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Finding a rapid, simple and precise method for determination of skim milk powder adulteration in non reconstituted milk

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Abstract

Nowadays, due to economically advantageous and milk shortages in some areas, adulteration in milk is growing up. One of these adulterations is depicting reconstituted milk as fresh milk or mix it with fresh milk. If the milk is heated at high temperature it would take caramel flavor and becomes brown (Maillard reaction) which causes unwanted traits. However, this reaction does not occur in the pasteurization process but it takes place in dehumidifying the milk. The amount of skim milk powder added can be measured in different ways. But finding a precise method is necessary. In the current study determination of hydroxy methyl furfural by colorimetry, RP- HPLC and UV-Visible Spectrophotometer were done on the base of Maillard reaction. Besides these, chemical analysis, measuring the Milk Reducing Substance, titration with nitric acid and determination the absorbance of samples at visible and UV wavelength were done. As expected we concluded that measuring the hydroxy methyl furfural by RP- HPLC method is the most precise method. But beside these we can say titration with nitric acid and for the next priority measuring the percent of fat (if the non-fat dry milk is used), dry matter and ash and also measuring the hydroxy methyl furfural by colorimetry are sensitive too.

Introduction

Food and nutrition are undoubtedly the most important topics in today's world and milk is one of the most important foods in the nature. Milk is the only food that can provide most of the nutrients for the body. It contains valuable proteins, carbohydrates, fats and minerals, especially calcium, phosphorus and different vitamins (Guetouache *et al*., 2014). Currently, in most of the countries that consume large quantities of milk and its products, calcium is mainly supplied from milk (Wattiaux, 2003). Today, due to economic changes and low price of dry milk compared with fresh milk and also lack of fresh milk in some seasons, some factories use dry milk to produce reconstituted milk and mix it with fresh milk. When milk is exposed to prolonged heat, many chemical changes occur. The two major changes are browning and releasing the regenerative compounds. Non-enzymatic browning reaction (Maillard reaction) occurs in a wide range of foods such as breads, biscuits, meat and etc and its products enter into the daily diet of the population (Chichester, 1986). Maillard reaction reduces the nutritional value and digestibility of protein in food (Hurrell, 1990) and also leads to the degradation of ascorbic acid and reduces absorption of minerals in the diet (Jing and Kitts, 2002). Maillard reaction that occurs even at low temperatures is relatively slow in high-moisture foods, but it is the predominant reaction at ambient temperatures in foods with low moisture content, such as milk or whey powders (Labuza and Saltmarch, 1982). Metabolite of the Maillard reaction is toxic to the body and can lead to symptoms such as weight loss, hepatomegly, liver necrosis and nephromegaly (Labuza and Saltmarch, 1982). If the milk is heated at high temperature due to the lactose reaction with amine groups it takes caramel flavor and becomes brown (Maillard reaction) which are unwanted traits in milk. However, this reaction does not occur in the pasteurization but it occurs in dehumidifying the milk (Henle *et al.*, 1991). So, due to adulteration of fresh milk by adding reconstituted milk or the selling of reconstituted milk as fresh milk, it is better to find a way to measure the amount of skim milk powder added to the fresh milk. A lot of methods have been examined such as: Cardwell and Herzer (1958) offered a colorimetric method for the determination of non-fat dry milk in fresh milk. Cardwell and Herzer (1958) and Lin *et al.* (1991) found that there is a linear relationship between the amount of milk regenerative protein and non-fat dry milk in fresh milk. Abu-Lehia and Abu-Tarboush (1994) measured

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different level of dry milk in pasteurized milk and yoghurt by Thiobarbituric acid and regenerative agent methods. Resmini *et al*. (1992) measured furosine by HPLC method as a way to measure the amount of dry milk. Rong-fa *et al.* (2005), used flourimetry method for determination the Maillard products in milk. When legislation prohibits selling reconstituted milk as fresh milk or mix with fresh milk and also due to disadvantages mentioned above, finding out the amount of non-fat dry milk in milk daily used is important. But we need a rapid and sensitive test. The current study is designed to compare different methods in order to establish the more rapid and sensitive way to measure the amount of skim milk added to the fresh milk.

Materials and Methods

Sample preparation

In this study, three samples of non-fat dry milk with different production dates were tested. 1 g of sample was brought to a volume of 10 mL using distilled water. Then they were heated in a water bath at 63ºC for 30 minutes to be pasteurized. The ratios of 3, 4, 5, 10 and 20% of pasteurized and UHT-treated milk (with no usage of dry milk in their production process) were replaced by this reconstituted milk. These were performed in duplicate.

Chemical analysis

The amount of protein and fat were measured by Kjeldahl and Gerber method in order (AOAC, 1996; ISO, 2008). Dry matter and ash of the samples were determined by standard methods (Pomeranz and Meloan, 1994; ISO, 2010). Dry matter (%w/w) was determined by drying 10 g of the sample at 105^{o}C to constant weight and ash contents were performed at 500^{o}C (%w/w).

Determination of hydroxy methyl furfural by colorimetry (HMF-C)

Using the method of Keeney and Bassette (1959) as modified by Della-Monica *et al.* (1968) and Ferrer *et al*. (2002), 10 mL of samples mixed with 5 mL of 0.3 mol.L^{-1} oxalic acid (freshly prepared daily) in a sealed tube to prevent evaporation. The tube was heated in a water bath at 100ºC for an hour. After rapid cooling in ice, 5 mL of a 400 g/L (w/v) trichloroacetic acid (TCA) solution was added, and the mixture was stirred thoroughly for 5 minutes. It was then centrifuged at 2000 g for 15 mins. The supernatant was filtered through Watman filter paper (42). Then 1 mL thiobarbituric acid (TBA) (0.05 mol.L^{-1}) was added to 4 mL filtered sample and stirred well. After, the sample was heated in a water bath at 35ºC for 40 minutes. After rapid cooling in ice, the absorbance of the samples was measured at 433 nm wavelength. In the blank sample, distilled water was used instead of milk. In order to measure the amount of HMF, the below formula, which was calculated by using different concentration of purred HMF instead of sample in this method, was used.

$$HMF\ (\mu M) = (30.08 \times Abs_{443}) - 0.13$$

Determination of hydroxy methyl furfural by RP-HPLC (HMF-H)

The sample was prepared as mentioned for HMF-C. But the samples were filtered through a syringe filter. The HPLC system consisted of binary pump, degasser, sampling valve, 20 µl sample loop, column oven, photo-diode array detector and computing integrator connected to a PC, all from Knauer (Berlin, Germany). Separations were carried out at room temperature using sodium acetate buffer (0.08M) adjusted with acetic acid to pH 3.6 at a flow rate of 1ml/min as the mobile phase. The mobile phase delivered under isocratic condition through the analytical column, Spheriosorb ODS-2 analytical column (25 × 0.40 cm, 5 µm particle size) (C18) (Morales, 1994). Detection in wavelength gradient at 280 nm was carried out and the volume of injection was 20 µl. The HMF was completely separated out in 11.23 mins and the run time was 13 mins. The external calibration curve was used for quantification of HMF.

Determination of HMF by uv-visible spectrophotometer

5 mL of milk sample was centrifuged at 10000 rpm for 10 minutes and the 4.5 mL supernatant liquid was obtained. It was done for one more time and the 4 mL supernatant liquid was obtained. 0.5 mL of Carrez Solution I (150 mg/mL potassium ferrocyanide) was added to the 4 mL sample and mixed well. 0.5 mL of Carrez Solution II (300 mg/mL zinc acetate) was then added to the sample and mixed well. Then it was centrifuged again at 10000 rpm for 10 minutes and 4mL supernatant liquid was obtained. Then it was divided to 2 tubes (2 mL in each tube). 2 mL distilled water was added to one of them and 2 mL sodium bisulfite (2 g.L^{-1}) was added to the other (to neutralize the absorbance the HMF, this tube was used as blank). The absorbance of the samples was measured at 284 and 336 nm wavelength. Then in order to measure the amount of HMF the below formula was used (DIN 10751-1, 1992; Kreuziger Keppy and W. Allen, 2009).

$$HMF\ (mg/Kg) = (Abs_{284} - Abs_{336}) \times 149.7 \times 5 \times \text{dilution factor / dry milk (gr)}$$

Measuring the milk reducing substance (MRS)

A modification of Chapman and McFarlane (1945) method was used. 5 mL of urea solution (10 $mol.L^{-1}$) was added to 1 mL sample and stirred well. Then 3 mL of potassium ferricyanide solution (1.2 g/L) was added to the mentioned solution. After, the sample was heated in a water bath at 70ºC for 40 minutes and cooled to 32˚C. Then 2 mL of ammonium sulphate (10 $mol.L^{-1}$) and 0.4 mL of tricholoroacetic acid (400 g/L) were added in order. The solution was filtered through Watman filter paper (40). 2 mL of filtered sample was mixed with 2 mL of distilled water and placed in a water bath 32.3˚C for 5 minutes. Finally 0.5 mL of ferric chloride (2 g/L) was added and stored at room temperature for 30 minutes to ensure that the reaction is complete. The absorbance of the samples was measured at 610 nm wavelength. In the blank sample, distilled water was used instead of milk. In order to measure the amount of reducing substance, the below formula, which was calculated by using different concentration of cysteine instead of sample in this method, was used.

$$MRS\ (moles \times cysteine) = (1.4 \times Abs_{610}) + 1.07$$

Titration with nitric acid

10 mL of sample was titrated by nitric acid (60%) until the appearance of yellow color. The aromatic rings of phenylalanine, tyrosine and tryptophan under the effect of nitric acid form yellow-colored nitro derivatives (xanthoproteic reaction) (Bankowski *et al.*, 2013). Preheat treatments and evaporation causes the denaturation of proteins in milk even β-lactoglobulin and α-lactalbumin (Singh and Creamer, 1991)

Determination the absorbance of samples at visible and UV wavelength

The samples were diluted 1 to 1000. Then the absorbance of the samples was measured at 700 nm and 240 nm wavelength and the results were multiplied in the thousand. The mechanism is the turbidity difference between homogenized milk and non-homogenized milk (Lin *et al*., 1991).

Statistical analysis

All samples were examined in triplicate. The results were analyzed using one-way analysis of variance and the statistical significance of differences between mean values was analyzed by Duncan's multiple range tests (Howell, 2002). Analysis was performed using a SPSS package (SPSS 16 for windows, SPSS Inc, Chicago, IL, USA).

Table 1. The result of chemical analysis of pasteurized milk with different amount of nonfat dry milk (Mean ± SD)

Non-fat dry milk (%)	Protein (%)	Fat (%)	Dry matter (%)	Ash (%)
0	2.985±0.007^{a}	1.835±0.021^{a}	9.870±0.017^{a}	0.750±0.000^{a}
4	2.997±0.010^{a}	1.812±0.019^{ab}	9.895±0.011^{ab}	0.758±0.010^{ab}
5	2.988±0.007^{a}	1.797±0.012^{b}	9.905±0.007^{b}	0.763±0.008^{b}
10	2.985±0.011^{a}	1.538±0.016^{c}	9.960±0.017^{c}	0.790±0.006^{c}
20	2.992±0.012^{a}	1.370±0.021^{d}	10.127±0.010^{d}	0.808±0.004^{d}

The different letters in each column show statistically significant differences ($p< 0.05$).

Table 2. The result of chemical analysis of UHT-treated milk with different amount of nonfat dry milk (Mean ± SD)

Non-fat dry milk (%)	Protein (%)	Fat (%)	Dry matter (%)	Ash (%)
0	3.005±0.007^{a}	2.060±0.000^{a}	9.957±0.010^{a}	0.755±0.007^{a}
4	3.013±0.008^{a}	2.022±0.017^{ab}	9.975±0.011^{a}	0.758±0.007^{a}
5	3.022±0.012^{a}	2.018±0.012^{b}	10.025±0.007^{b}	0.773±0.005^{b}
10	3.012±0.008^{a}	1.823±0.020^{c}	10.075±0.022^{c}	0.793±0.005^{c}
20	3.013±0.012^{a}	1.658±0.019^{d}	10.203±0.016^{d}	0.807±0.005^{d}

The different letters in each column show statistically significant differences ($p< 0.05$).

Results

Chemical analysis results were shown in tables 1 and 2 and the results of the other methods to find out the amount of nonfat dry milk were shown in tables 3 and 4. In the HPLC analysis for the linearity assessment, a seven point calibration of HMF curve was constructed. The concentration ranges were 0.01-100 µg/ml. The linear regression equation used was Y=1.3562X + 5.0921, where Y is the peak height and X is the HMF concentration. The correlation coefficient was 0.9998. The validation parameters are represented in Table 5.

Linearity was determined in concentration range of 0.01-100 µg/ml and correlation coefficient (R^2) of 0.9998 was found indicating a good linear relationship between concentration and peak area.

The limit of detection (LOD) and limit of quantification (LOQ) were determined by following formula: LOD= 3.3 SD/m, LOQ= 10 SD/m where

Table 3. The result of different method to measure the amount of skim milk added to the pasteurized milk (Mean ± SD)

Non-fat dry milk (%)	HMF-C (μ mol.L^{-1})	HMF-H (μ mol.L^{-1})	HMF-uv visible (mg/Kg)	MRS (moles × cysteine)	Nitric acid (mL)	Abs_{700nm}	Abs_{240nm}
0	1.209±0.021^{a}	1.085±0.007^{a}	0.220±0.014^{a}	1.181±0.002^{a}	6.650±0.071^{a}	77.500±4.950^{a}	923±2.828^{a}
3	*	1.398±0.015^{b}	*	*	6.530±0.081^{a}	*	*
4	1.219±0.044^{a}	1.601±0.008^{c}	*	*	6.300±0.110^{b}	*	*
5	1.454±0.084^{b}	1.781±0.012^{d}	*	1.175±0.003^{a}	6.217±0.075^{bc}	74.330±2.066^{a}	921.500±4.637^{a}
10	2.176±0.098^{c}	2.623±0.015^{e}	0.225±0.014^{a}	1.206±0.005^{b}	6.050±0.105^{c}	62.500±1.643^{b}	848.170±4.491^{b}
20	3.314±0.138^{d}	3.342±0.013^{f}	0.418±.002^{b}	1.201±0.031^{b}	5.417±0.117^{d}	53.670±1.366^{c}	742.170±15.092^{a}

The different letters in each column show statistically significant differences ($p< 0.05$).

*Due to no statistically significant differences ($p< 0.05$) in higher percent of skim milk added to the pasteurized milk, there was no need to do lower percent.

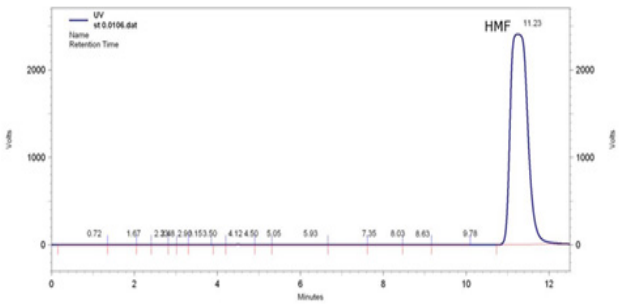

Figure 1. Chromatogram of an HMF standard solution (HMF concentration is 1.06 μM). Run time=13 mins and retention time for HMF=11.23 mins

SD is residual standard deviation of regression line and m is the slope of regression line. Intra-day precision was determined by replicate analysis (n = 10) of the spiked sample on the same day and inter-day precision was determined by replicate analysis of the spiked sample on ten consecutive days. Assay precision was expressed as the relative standard deviation (RSD%). Intra-day and inter-day precisions for different concentration were between 1-2% and 1-5%, respectively. The recoveries for spiked samples at different concentrations (0.01-100μg/ml) were between 95-105%. These data are in acceptable ranges described in the AOAC manual for peer-verified methods.

According to the results, HPLC method was the most sensitive tests that can even determine the low amount of skim milk powder. Notably, there was no statistically significant difference between skim milk powders with different production dates.

Discussion

Today a lot of adulterations take place in the milk industry. Common adulterants are adding water, urea, detergent and starch to the milk (Swathi and Kauser, 2015). One of them is depicting reconstituted milk as fresh milk or mixing it with fresh milk. It should be mentioned that it is not an adulteration until legislation prohibits such practice. In this situation, it is necessary to find a way to estimate the amount of milk powder in fresh milk. Methods, using to detect the amount of reconstituted milk are based on the molecular structure changes (especially protein and carbohydrate changes) which come from preheat treatments and evaporating milk (Elversson and Millqvist-Fureby, 2005; Fanga *et al.,* 2012). So many methods can be used, but choosing a rapid, simple, precise and sensitive method is necessary. According to the results of this study using HPLC method to measure the amount of skim milk, on the basis of the amount of hydroxy methyl furfural producing in Maillard reaction, is more reliable than the others. But this method is too expensive. The result showed that some other methods such as titration with nitric acid, measuring the amount of hydroxy methyl furfural by colorimetry are sensitive too. But in contrast of HPLC they are less sensitive. The HPLC method can measure less than 3% reconstitude milk in fresh milk and for the second priority the titration

Table 4. The result of different method to measure the amount of skim milk added to the UHT-treated milk (Mean ± SD)

Non-fat dry milk (%)	HMF-C (μ mol.L^{-1})	HMF-H (μ mol.L^{-1})	HMF-uv visible (mg/Kg)	MRS (moles × cysteine)	Nitric acid (mL)	Abs_{700nm}	Abs_{240nm}
0	2.006±0.042^{a}	1.720±0^{a}	0.285±0.007^{a}	1.223±0.002^{a}	6.150±.071^{a}	96±0.414^{a}	1068±5.657^{a}
3	*	2.200±0.025^{b}	*	*	6.067±0.082^{a}	*	*
4	2.061±0.052^{a}	2.325±0.021^{c}	0.295±0.009^{a} ^{b}	*	5.800±0.089^{b}	94.670±2.066^{a}	*
5	2.477±0.091^{b}	2.592±.022^{d}	0.300±0.014^{b}	1.217±0.004^{a}	5.633±0.121^{c}	92.170±1.472^{b}	1066.830±13.877^{a}
10	3.364±0.128^{c}	3.497±0.015^{e}	0.437±0.002^{c}	1.244±0.004^{b}	5.4±0.089^{d}	80.170±1.472^{c}	952.330±5.125^{b}
20	4.397±0.157^{d}	4.665±0.026^{f}	0.583±0.003^{d}	1.297±0.032^{c}	4.95±0.105^{e}	68.170±0.408^{d}	848.170±7.389^{c}

The different letters in each column show statistically significant differences ($p< 0.05$).
*Due to no statistically significant differences ($p< 0.05$) in higher percent of skim milk added to the UHT-treated milk, there was no need to do lower percent.

Table 5. Validation parameters data for the HMF quantified in the milk samples.

Linearity		Sensitivity		Precision		
Concentration range(μg/ml)	R^2	LOD (μg/ml)	LOQ (μg/ml)	Intra-day (RSD %)	Inter-day (RSD%)	(%)Recovery
0.01-100	0.9998	0.028	0.185	1-2	1-5	95-105

with nitric acid is sensitive with the sensitivity of 4% reconstituted milk added. During preheat treatments and evaporating milk, the denaturation of milk protein takes place. It causes more reaction of aromatic rings of phenylalanine, tyrosine and tryptophan under the effect of nitric acid to form yellow-colored nitro derivatives. Measuring the amount of hydroxy methyl furfural by colorimetry with the sensitivity of 5% reconstituted milk added can be applicable too. Measuring the amount of hydroxy methyl furfural by UV-Visible Spectrophotometer and measuring the absorbance at visble wavelength is applicable for UHT milk with the sensitivity of 5%. The determination of ultra violet and visible spectra (700 to 240 nm) is also recommended as sensitive methods by Madkour and Moussa (1989). They reached to this conclusion that the sensitivity is 2.5% reconstituted milk added (Madkour and Moussa, 1989). Chemical analysis such as measuring the percent of fat (if the non-fat dry milk is used), dry matter and ash with the sensitivity of 5% can be recommended too. Even though adulteration of fresh milk by adding reconstituted milk or the selling of reconstituted milk as the fresh product is important due to reasons mentioned before, but there is a little research in order to detect the amount of reconstituted milk added. Rong-fa *et al.* (2005), who showed the FAST (fluorescence of advanced Maillard products and soluble Tryptophan) method is a suitable, simple and rapid method to detect and monitor whether milk adulteration has occurred, believe that when legislation prohibits such practice, the detection of reconstituted milk depicted as fresh milk or mixed with fresh milk, becomes an analytical problem.

Conclusion

Determination of hydroxy methyl furfural by RP-HPLC is the more reliable method than the others. Titration with nitric acid, measuring the percent of fat (if the non-fat dry milk is used), dry matter and ash, hydroxy methyl furfural by colorimetry are also sensitive and can be mentioned as applicable methods.

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Conflict of interest statement

The authors declare that they have no conflict of interest.

References

Abu- Lehia, I. H. and Abu- Tarboush, H. M. 1994. Detection of dry milk in pasteurized milk and yogurt. Journal of King Saud University – Science 6(1): 41-49.

AOAC. 1996. Nonprotein nitrogen in whole milk. Kjeldahl method, method No. 991,21. Gaithersburg, MD: USA.

Bankowski, E., Galewska, Z., Gogiel, T., Małkowski, A., Romanowicz, L., Sobolewski, K. and Wolanska, M. 2013. Biochemistry workbook. Uniwersytet Medyczny Bialymstoku.

Cardwell, J. T. and Herzer, F. H. 1958. A study of factors which variation in the protein reducing value of fluid milk. Journal of Dairy Science 41: 702-703.

Chapman, R. A. and McFarlane, W. D. 1945. A colorimetric method for the estimation of reducing groups in milk powders. The Canadian Journal of Research 23(3): 91-99.

Chichester, C. O. 1986. Advances in Food and Nutrition Research, p. 79. Boston: Academic Press.

Della-Monica, E. S., Craig, J. C. and Calhoum, M. J. 1968. Error in the analysis of Hydroxymethylfurfural in processed milk. Journal of Dairy Science 51(3): 352-355.

DIN 10751-1. 1992. Determination of hydroxymethylfurfural- Winkler Photometric Method. Beuth: Berlin.

Elversson, J. and Millqvist-Fureby, A. 2005. Particle size and density in spray drying- Effects of carbohydrate properties. Journal of Pharmaceutical Sciences 94(9): 2049-2060.

Fanga, Y., Rogers, S., Selomulya, C. and Chen, X. D. 2012. Functionality of milk protein concentrate: Effect of spray drying temperature. Biochemical Engineering Journal 62: 101-105.

Ferrer, E., Alegria, A., Farre, R., Abellan, P. and Romero, F. 2002. High-performance liquid chromatographic determination of furfural compounds in infant formula: Changes during heat treatment and storage. Journal of Chromatography A 947(1): 85-95.

Guetouache, M., Guessas, B. and Medjekal, S. 2014. Composition and nutritional value of raw milk. Issues in Biological Sciences and Pharmaceutical Research 2(10): 115-122.

Henle, T., Walker, H., Krause, I. and Klostermeyer, H. 1991. Efficient determination of individual Maillrd compounds in heat-treated milk products by amino acid analysis. International Dairy Journal 1(2): 125-135.

Howell, D. 2002. Statistical Methods for Psychology, p. 324–325. Boston: Duxbury Press.

Hurrell, R. F. 1990. Influence of the Maillard reaction on the nutritional value of foods. In Finot, P. (Eds). The Maillard Reaction in Food Processing, Human Nutrition and Physiology, p. 245. Advances in life science, Birkhauser Verlag Basel.

ISO/IDF. 2008. Milk- Determination of fat content- Gerber butyrometers. Brussels: Belgium.

ISO/IDF. 2010. Milk, cream and evaporated milk- Determination of total solids content. Brussels: Belgium.

Jing, H. and Kitts, D. D. 2002. Chemical and biochemical properties of casein-sugar Maillard Reaction products. Food and Chemical Toxicology 40(7): 1007- 1015.

Keeney, M. and Bassette, R. 1959. Detection of intermediate compounds in the early stages of browning reaction in milk products. Journal of Dairy Science 42(6): 945-960.

Kreuziger Keppy, N. and W. Allen, M. 2009. The determination of HMF in honey with an evolution array UV-visible spectrophotometer. Thermo Fisher Scientific. Madison, WI: USA.

Labuza, T. P. and Saltmarch, M. 1982. Kinetics of browning and protein quality loss in whey powders during steady state and non steady state storage conditions. Journal of Food Science 47(1): 92-96.

Lin, T. C., Chen, I. J. and Ko, Y. C. 1991. The mechanism of determining the adulteration of whole milk with milk powder by spectrophotometry. Molecular Nutrition and Food Research 35(4): 351-358.

Madkour, M. A. and Moussa, B. M. 1989. The determination of milk powder added to whole milk. Nahrung 33(1): 77-82.

Morales, F. J. 1994. Determination de 5-Hidroximetilfurfural en leche tratada termicamiento. Madrid, Spain: Universidad Autonoma de Madrid, PhD Thesis.

Pomeranz, Y. and Meloan, C. E. 1994. Food Analysis Theory and Practice. 3rd ed. New York: Chapman Hall.

Resmini, P., Pellegrion, L., Masotti, F., Tirelli, A. and Partim, F. 1992. Detection of reconstituted milk powder in raw and pasteurized milk by HPLC of furosine. Scienza e Tecnica Lattiero-Casearia 43: 169-186.

Rong-fa, G., Dong-hong, L., Xing-gain, Y. and Kai, Y. 2005. Use of fluorometry for determination of skim milk powder adulteration in fresh milk. Journal of Zheijang University Science 6(11): 1101-1106.

Singh, H. and K. Creamer, L. 1991. Denaturation, aggregation and heat stability of milk protein during the manufacture of skim milk powder. Journal of Dairy Research 58(03): 269-283.

Swathi, J. K. and Kauser, S. 2015. A study on adulteration of milk and milk products from local vendors. International Journal of Biomedical and Advance Research 6(09): 678-681.

Wattiaux, M. A. 2003. Milk composition and nutritional

value. Babcock Institute for International Dairy Research and Development. University of winconsin, Madrid: Spain.