Official Analytical Chemists, AOAC (2000). Official methods of Analysis of AOAC international, 17 th ed. Published by AOAC international.

Australian Academy of Science (2004). Chemical preservation. Cited in: http://www.yahoo.com/ search. Publications / abstracts / september 2004.html.

Brown CA (1982). Aflatoxin M1 in milk. Food Technol. 34:228-231.

Cathey CG, Huang ZG, Sarr AB, Clement BA, Philips TD (1994). Development and evaluation of a mini-column assay for the detection of aflatoxin M1 in milk. J. Dairy Sci. 77:1223-1231.

Dragacci S, Fremy JM (1996). Application of immunoaffinity column clean up to aflatoxin M1 determination and survey in cheese. J. Food Protect. 12:59-65.

Dragacci S, Gleizes E, Fremy JM, Candlish AAG (1995). Use of immunoaffinity chromatography as a purification step for the determination of aflatoxin M1 in cheeses. Food Addit. Contam. 12(1):59-65.

Edward SK (1981). Handbook of meat analysis, 2 nd. ed. Avery publishing group inc., Wayne, New Jersy.

Egmond VHP (1991). Mycotoxins. In: Monograph on residues and contaminants in milk and milk products, International Dairy Federation, p. 131.

Egmond VHP (1989). Current situation on regulations for mycotoxins. Overview of tolerances and status of standard methods of sampling and analysis. Food Addit. Contam. 6(2):139-88.
Fang H, You-Zhao H, Lian L, Guo-Ni F, Hai-Yang X, Wu-Er G (2008). Determination of benzoic acid and sorbic acid in food products using electro-kinetic flow analysis–ion pair solid phase extraction–capillary zone electrophoresis. Anal. Chim. Acta 6(18):79-85.
Foley J, Buchley J, Murphy MF (1974). Commercial testing and product control in the dairy industry. University College, Cork.
Food and Agricultural Organization, FAO (1957). Report on the meeting of experts on the use of H2O2 and other preservatives in milk. FAO 157/11/8655.
Glaesser H (1989). Use of nitrate in cheese production. Dairy Ind. Int. 54:19-233.
Glass KA Kaufman KM, Johnson EA (1998). Survival of bacterial pathogens in pasteurized process cheese slices stored at 30ºC. J. Food Prot. 61(3):290-294.
Haddad LM, Winchester JF (1990). Clinical management of poisoning and drug overdose. W. B. Saunders Company, Philadelphia, PA, 19106.
Heeschen WH, Blüthgen A (1991). Veterinary drugs and pharmacologically active compounds. Monograph on Residues and Contaminants in Milk and Milk Products. IDF, Brussel.
Iswariah V, Guruswami MN (1973). Pharmacology and pharmacotherapeutics (P Varadacharya and Co, India), pp. 416, 427, 491.
Jahed KR Gh (2007). Chemical contaminants in milk and public health concerns: A review. Int. J. Dairy Sci. 2(2):104-115.
Lawley R, Curtis L, Davis J (2008). The Food Safety Hazard Guidebook ,Food Safety Info, London, Ukm.
Ling ER (1963). A text book of dairy chemistry. 3rd ed. Chapman and Hall LTD, London.
Mohanan S, Thomas PG, Lilly I, Laila M, Dominiand BI (2002). A new ultrasonic method to detect chemical additivesin branded milk. Indian Acad. Sci. 59(3):525-529.
Pien J, Desirant J, Iafontaine D (1953). Detection of hydrogen peroxide in milk. Ann. Falsif, Fraudes, Paris, Dairy Sci. Abst. 46:539/540, 416-426.
Resmini P, Saracchi S, Bernardi G, Panari G (1988). Determination of spinacine (6-carboxy 1,2,3,4-tetrahydroimidazopyridine) in cheese to estimate the formaldehyde used in cheese making. Scienza-e-Tecnia-Lattiero-Casearia, 39(1):7-19.
Riedel de-Haen AG (1997). Enzymeimmunoassay zur quantitative bestimung von aflatoxin M1, ELISA system, diagnostic catalog, p. 2.
Satoskar RS, Bhanderkar SD, Ainapure SS (1999). Pharmacology, 16th Ed. Popular Prakashan, Mumbai, p. 591.
Schroeter LC (1966). Sulfur dioxide. Applications in foods, Bevearges and pharmaceuticals- pergamon, Oxford.
Skovgaard N (1992). Microbiological aspects and technological need: technological needs for nitrates and nitrites, Food Addit. Contam. 9(5):391-397.
Sundin GW (2003). Tetracycline. Encyclopedia of Agrochemicals, John Wiley & Sons, New Jersey, pp.1521-1522.
Tenant DR (1997). Food Chemical Risk Analysis. Blakie Academic and Professional. Chapman and Hall. UK. 1 st. Ed., pp. 381-398.
Wood GE (1991). Aflatoxin M1. In: Mycotoxins and Phytotoxins (chapter 6). Sharma, R.P. and Salunkhe, D.K. (Eds). London, CRC Inc., pp. 145-163.
Wynther BA, Meredith WB (1927). Foods: Their composition and analysis. 7th Ed., Charles, Griffin and Company, Ltd., London.