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Implementation of Syber Green Based Real-Time Pcr for Detection of Cow's Milk in Buffalo's Milk and its Products

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ABSTRACT

The widely buffalo's milk and its products consumption in Egypt, in additional to high nutritional values of buffalo's milk make these products target for potential adulteration with different types of milk, especially cow's milk. In the present study, 100 samples of buffalo's milk and its products were randomly collected and investigated for the presence of cow's milk by using SYBER green-based real-time PCR. Samples were collected from local market in Mansoura city, Egypt. Raw buffalo's milk, yogurt, fresh Domiati cheese and pickled Domiati cheese samples were found to contain cow's milk, the presence rate of cow's milk in raw buffalo's milk more than yogurt and Domiati cheese. To determine the sensitivity limit of SYBER green- based real-time PCR using experimental samples of buffalo's milk, buffalo's yogurt and buffalo's Domiati cheese, including different concentrations of cow's milk (0.5, 1, 2, 5, 10, 20, 30 and 40%) which revealed the efficiency of method detection limit till reached 0.5% of cow's milk in buffalo's milk and its products. However the detection limit of cow's milk in buffalo's yogurt and cheese were 1 and 2% respectively.

Keywords: SYBER green-based real-time PCR- Dairy products–Buffalo's milk- Cow's milk

INTRODUCTION

The food adulteration was defined in 2009 by the food and drug administration as "the fraudulent, deliberate addition or substitution of a substance in a final product in order to increase the superficial product value or decreasing the cost of its manufacture," and may often include public safety influences though the toxins, allergens and hygienic risks unknown addendum (Wheatley& Spink, 2013). Dairy products occupy the second position in the list of adulterated food products according to FDA (Moore *et al*., 2012).

The most of retail dairy products are manufactured from cow's milk. Dairy products made from water buffalo milk considered as characteristic properties of sensory, primarily the flavor and colour. In addition, the high concentration of total solid and fat, compare with cow milk (Bonfatti, *et al.,* 2013). It is of important to government authorities and industrialists and consumers to have a sensitive, fast, simple and accurate method for detection of adulteration by cow's milk.

Many methods have been enhanced to determine adulteration of species in milk and dairy products involving chromatography, immunological and molecular methods (Mayer, 2005). The method for the official control to investigate bovine casein in milk and dairy products is depended on isoelectric focusing of gamma casein after proteolysis (EC Regulation No. 213/2001). Nevertheless the single species protein profile results a complex banding pattern and even low protein levels from other species will often overlap the species - specific bands, so reducing the sensitivity of detection level for this method (Lopez-Calleja *et al.*,2007).

Additional procedures presently applied are base on the protein fraction analysis, including Enzyme linked immuno Sorbent Assay (ElISA) (Hurley *et al.,* 2004; Lopez-Calleja *et al.,* 2007). However, these protocols may not always differentiate milk types from closely related species such as goat and sheep and buffalo and cow, in addition to are not usable to heat treated milk (Lopez-Calleja *et al.,* 2005).

Other methods based on analysis of protein for identification of species in dairy products, such as HPLC/ESI-MS (Chen *et al.,* 2004) and MALDI-TOFMS (Cozzolino *et al*., 2002) have been used, but these techniques are high coast and time consuming and therefore unseemly for routine analytical testing (Lockley and Bardsley, 2000; Woolfe and Primrose, 2004).

Other procedures based on fatty acid profiles and triglyceride determination by NanoESI-MS) (Mirabaud *et al*., 2007). Obviously, the sensitive of these methods directly proportional to fat content of milk and dairy products and therefore these methods unsuitable for assaying adulteration of skimmed milk and fat free dairy products (Mayer, 2005).

The usage of immunological and chromatography methods are not suitable for implementation in neoteric mass manufacture dairy industries, where the species adulteration of enormous number of dairy products samples needs to be evaluated in a well timed.

Methods based on polymerase chain reaction (PCR) have been enhanced as timekeeping, efficacious and effort-effective methods for the determination species adulteration in milk and dairy products (Bottero *et al*., 2002; Bottero *et al*.,2003; El-Rady and sayed, 2006; Rea *et al*., 2001). Particularly, the real-time PCR protocol has been newly utilized in different applications of analytic in food industries involving the detection of species adulteration (Lopez Calleja *et al*., 2007; Lopparelli *et al*., 2007; Zhang *et al*., 2007).

The aim of this study was to develop a real-time PCR method for cow and buffalo DNA identification in pure buffalo milk and buffalo dairy products.

MATERIALS AND METHODS

Collection of samples

A total of 100 samples of buffalo's milk and its products were randomly obtained from the domestic markets in the Mansoura city. These samples contained raw milk (n=30), yogurt (n=30), fresh Domiati cheese (n=20) and pickled Domiati cheese (n=20).

DNA extraction

1ml of milk sample was centrifuged at 3000g for 20 min to obtain somatic cells. The somatic cells were washed three times in PBS (1ml), centrifuged at 13.000g for 20 min and then resuspended in 250 µl of PBC. DNA extraction was performed according to the protocol lof Dneasy Blood and Tissue kit (Qiagen, Hilden, Germany), while the extraction of DNA from milk and dairy products were performed as described by animal tissue Protocol of Dneasy Blood and Tissue kit (Qiagen, Hilden, Germany).

Real-time PCR reactions

A set of forward and reverse primer for cow's milk (DLOOP) and buffalo's milk (WB12S2) were designed from the literature (Lopez-Calleja *et al*., 2005 and Pegels *et al*., 2011). The sequences of primers, melting temperature and size of DNA products are presented in Table (1). The concentration of primers in the SYBER – green based real time PCR reaction was 300µM with for each primer, while the DNA template concentration was 10 ηg/reaction and the final PCR reaction volume of 25µL. Condition of thermal cycling were 94°C for 3 minutes followed by 94°C for 10 seconds and 61.1°C with detection of fluorescent for 40 cycles.

The detection limit of cow's milk determination

The accuracy of the protocol for determining cow's milk in buffalo's milk and its products was assessed at the following, buffalo's milk samples were included a different concentrations of cow's milk (40%, 30%, 20%, 10%, 5%, 2%, 1% and 0.5%). These samples of mixture were subjected to extraction of DNA and subsequent SYBER green real-time PCR.

Table 1. Oligonucletides used as PCR primers for identification of adulteration of buffalo's milk with cow's milk.

Species	Primers	Sequences of primers	Genes	Annealing Temperature	Amplicons	Melting temperature	References
B. Taurus (Cow's milk)	Forward (DLOOP)	5-AACCAAATATTACAAACACCACTAGCT-3	Bovine Mitochondria l D-Loop	61.1°C	77bp	75.8°C	Pegels *et al.*, 2011
	Reverse (DLOOP)	5-CCTTGCGTAGGTAATTCATTCTG-3					
B. bubalis (buffalo's milk)	Forward (WB12S2)	5-CTAG-AGGAGCCTGTTCTATAATCGATAA-3	Buffalo mitochondria l 12S rRNA	61.1°C	220bp	80 °C	Lopez-Calleja *et al.*, 2005
	Reverse (WB12S2)	5-TTCATAATAACTTTCGTGTTGGGTGT-3					

RESULTS

Sensitivity of the SYBER green- based real-time PCR to detect cow's milk in buffalo's milk and its products

To establish the SYBER green- based, real-time PCR detection limit assay, its ability to identify different percentages ranging from 0.5-100% of cow's milk in buffalo's milk was evaluated. As expected higher values of C_T were found associated with decline the concentration of cow's milk. This might be attributed to decline the DNA concentration extracted from somatic cells (Lopez-Calleja *et al.*, 2005; Fig. 1A). The RT-PCR examination using set of DLOOP primer was able to determine cow's milk concentration of approximately 0.5-100% in buffalo's milk with values of C_T from 31 to 14.96 respectively (Fig. 1A). The detection limit of cow's milk in buffalo's yogurt and cheese were significantly lower than buffalo's milk (Fig. 1B and1C). The detection limit of yogurt and cheese were 1 and 2% respectively. The detection limit in this study, was little lower than that reported by zarei *et al.*, (2016), who reported that the detection limit of cow's milk in buffalo's cheese , yogurt and milk 4, 2 and 1% respectively. As non adulterated dairy products are made for financial gain, substituting a high expensive type of milk with a less price type for less than 5% did not has negative economic effect (Maudet and Taberlet, 2001; Khanzadi *et al.*, 2013). The use of set of primer (DLOOP) for this study was suitable for detection of cow's milk, where the PCR reaction with set of primer produced the intended ampilicon, in addition to primer dimmers and non-specific products were not formed in PCR mixture as presented in Fig 2A, 2B and 2C. This primer was not able to amplify DNA products in DNA extracted from buffalo's milk so that fluorescence level might be not detected above background as shown in Fig. 1.

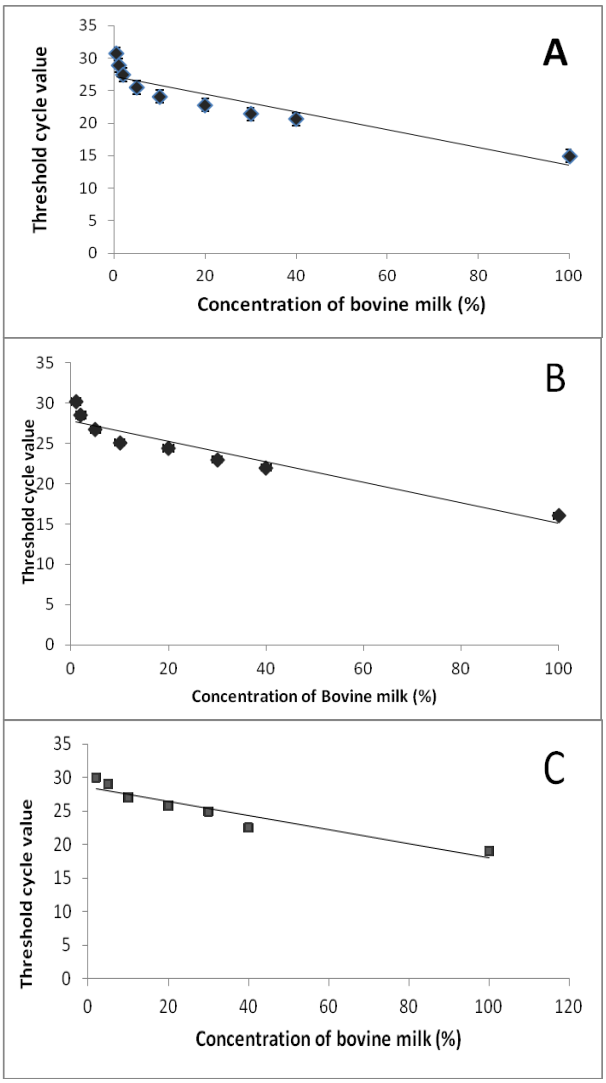

Fig. 1. Real time PCR identification of various concentration of cow's milk in buffalo's milk (A) and buffalo's yogurt (B) and buffalo's cheese (C). Values of C_T are averages of 3separate estimation, and error bars present ±SE.

The curve of melt temperature of the RT-PCR reaction within set of DLOOP primer indicated non-product amplification and no primer dimer (Fig. 2). The set of BDLOOP primer in reaction of singleplex at a concentration of 300µM was used to amplify a 77 bp amplicon (Fig.3). There are not cross reactions with DNA template of buffalo's milk and for that reason, BDLOOP primer is suitable for the application of this procedure to detect mtDNA of bovine milk in buffalo's milk and its products. The present results are in agreement with the results of Pegels *et al*., (2011).

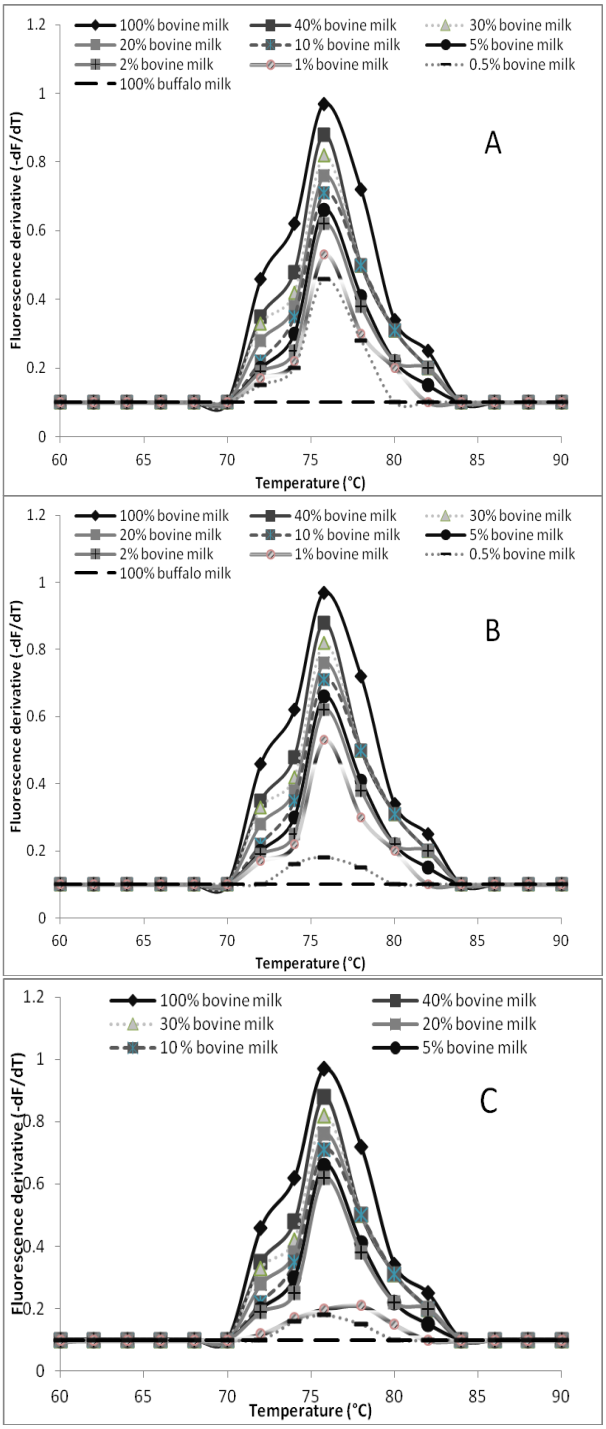

Fig. 2. Curves of DNA melting following RT-PCR analysis of different concentration of cow's milk in buffalo's milk (A) and yogurt (B) and cheese (C).

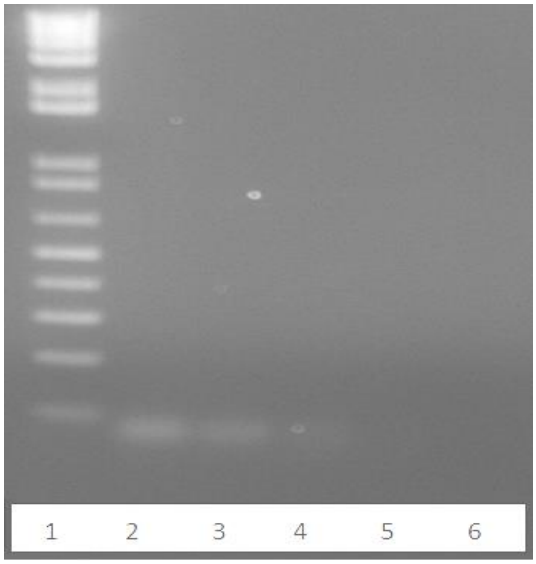

Fig. 3. Agrose gel electrophoresis (2%) of PCR amplicon was amplified with DLOOP primer pair and DNA template of bovine and buffalo milk sample. Lane 1: 1Kb Plus DNA ladder; Lane 2: 2% bovine milk sample singleplex; Lane 3: 1% bovine milk sample; Lane 4: 0.5% bovine milk sample; Lane 5:100% buffalo's milk sample; Lane 6: 40% ;buffalo's milk sample..

Sensitivity of the SYBER green- based real-time PCR to detect buffalo's milk and its products

The real-time PCR based on SYBER green with set of buffalo's primer pairs (WB12S2) detection limit assay, its ability to identify different percentages ranging from 0.5-100% of buffalo's milk was assessed. As expected lower concentrations of buffalo's milk were found associated with higher values of C_T. This might be attributed to decline the DNA concentration extracted from somatic cells (Lopez-Calleja *et al.*, 2005; Fig. 4). This primer was capable of amplify amplicon in DNA extracted from buffalo's milk so that fluorescence concentration could be investigated above background as show in Fig. 4. The RT-PCR examination using set of WB12S2 primer was able to determine buffalo's milk concentration of approximately 0.5-100% with values of C_T from 29.33 to 14.73 respectively (Fig. 4).

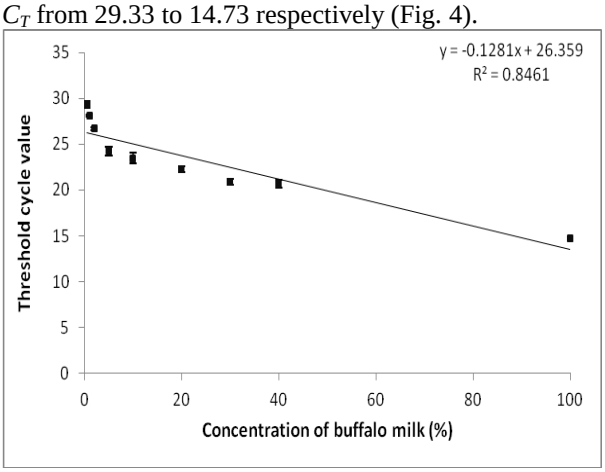

Fig. 4. Real time PCR identification of various concentration of buffalo's milk.Values of CT are averages of 3separate estimation, and error bars present ±SE.

The use of set of primer (WB12S2) for this study was suitable for detection of buffalo's milk, where the PCR reaction with set of primer produced the intended ampilicon, in addition to primer dimmers and non-specific products were not formed in PCR mixture as presented in Fig.(5). This primer was not able to amplify DNA products in DNA extracted from cow's milk so that fluorescence level might be not detected above background as shown in Fig. (4).

The melt temperature curve of the RT-PCR reaction within set of WB12S2 primer indicated non-product amplification and no primer dimer (Fig. 2). The set of WB12S2 primer in reaction of singleplex at a concentration of 300µM was used to amplify a 220 bp amplicon (Fig.6). There are not cross reactions with DNA template of cow's milk and for that reason, BDLOOP primer is suitable for the application of this procedure to detect mtDNA of buffalo's milk in other dairy products.

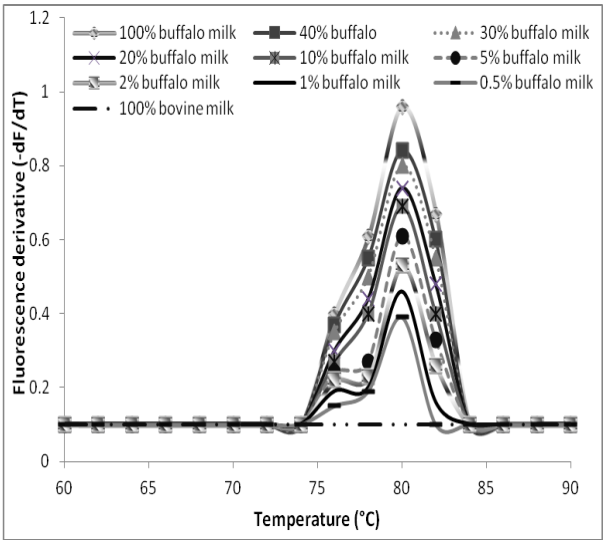

Fig. 5. Curves of DNA melting following RT-PCR analysis of different concentration of buffalo's milk.

Identification of cow's milk in raw buffalo's milk and its products were collected from retail trade in Mansoura city:

Cow's milk was detected in 67 of 100 raw buffalo's milk and its products samples collected from local market of Mansoura city. Twenty eight of 40 raw buffalo's milk samples (70%) (Table 2), nineteen of 30 yogurt samples (63.33%) (Table 3) and twenty of 30 Domiati cheese samples (67.7%) (Table 4). The present study shows that cow's milk was widely associated with raw buffalo's milk and its products in the Mansorua city. It was suggested that poor quality control practices could aid the adulteration of cow's milk in this area. The animal species identification in milk and dairy products is receiving increasing interest, because consumers are more interested in origin of food and also for health based reasons. This results are consistent with Darwish *et al* (2009) who, reported bovine milk in eight of 21 raw buffalo's milk samples, as well as and also Abdelfatah *et al.,* (2015), who, found cow's milk associated with twenty five of 50 buffalo's milk samples and seventeen of 50 samples of buffalo's butter in Sharkia Governorate, Egypt. And also other research carried out PCR techniques for detection different milk types in several dairy products (Colak, *et al.,* 2006; Zelennkova, *et al.,* 2009; Stanciuc and Rapeanu, 2010; Khanzadi *et al.,* 2013)

Table 2. Detection of cow's milk in raw buffalo's milk samples were collected from local market of Mansoura city

Number of samples	Presence of cow's milk		CT (Cow's milk)	Concentration of cow's milk*	Fat (%)	Appearance
	Declared	Detected				
1	No	Yes	25.29	13.5	6	Normal
2	No	Yes	23.98	23	5.5	Normal
3	No	Yes	23.25	28.3	5.7	Normal
4	No	Yes	26.21	6.8	6.8	Normal
5	No	Yes	25.80	9.8	6.1	Normal
6	No	Yes	24.67	18	6.3	Normal
7	No	Yes	26.02	8.2	6.6	Normal
8	No	Yes	22.61	33	5.7	Normal
9	No	Yes	21.37	42	5.8	Normal
10	No	No	-	-	6.7	Normal
11	No	Yes	26.05	8	6.8	Abnormal
12	No	Yes	22.75	32	5.6	Normal
13	No	No	-	-	5.9	Normal
14	No	Yes	22.06	37	5.6	Normal
15	No	Yes	24.95	16	6.8	Normal
16	No	No	-	-	5.8	Normal
17	No	Yes	25.22	14	6.3	Normal
18	No	Yes	24.71	17.7	6.6	Abnormal
19	No	No	-	-	6.5	Normal
20	No	Yes	21.23	43	5.8	Normal
21	No	No	-	-	7	Normal
22	No	Yes	21.92	38	5.5	Normal
23	No	Yes	20.96	45	5.6	Normal
24	No	Yes	22.88	31	6.1	Normal
25	No	No	-	-	5.5	Normal
26	No	No	-	-	5.5	Abnormal
27	No	No	-	-	5.7	Normal
28	No	Yes	21.51	41	5.8	Normal
29	No	Yes	23.85	24	6.1	Normal
30	No	No	-	-	6.2	Normal
31	No	No	-	-	5.7	Normal
32	No	Yes	22.02	37.3	5.5	Normal
33	No	Yes	22.46	34.1	5.5	Normal
34	No	No	-	-	6.1	Normal
35	No	Yes	24.19	21.5	6.4	Normal
36	No	Yes	25.55	11.6	6.3	Abnormal
37	No	Yes	21.62	40.2	5.7	Normal
38	No	No	-	-	5.8	Normal
39	No	Yes	24.77	17.3	6.2	Normal
40	No	Yes	24.85	16.7	6.3	Normal

Normal samples (%)= 30%

Adulterated samples (%)= 70%

***Determination within the relationship between C_T and concentration of cow's milk according to the following equation: C_T=(-0.1372 x Concentration of cow's milk)+27.21**

****Appearance means the degree of milk colur (white for buffalo's milk and yellowish white for cow's milk)**

Table 3. Detection of cow's milk in buffalo's yogurt samples were collected from local market of Mansoura city

Number of samples	Presence of cow's milk Declared	Detected	C_T (Cow's milk)	Concentration of cow's milk	Appearance
1	No	No	-	-	Normal
2	No	No	-	-	Normal
3	No	Yes	24.19	28.3	Abnormal
4	No	No	-	-	Normal
5	No	Yes	26.54	9.8	Normal
6	No	Yes	25.50	18	Normal
7	No	Yes	26.74	8.2	Normal
8	No	Yes	23.60	33	Normal
9	No	No	-	-	Normal
10	No	No	-	-	Normal
11	No	Yes	26.76	8	Normal
12	No	No	-	-	Normal
13	No	No	-	-	Normal
14	No	Yes	23.10	37	Normal
15	No	Yes	25.75	16	Abnormal
16	No	Yes	26.26	12	Abnormal
17	No	Yes	26.01	14	Normal
18	No	No	-	-	Normal
19	No	No	-	-	Normal
20	No	Yes	22.34	43	Normal
21	No	Yes	25.85	15.2	Normal
22	No	Yes	22.97	38	Normal
23	No	No	-	-	Normal
24	No	No	-	-	Normal
25	No	Yes	25.46	18.3	Normal
26	No	Yes	25.25	20	Normal
27	No	Yes	26.13	13	Normal
28	No	Yes	22.59	41	Normal
29	No	Yes	24.74	24	Abnormal
30	No	Yes	24.87	23	Abnormal

Normal samples (%)= 36.7%
Adulterated samples (%)= 63.3%

***Determination within the relationship between C_T and concentration of cow's milk according to the following equation: C_T=(-0.1264 x Concentration of cow's milk)+27.775**

****Appearance means the degree of milk colur (white for buffalo's yogurt and yellowish white for cow's yogurt)**

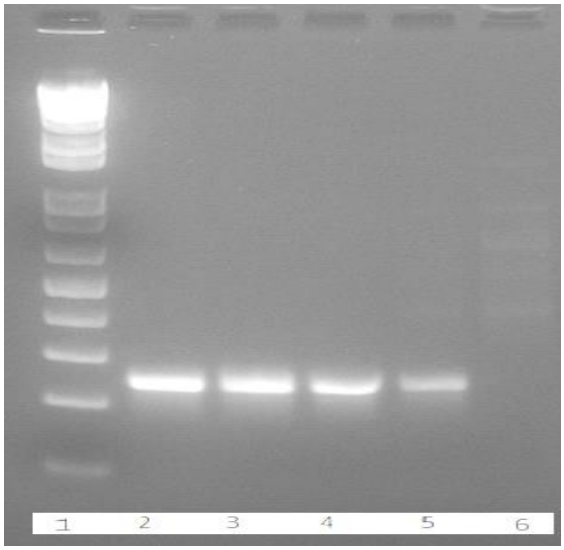

Fig. 6. Agrose gel electrophoresis (2%) of PCR amplicon was amplified with WB12S2 primer pair and DNA template of bovine and buffalo milk sample. Lane 1: 1Kb Plus DNA ladder; Lane 2: 5% buffalo's milk sample singleplex; Lane 3: 2% buffalo's milk sample; Lane 4: 1% buffalo's milk sample; Lane 5:0.5% buffalo's milk sample; Lane 6: 100% ;buffalo's milk sample..

Table 4. Detection of cow's milk in buffalo's cheese samples were collected from local market of Mansoura city

Number of samples		Presence of cow's milk Declared	Detected	C_T (Cow's milk)	Concentration of cow's milk	Appearance
1		No	Yes	27.51	10.2	Normal
2		No	Yes	27.17	13.4	Normal
3		No	Yes	23.82	45	Normal
4		No	No	-	-	Normal
5		No	No	-	-	Normal
6		No	No	-	-	Normal
7		No	Yes	23.90	44.3	Abnormal
8		No	Yes	24.78	36	Normal
9	Fresh	No	No	-	-	Normal
10	Domiati	No	No	-	-	Normal
11	cheese	No	Yes	25.62	28	Abnormal
12		No	No	-	-	Normal
13		No	Yes	22.87	54	Abnormal
14		No	Yes	24.46	39	Normal
15		No	Yes	25.73	27	Abnormal
16		No	Yes	26.68	18	Normal
17		No	Yes	24.99	34	Normal
18		No	No	-	-	Normal
19		No	No	-	-	Normal
20		No	Yes	24.04	43	Normal
21		No	Yes	26.52	19.5	Normal
22		No	Yes	24.04	43	Normal
23		No	Yes	-	-	Normal
24	Pickled	No	Yes	26.74	17.5	Normal
25		No	No	-	-	Normal
26	Domiati cheese	No	Yes	24.46	39	Abnormal
27		No	Yes	25.73	27	Normal
28		No	Yes	24.67	37	Normal
29		No	No	-	-	Normal
30		No	Yes	24.14	42	Normal

Normal samples (%)= 33.3%
Adulterated samples (%)= 66.7%

***Determination within the relationship between C_T and concentration of cow's milk according to the following equation: C_T=(-0.1059 x Concentration of cow's milk)+28.589**

****Appearance means the degree of milk colur (white for buffalo's cheese and yellowish white for cow's cheese).**

CONCLUSION

It could be deduced that the SYBER green -based real-time PCR method is potentially dependable protocol for adulteration of buffalo's milk and its products with cow's milk. Furthermore, this procedure can investigate adulterated buffalo's milk, buffalo's yogurt and buffalo's cheese mixed with bovine milk with detection limit 0.5, 1 and 2% respectively. The use of previous protocol (real-time PCR) is appropriate for routine experiment for adulteration of buffalo's milk and its products to protect consumers and manufacturer from this fraudulence which is presented as a common practice in local market in Mansoura city as cleared through this study.

REFERENCES

Wheatley, V. M. and Spink, J. (2013). Defining the public health threat of dietary supplement fraud. Comprehensive Reviews in Food Science and Food Safety, 12(6): 599-613.

Moore, J. C., Spink, J. and Lipp, M. (2012). Development and application of a database of food ingredient fraud and economically motivated adulteration from 1980 to 2010. Journal of Food Science, 77(4): 118-126.

Bonfatti, V., Giantin, M., Rostellato, R., Dacasto, M. and Carnier, P. (2013). Separation and quantification of water buffalo milk protein fractions and genetic variants by RP-HPLC. Food Chemistry, 136(2): 364-367.

EC Regulation No. 213/2001 (2001). Commission Regulation (EC) No. 213/2001 of the Commission of 9 January Annex XV, Reference method for the detection of cows' milk and caseinate in cheeses from ewes' milk, goats' milk or buffalos' milk or mixtures of ewes' and buffalos' milk. Official Journal of the European Commission, February 7, 2001, 1–99.

Mayer, H. K. (2005). Milk species identification in cheese varieties using electrophoretic, chromatographic and PCR techniques. International Dairy Journal, 15(6-9): 595-604.

Lopez-Calleja, I. M., Gonzalez, I., Fajardo, V., Hernandez, P. E., Garcia, T. and Martin, R. (2007). Application of an indirect ELISA and a PCR technique for detection of cows' milk in sheep's and goats' milk cheeses. International Dairy Journal, 17: 87–93.

Lopez-Calleja, I., Alonso, G., Fajardo, V., Rodriguez, M. A., Hernandez, P. E. and Garcia, T. (2005). PCR detection of cows' milk in water buffalo milk and mozzarella cheese. International Dairy Journal, 15: 1122–1129.

Hurley, I. P., Ireland, H. E., Coleman, R. C. and Williams, J. H. H. (2004). Application of immunological methods for the detection of species adulteration in dairy products. International Journal of Food Science and Technology, 39: 873–878.

Chen, R. K., Chang, L. W., Chung, Y. Y., Lee, M. H. and Ling, Y. C. (2004). Quantification of cow milk adulteration in goat milk using high-performance liquid chromatography with electrospray ionization mass spectrometry. Rapid Communications in Mass Spectrometry, 18: 1167–1171.

Cozzolino, R., Passalacqua, S., Salemi, S. and Garozzo, D. (2002). Identification of adulteration in water buffalo mozzarella and in ewe cheese by using whey proteins as biomarkers and matrix-assisted laser desorption/ionization mass spectrometry. Journal of Mass Spectrometry, 37, 985–991.

Lockley, A. K. and Bardsley, R. G. (2000). DNA-based methods for food authentication. Trends in Food Science and Technology, 11: 67–77.

Woolfe, M. and Primrose, S. (2004). Food forensics: Using DNA technology to combat misdescription and fraud. Trends in Biotechnology, 22: 222–226.

Mirabaud, S., Rolando, C. and Regert, M. (2007). Molecular criteria for discriminating adipose fat and milk from different species by NanoESI MS and MS/MS of their triacylglycerols: Application to archaeological remains. Analytical Chemistry, 79: 6182–6192.

Bottero, M. T., Civera, T., Anastasio, A., Turi, R. M. and Rosati, S. (2002). Identification of cow's milk in ''buffalo'' cheese by duplex polymerase chain reaction. Journal of Food Protection, 65: 362–366.

Bottero, M. T., Civera, T., Nucera, D., Rosati, S., Sacchi, P.and Turi, R. M. (2003). A multiplex polymerase chain reaction for the identification of cows', goats' and sheep's milk in dairy products. International Dairy Journal, 13: 277–282.

El-Rady, A. and Sayed, M. (2006). Identification of milk source by polymerase chain reaction-restriction fragment length polymorphism analysis. Journal of Rapid Methods and Automation in Microbiology, 14: 146–155.

Rea, S., Chikuni, K., Branciari, R., Sangamayya, R., Ranucci, D.and Avellini, P. (2001). Use of duplex polymerase chain reaction (duplex-PCR) technique to identify

bovine and water buffalo milk used in making mozzarella cheese. Journal of Dairy Research, 68: 689–698.

Lopparelli, R. M., Cardazzo, B., Balzan, S., Giaccone, V. and Novelli, E. (2007). Real-Time TaqMan Polymerase chain reaction detection and quantification of cow DNA in pure water buffalo mozzarella cheese: Method validation and its application on commercial samples. Journal of Agricultural and Food Chemistry, 55: 3429–3434.

Zhang, C. L., Fowler, M. R., Scott, N. W., Lawson, G. and Slater, A. (2007). A TaqMan real-time PCR system for the identification and quantification of bovine DNA in meats, milks and cheeses. Food Control, 18: 1149–1158.

Khanzadi, S., Jamshidi, A., Razmyar, J. and Mohsenzadeh, M. (2013). PCR-based detection of cow and goat milk in sheep milk and dairy products marketed in Mashhad city of Iran. Iranian Journal of Veterinary Medicine, (4):257–62.

Maudet, C. and Taberlet, P.(2001). Detection of cows' milk in goats' cheeses inferred from mitochondrial DNA polymorphism. Journal of Dairy Research, 68(2):229–35.

Zarei, M., Maktabi, S. and Nasiri, M. (2016). Fraud identification of cow's milk in buffalo's milk and it's products using the polymerase chain reaction. Journal of Health Science, 8(4):28-33.

استخدام نظام يعتمد علي تفاعل السلسلة المتبلمر في الوقت الحقيقي للكشف عن تواجد اللبن البقري في اللبن الجاموسي و منتجاته

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نظراً لاستخدام اللبن الجاموسي ومنتجاته بشكل كبير في جمهورية مصر العربية وبالإضافة إلي القيمة الغذائية المرتفعة للبن الجاموسي ومنتجاته، مما يجعلها عرضة للغش بإضافة أنواع أخري من الألبان وعلي وجه الخصوص اللبن البقري . استهدفت الدراسة جمع ١٠٠ عينة من اللبن الجاموسي ومنتجاته عشوائيا وتم استخدام نظام يعتمد علي تفاعل السلسلة المتبلمر في الوقت الحقيقي للكشف عن تواجد اللبن البقري في اللبن الجاموسي ومنتجاته. تم تجميع العينات من الأسواق المحلية في مدينة المنصورة واشتملت العينات علي اللبن الخام (٤٠ عينة) واللبن الزبادي (٣٠ عينة) والجبن الدمياطي الطازج (١٥ عينة) والجبن الدمياطي المخزن (١٥ عينة) وكان معدل تواجد اللبن البقري في اللبن الجاموسي الخام أعلي مقارنة باللبن الزبادي والجبن . تم تحديد مدي حساسية الطريقة المتبعة في الكشف عن اللبن البقري وذلك باستخدام لبن جاموسي ومنتجات ألبان مصنعة من اللبن الجاموسي وتحتوي علي تركيزات معلومة من اللبن البقري (٠.٥، ١، ٢، ٥، ١٠، ٢٠، ٣٠، ٤٠ %) وكان معدل حساسية الطريقة وصل إلي حد القدرة علي كشف إضافة اللبن البقري إلي اللبن الجاموس الخام بمعدل ٠.٥% في حالة اللبن الجاموسي الخام بينما انخفض معدل الكشف عن اللبن البقري في الزبادي والجبن المصنع من اللبن الجاموسي ليصل إلي ١ و ٢% علي الترتيب.