Original Research

PCR Based Assay for the Detection of Cow's Milk Adulteration in Buffalo Curd

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Abstract

This study evaluated the applicability of PCR based assay for the detection of cow's milk adulteration in buffalo curd. Two sets of species-specific universal primers, resulting 272bp (cow) and 242bp (buffalo) amplified fragments were used. The sensitivity of the PCR method was determined with both types of milk and curd samples prepared using pure buffalo milk with a series of defined incorporation of cow milk. The fat%, MSNF% and pH parameters of both prepared curd samples and market buffalo curd samples were compared with SLSI buffalo curd values. Six different curd brands labelled as "buffalo curd" in triplicate were used to determine the applicability. Undeclared cow's milk was detected in four curd brands, while two brands were confirmed to be pure buffalo curd. Results revealed that mixing of cow's milk significantly influenced the milk fat % of the samples. The PCR assay used here, is high sensitive and applicable technique to detect the cow's milk adulteration in buffalo curd.

Key words: Buffalo Curd, Cow's Milk, Food Adulteration, PCR

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Introduction

Curd originally made from buffalo is popular in Sri Lanka as typical traditional dairy product which is now made using less expensive and nutritious cow's milk. Therefore, species identification is an important issue in such a product to avoid unfair competition and assure consumers of accurate labeling as food safety requirements. Currently, several techniques and methods are used for the species identification of milk and milk products. However, those methods are not reliable to distinguish milk from closely related species and also result a lower sensitivity for the thermally treated foodstuffs

(Bottero *et al*., 2002; Darwish *et al*., 2009 and Maskova *et al*., 2006). Hence, there is a need of developing an accurate method to detect cow's milk adulteration in buffalo curd. Molecular techniques have been recently applied for species identification and differentiation. Those methods are more reliable, sensitive and fast (Bottero *et al*., 2002 and Darwish *et al*., 2009). Among molecular techniques, PCR (Polymerase Chain Reaction) provides an alternative path to detect the adulterants of foreign milk to original milk by analyzing the mitochondrial DNA which is most widely used for the identification of species of origin in milk (Bottero *et al*., 2002; Cheng *et al*., 2006; Darwish *et al*., 2009; Maskova *et al*., 2006; Khanzadi *et al.*, 2013 and Osman *et al.*, 2014).

Physicochemical and microbiological aspects are also important factors that reflect the quality and acceptability of the curd. The specifications for compositions and microbiological tolerance limits of curd are confirmed by the Sri Lanka Standard Institution (SLSI), the apex body for national standardization of food products. Therefore, studies in the field of quality assessment of curd marketed by local producers are required to create the confidence among consumers. As per the SLSI standards, buffalo curd should comply with the requirements specified in Table 1.

Table 1: Acceptable SLSI values for buffalo curd

Characteristics	Buffalo Curd
Milk fat, percent by mass, min.	7.5
Milk solid non-fat, percent by mass, min.	8.5
pH, max.	4.5
E. coli	Absent in 1 g

Source: (*Sri Lanka Standards, 1988).*

Therefore, the current study was designed to evaluate the PCR assay to detect the adulteration of cow's milk in buffalo curd and also to detect the effect of mixing of milk on Fat %, pH and MSNF (Milk Solid Non Fat) % of buffalo curd and to determine the physicochemical (Fat %, pH and MSNF%) and microbiological (*E. coli*) properties of buffalo curd available at supermarkets and small scale markets at municipal council area of Kandy, Sri Lanka.

Material and Method

Sampling

Pure cow and buffalo milk samples were collected from Experimental Farm of Veterinary Research Institute, Gannoruwa, Sri Lanka to evaluate the sensitivity of the PCR assay and to determine the effect of milk mixing on buffalo curd. Lactoscan (Milktronic, Bulgaria) readings were taken to determine the quality of collected milk samples. Model milk and curd samples were prepared by addition of different percentages of cow milk to the buffalo milk. According to the percentages of 100, 50, 25, 10, 5, 1, 0 (v/v),

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samples of cow's milk were added in the buffalo milk to prepare a final volume of 20 ml. Cow and buffalo milk were mixed according to the same percentages as final volume of 400ml to prepare the curd samples.

Sample Collection

Buffalo curd samples from six different brands with three different batches from each brand (6 × 3 = 18) available at super markets and small scale markets in Kandy municipal council area were randomly collected to evaluate the applicability of PCR assay. Physicochemical properties (pH, fat %, MSNF %) and microbiological properties (*E. coli*) were analyzed to determine the quality of market curd brands.

Extraction of DNA from Milk and Curd Samples

DNA was extracted from prepared milk, curd series and buffalo curd samples collected from the market using PureLink Genomic DNA Mini Kit (Invitrogen, USA) protocol as indicated by the manufacturer. DNA was quantified by using Nano-drop. DNA extraction of milk and curd samples was done after prior separation of the cells from the food matrix. Therefore, prior separation was done according to the method described by Monnet and Matijasic (2012) with some modifications. For separation of cells from milk samples, 2 ml of each milk sample was mixed with 10 ml of 1% peptone water then vortexed the samples. Two milliliters of each milk sample was put in to sterile eppendorf and then the samples were centrifuged for 10 minutes at 10000 rpm. Finally, the liquid portion was discarded and DNA extraction procedure was continued. For separation of cells from prepared curd samples and market curd samples, 2 g of each curd sample was mixed with 2% sodium citrate solution. Two grams of each curd sample was taken after removing the fat layer and mixed the samples well. Then, samples were homogenized using paddle blender (Minimix wcc). Samples were put in to sterile centrifuge tubes and centrifuged for 10 minutes at 6000 rpm. Then, the liquid portion was discarded. Ten milliliters of normal saline was added to each centrifuge tube for wash the sample and centrifuged for 10 minutes at 6000 rpm. Finally 1 ml of PBS buffer was added to each centrifuge tube and vortexed the samples after discarding the liquid portion. After then the DNA extraction procedure was continued.

200 µL from each sample was put in to sterile eppendorf. Then, 20 µL of proteinase-K and 20 µL of RNAse were added. The samples were vortexed well and kept 2 minutes at room temperature. 200 µL of genomic lysis buffer was added to each eppendorf tube and then vortxed and incubated for 10 minutes at 55ºC. Later, 200 µL ethanol was added and vortexed the samples for 5 seconds. Solution was transferred to spin column with collection tube from the each eppendorf tube and centrifuged for 1 minute at 10000 rpm and kept for 1 minute at room temperature. After that, 500 µL from wash buffer-1 was added to each sample and centrifuged for 1minute at 10000 rpm and then discarded the collection tube and set the spin

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column to another collection tube. Then, 500 µL from Wash buffer-2 was added to samples and centrifuged for 3 minutes at 10000 rpm and then discarded the collection tube and set the spin column to sterile eppendorf tube. 100 µL of genomic elution buffer was added to each eppendorf and incubated for 1 minute at room temperature and then centrifuged for 1 minute at 10000 rpm. Finally, spin column was discarded and stored in the eppendorf with DNA at -20ºC until further use for PCR.

Amplification Procedure of PCR

Two universal oligonucleotide primers were used for PCR amplification as listed in Table 2.

Table 2: PCR oligonucleotide primers targeting mitochondrial DNA

Primer Sequence	Product Size
Cattle F- 5' GACCTCCCAGCTCCATCAAACATCTCATCTTGATGAAA 3'	272 bp
R-5' CTAGAAAAGTGTAAGACCCGTAATATAAG 3'	
Buffalo F- 5' TAGGCATCTGCCTAATTCTG3'	242 bp
R- 5' ACTCCGATGTTTCATGTTTCT 3'.	

F= forward, R = reverse, bp= base pair

According to the PCR amplification method described by Rajapaksha *et al*. (2003) with some modification, PCR assay was conducted to amplify the target DNA in milk and curd samples. Three micro liters of DNA were added to 22 µL of the PCR mixture, containing 16.6 µL Sigma H_2O, 3 µL PCR buffer, 0.6 µL dNTP, 1 µL from each primer (forward and reverse) and 0.3 µL of Taq DNA polymerase. The amplification cycles were carried out in the PCR system (ProFlex, USA). Reaction conditions were optimized at 95ºC for 3 minutes as initial denaturation, followed by 95ºC for 1 minute as denaturation, 35 cycles of 55ºC for 1 minute as annealing stage for samples with cattle primer and 35 cycles of 58ºC for 1 minute as annealing stage for samples with buffalo primer, 72ºC for 1 minute as extension and finally 72ºC for 7 minutes as final extension. For each PCR amplification, both pure cow and buffalo milk samples were used as positive controller and distilled water was used as negative controller.

Agarose Gel Electrophoresis

The PCR products (8 µL) were resolved in 1.5% agarose gel containing 150 ml TBE buffer at 100 volts for 75 minutes and stained with 10 µL of ethidium bromide. Visualization was achieved through the UV transilluminator (Trance illuminator, Vilber lourmat, France) and the photographic documentation was accomplished using SONY digital camera (Digital still camera, DSC.W690, China). To ensure the amplification, PCR products were visualized with 100bp DNA ladder as a marker. The presence of 272bp DNA fragment indicated the presence of cow's milk and if not, presence of 242bp DNA fragments indicated the presence of buffalo milk.

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Analysis of Market Curd Samples

Chemical (pH, fat % and MSNF %) and microbiological analysis (*E. coli*) were done according to standard methods in AOAC, 2005.

Results and Discussion

Specificity of the Primers

As shown in Fig. 1, the oligonucleotide primers targeting mitochondrial DNA of cow and buffalo were specifically amplified producing 272bp and 242bp amplicons, respectively. No amplicons were found both in negative controls (NC1 and NC2) and lane 3 and 4. This confirmed and ensured that these primers are species specific.

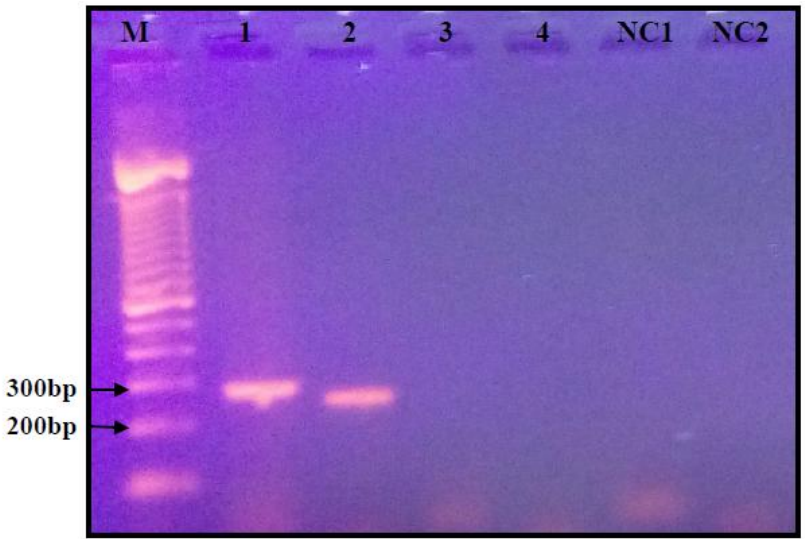

Fig. 1: The species specificity of the cow and buffalo primers

M:100bp molecular ladder (Invitrogen, USA), **Lane 1:** Pure cow template at 272bp amplicons. **Lane 2:** Pure buffalo template at 242bp amplicons. **Lane 3:** Pure cow template with buffalo primers. **Lane 4:** Pure buffalo template with cattle primers. **NC1** (negative control): Distilled water with cattle primers. **NC2:** (negative control): Distilled water with buffalo primers.

Sensitivity of the PCR Assay

The sensitivity of the PCR assay is shown in Fig. 2 and 3. Both prepared milk and curd samples were used to determine the specificity of the PCR assay. As per the results of the PCR assay, the minimum detection limit was 1% in the present study. The observed sensitivity limit was within the reported values of other experiments. Most research studies reported that the PCR technique used for the estimation of undeclared mixing of cow's milk detection limit was within the range of 0.1% - 5% (Cheng *et al.*, 2006; Darwish *et al.*, 2009; Maskova *et al.*, 2006; Zarei *et al.*, 2016a and Zarei *et al.*, 2016b). Cheng *et al.* (2006) reported that sensitivity of the PCR method used in the study was 0.1% for the detection of cow's

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milk adulteration in goats' milk. Darwish *et al.* (2009) also showed the detection threshold was 0.5% for the study of 0.5% adulteration of cow's milk in buffalo milk.

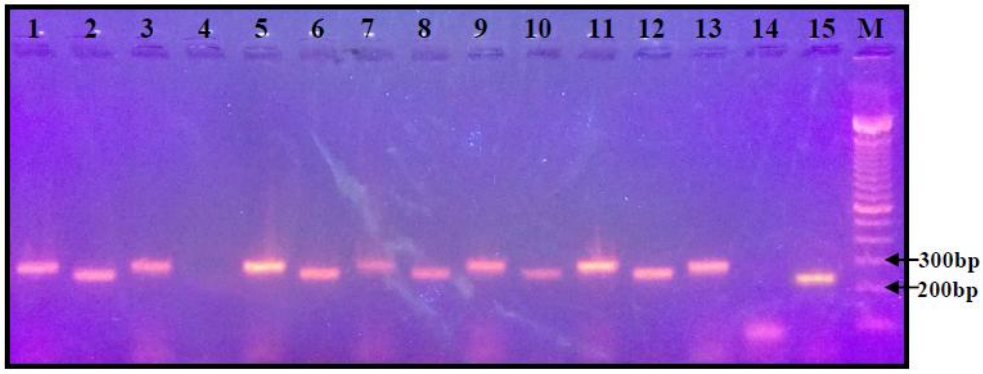

Fig. 2: Sensitivity of the PCR assay with prepared milk samples

Lanes 1 & 2: Positive cow and buffalo control. **Lane 3:** Pure cow milk template at 272bp amplicons. **Lane 4:** Pure cow milk template with buffalo primers. **Lane 14:** 0% cow milk template (pure buffalo milk) with cattle primers. **Lane 15:** 0% cow milk template (pure buffalo milk) at 242bp amplicons. **Lanes 5, 7, 9, 11, 13:** Milk templates with cow milk incorporation of 50%, 25%, 10%, 5% & 1% respectively at 272bp amplicons with cattle primers. **Lanes 6, 8, 10, 12:** Milk templates with cow milk incorporation of 50%, 25% 10%, & 5% respectively at 242 amplicons with buffalo primers. **M:** 100bp molecular ladder (Invitrogen, USA).

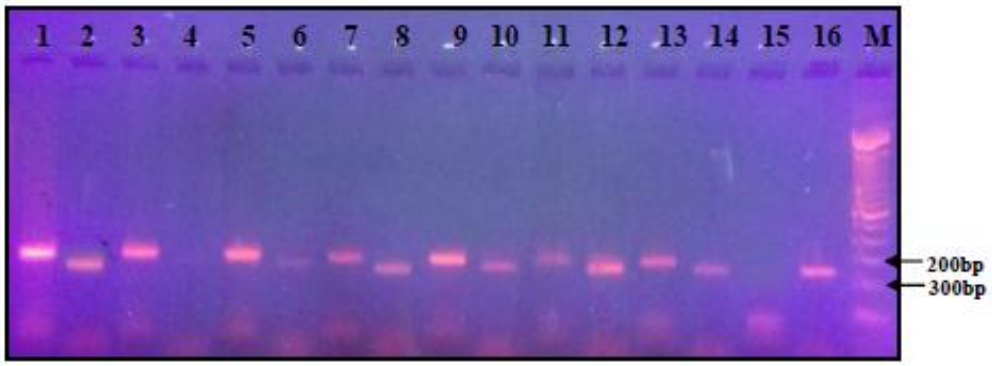

Fig.3: Sensitivity of PCR assay with prepared curd samples

Lanes 1 & 2: Positive cow and positive buffalo controllers. **Lane 3:** Pure cow curd template at 272bp amplicons. **Lane 4:** Pure cow curd template with buffalo primers. **Lanes 5, 7, 9, 11, 13:** templates with cow milk incorporation of 50%, 25%, 10%, 5% & 1% respectively at 272bp amplicons with cattle primers. **Lanes 6, 8, 10, 12, 14:** Curd templates with cow milk incorporation of 50%, 25%, 10%, 5% & 1% respectively at 242bp amplicons with buffalo primers. **Lane 15:** Pure buffalo curd template (0% cow milk) with cattle primers. **Lane 16:** Pure buffalo curd template (0% cow milk) at 242bp amplicons. **M:** 100bp molecular ladder (Invitrogen, USA).

For detecting the adulteration of foreign milk in dairy products, 1% sensitivity was detected for cow's milk adulteration in goat and sheep cheese (Maskova *et al.*, 2006). Zarei *et al.* (2016a) also used the multiplex PCR method and detected the 2% sensitivity for cow's or goats' milk in sheep yoghurt and 4%

detection limit for sheep cheese adulterated with cow's or goats' milk. Zarei *et al.* (2016b) also reported 1%, 2% and 4% detection limit of cow's milk in buffalo milk, buffalo yogurt and buffalo cheese respectively. The non-authentic dairy products are produced by mixing less costly milk with expensive milk to gain financial benefits. Five percent of detection limit was considered as sufficient for detection of undeclared milk components because the adulteration of milk less than 5% did not have any economic effect (Maskova *et al.*, 2006; Darwish *et al.*, 2009 and Zarei *et al.*, 2016a). Therefore, the sensitivity of the PCR assay (1%) used in the present study was sufficient for the determination of adulteration of cow's milk in buffalo curd.

Applicability of the PCR Assay

The Fig. 4 and Table 3 shows the grading of six buffalo curd brands available in the market based on the PCR assay. The different buffalo curd brands available in the market were used to evaluate the applicability of the PCR assay. Results revealed that, two market brands (D and E) were confirmed to be with pure buffalo curd while undeclared cow's milk was detected in other four brands. The positive and negative controllers were used to confirm the no cross contamination taking place or no any amplification was resulted during the process.

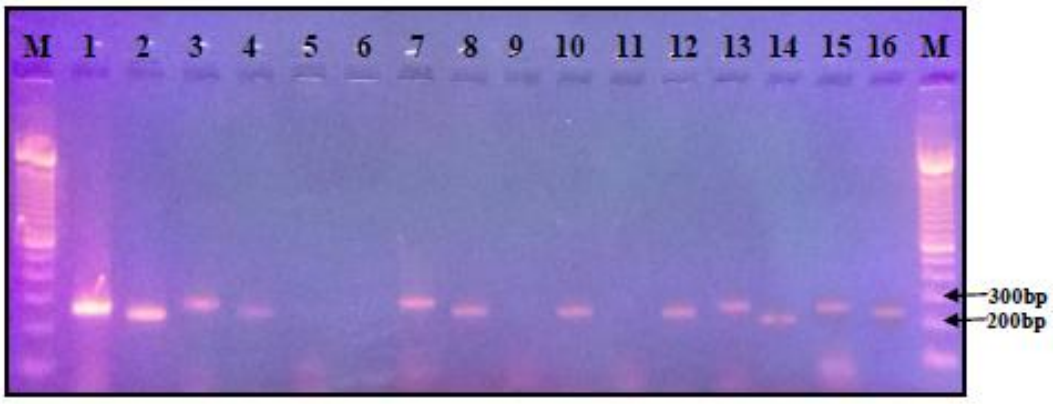

Fig. 4: Determination of the applicability of PCR reaction with market buffalo curd samples.

M: 100bp molecular ladder (Invitrogen, USA). **Lanes 1 & 2:** Positive cow and buffalo control. **Lanes 3 & 4:** Market buffalo curd brand A with cattle and buffalo primers respectively producing 272bp amplicons and 242bp amplicons. **Lanes 7 & 8:** Market buffalo curd brand C with cattle and buffalo primers respectively producing 272bp amplicons and 242bp amplicons. **Lanes 9 & 10:** Market buffalo curd brand D with cattle and buffalo primers respectively, producing no bands and 242bp amplicons. **Lanes 11 & 12:** Market buffalo curd brand E with cattle and buffalo primers respectively, producing no bands and 242bp amplicons. **Lanes 13 & 14:** Market buffalo curd brand F with cattle and buffalo primers respectively, producing 272bp amplicons and 242bp amplicons. **Lanes 15 & 16:** Market buffalo curd brand B with cattle and buffalo primers respectively, producing 272bp amplicons and 242bp amplicons. **Lanes 5 & 6:** (NC) Distilled water with cattle and buffalo primers. **M:** 100bp molecular ladder (Invitrogen, USA).

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According to the Fig. 4, positive cow and buffalo control resulted the amplified products giving 272bp and 242bp, respectively. Negative control did not show any amplified product for both primer pairs. This confirmed that there was no any cross contamination or amplification failure took place in the reaction.

Table 3: Grading of buffalo curd available at the market based on the PCR assay

Buffalo Curd	Presence of Cow Milk	
	Detected	**Declared**
Brand A	No	Yes
Brand B	No	Yes
Brand C	No	Yes
Brand D	No	No
Brand E	No	No
Brand F	No	Yes

Effect of Mixing of Both Buffalo and Cow's Milk on Fat%, pH and Solid Non Fat % of Prepared Curd Samples

The Table 4 indicates the milk composition of cow and buffalo milk that were used to prepare the model curd samples. According to values indicated in Table 4, both milk types were satisfied with the standards required for pure cow and buffalo milk as reported by Vishweshwar *et al.* (2005). This revealed that all the prepared curd was developed using good quality cow and buffalo milk.

Table 4: Milk composition of pure cow and buffalo milk that used to prepare curd samples

Pure milk	Fat (%)	MSNF (%)	Protein (%)	Acidity(%)	pH
Cow	4.46	8.27	3.28	0.18	6.5
Buffalo	7.06	9.43	3.5	0.13	6.4

Effects of Mixing of Cow's Milk on Fat % in Prepared Curd

The curd prepared using pure buffalo milk with defined incorporation of cow's milk was used to determine the effect of mixing of cow's milk on buffalo curd and the results are shown in Fig. 5. With the increasing of cows' milk percentage, milk fat % of curd was reduced and it was deviated from SLSI requirement of fat % by mass for buffalo curd. Normally, milk fat % of cow milk is lower than buffalo milk (Vishweshwar *et al*., 2005). The reduction of fat % may be due to increasing level of cow's milk in buffalo curd. The fat % of sample 1 (pure cow milk), sample 2 (50% cow milk) and sample 3 (25% cow milk) were below the level of SLSI requirement for buffalo curd fat %, while other samples with 10%, 5%, 1% and 0% cow's milk percentages were up to the level of SLSI standards (minimum 7.5%).

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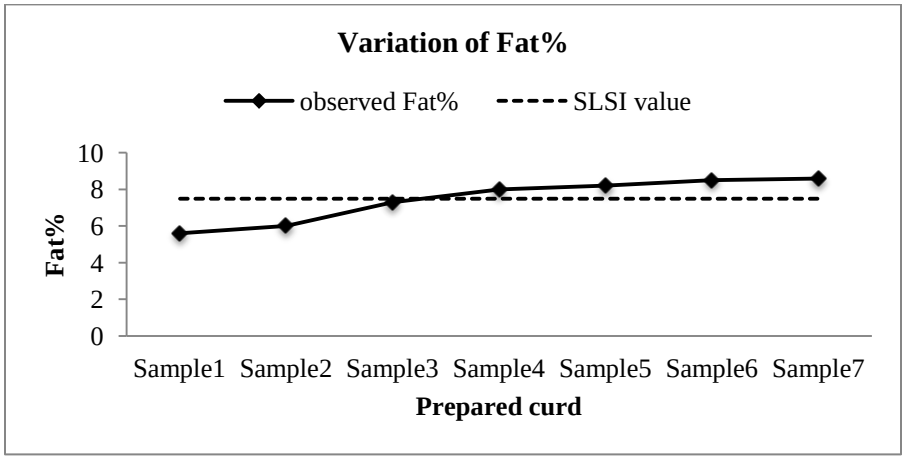

Fig. 5: The changes of Fat % in the prepared curd (Sample 1 = 100% cow milk, Sample 2 = 50% cow milk, Sample 3 = 25% cow milk, Sample 4 = 10% cow milk, Sample 5 = 5% cow milk, Sample 6 = 1% cow milk, Sample 7 = 0% cow milk)

Effect of Mixing of Cow's Milk on MSNF % in Prepared Curd

The changes of MSNF % of prepared curd samples with MSNF % of SLSI requirement for buffalo curd are shown in Fig. 6. The MSNF % of all prepared curd samples were up to the level of SLSI standards for buffalo curd (minimum 8.5%). The highest MSNF % was reported by sample 1 (20.98%) which was a pure cow curd. The lowest MSNF % was given by sample 7 (17.58%) which was prepared by pure buffalo milk.

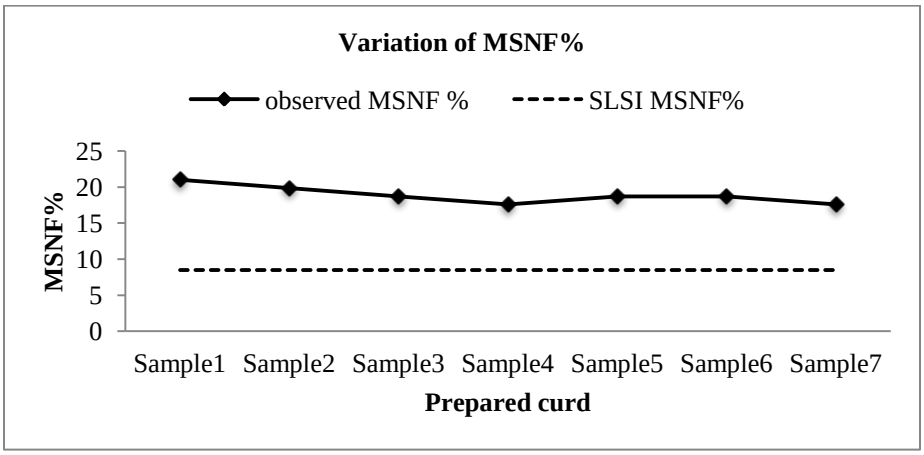

Fig. 6: The change of MSNF% in prepared curd (Sample 1 = 100% cow milk, Sample 2 = 50% cow milk, Sample 3 = 25% cow milk, Sample 4 = 10% cow milk, Sample 5 = 5% cow milk, Sample 6 = 1% cow milk, Sample 7 = 0% cow milk

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Effect of Mixing of Cow's Milk on pH in Prepared Curd

The change of pH of prepared curd samples with pH of SLSI requirement for buffalo curd is shown in Fig. 7. The pH of all prepared curd samples was up to the level of SLSI standards for buffalo curd (maximum 4.5). The highest pH was given by sample 5 (4.36), and it was a mixed curd sample with incorporation of 5% cow's milk in to buffalo curd. The lowest pH was observed in the Sample No. 2 (4.16), and it was a mixed curd sample with incorporation of 50% cow's milk in to buffalo curd.

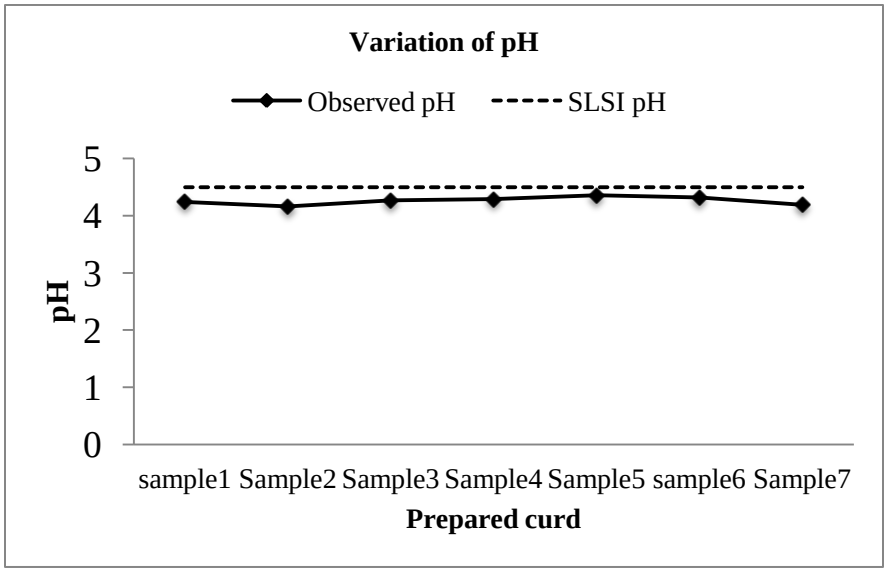

Fig. 7: The change of pH in prepared curd **(**Sample 1 = 100% cow milk, Sample 2 = 50% cow milk, Sample 3 = 25% cow milk, Sample 4 = 10% cow milk, Sample 5 = 5% cow milk, Sample 6 = 1% cow milk, Sample 7 = 0% cow milk)

Determination of the Quality of Buffalo Curd Brands Available in the Market the Change of Milk Fat % of Buffalo Curd Brands Available in the Market

Fig. 8 shows the change of fat % of market buffalo curd with SLSI standards. The mean fat % of buffalo curd brands A, B, C, D, E and F were 6.65, 8.40, 5.47, 7.67, 5.07 and 5.40, respectively. Fat % of buffalo curd brand A, C, E and F were less than the acceptable fat % level (minimum 7.5%). According to PCR results, curd brand E was confirmed to be with pure buffalo curd, but the fat % was below the SLSI standards. The reason for lower fat % may be due to adulteration of milk with water, partial skimming of milk before curd preparation or usage of low quality milk which is low in fat (Darwish *et al.*, 2009 and Weerasekara *et al.*, 2010) but not with adulteration by cow's milk. Brand A, C and F were mixed curd types as confirmed by PCR assay. Due to adulteration of cow's milk, the fat % was below the accepted level. Only brand B and D were complied with the SLSI requirements for fat %. Brand B showed an adequate fat % that met the requirement of SLSI standard for buffalo curd even though it was detected as

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a mixed sample according to the PCR results. The reason may be the use of quality raw cow and buffalo milk with higher fat %.

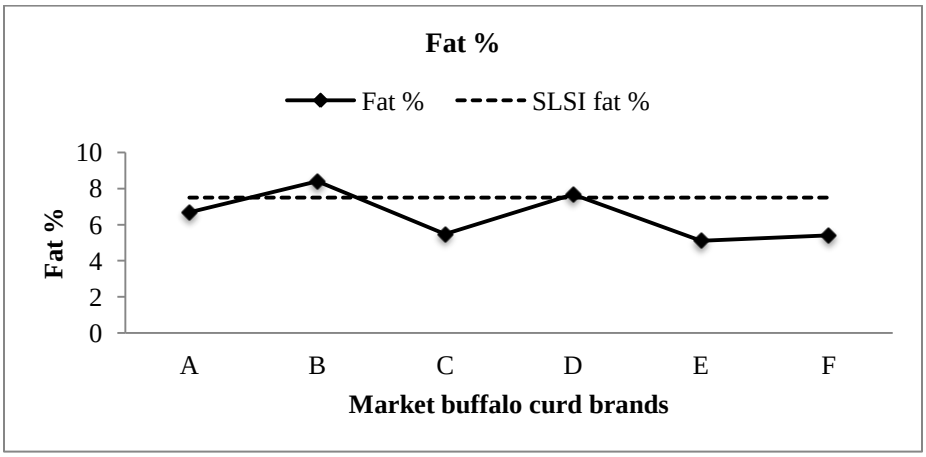

Fig. 8: The changes of Fat % of market buffalo curd with SLSI standards

MSNF % of Buffalo Curd Brands

As shown in Fig. 9, the mean MSNF % of market buffalo curd brands A, B, C, D, E and F were 11.83, 9.43, 10.62, 10.92, 11.02 and 13.62 respectively. All the values are higher than the SLSI standards for MSNF % therefore tested brands were up to the level of SLSI requirement (minimum 8.5%). The highest MSNF % was given by brand F (13.62%) and lowest MSNF % was given by brand B (9.43%). Both brands were recognized as mixed brands according to result of the PCR. The brand D and E that pure buffalo curd according to the PCR result, given MSNF % as 10.92% and 11.02% respectively.

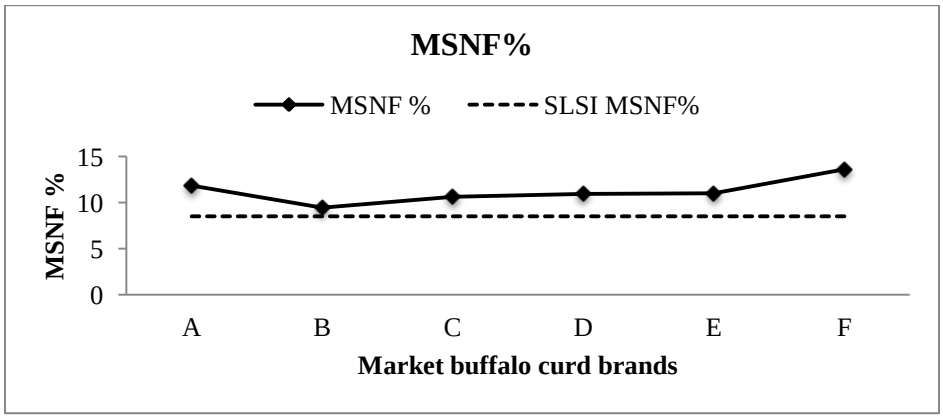

Fig. 9: The changes of MSNF % of market buffalo curd with SLSI standards

pH of Buffalo Curd Brands

Fig. 10 shows the changes of pH of market buffalo curd with SLSI standards. The mean pH of brand A, B, C, D, E and F was 3.67, 3.71, 3.69, 3.69, 3.83 and 3.54 respectively. This confirmed that all market curd brands tested were up to the SLSI pH level for buffalo curd. The study carried out by Weerasekara *et*

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al. (2010) showed the similar results close to the present study. They showed that only fat % was below the level of SLSI standard for curd and both MSNF % and pH were up to the level of SLSI standards.

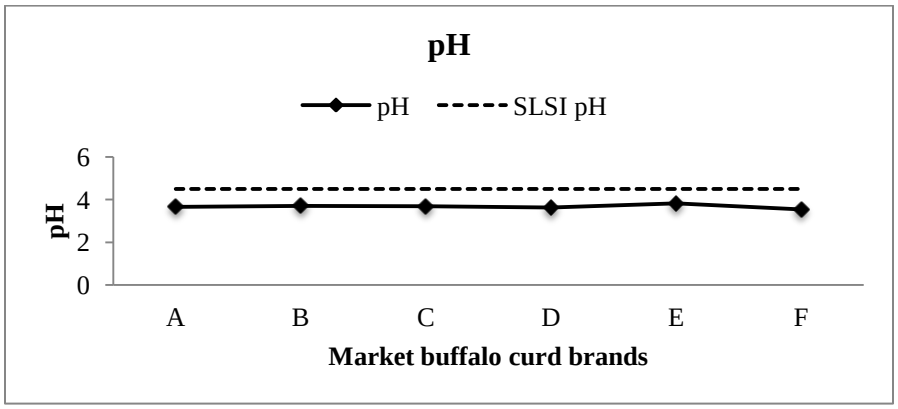

Fig. 10: The changes of pH of market buffalo curd with SLSI standards

E. coli of Buffalo Curd Brands

According to the results shown in the Table 5, there was no any detection of *E. coli* in market buffalo curd brands. All the six market buffalo curd brands were up to SLSI level of *E. coli* limit for buffalo curd (absent in 1 g). This confirmed that hygienic quality of curd brands available at the market was in good condition and acceptable for consumption.

Table 5: The results of *E. coli* analysis of buffalo curd with SLSI standards

Buffalo Curd Brand	SLSI limit/g	Observed *E. coli*/g
Brand A	absent in 1 g	0
Brand B	absent in 1 g	0
Brand C	absent in 1 g	0
Brand D	absent in 1 g	0
Brand E	absent in 1g	0
Brand F	absent in 1g	0

Conclusion

The PCR assay is sensitive enough to detect the cow's milk in buffalo curd with a detection limit of 1% and thus, it is an applicable technique for fraud identification of undeclared cow's milk in buffalo curd. Two third of the studied buffalo curd brands available in the market are adulterated with cow's milk. The mixing of milk has an effect on fat % of the curd, when cow's milk adulteration in to buffalo curd. MSNF %, pH and *E. coli* of the buffalo curd available at the market comply with SLSI standards for buffalo curd, except fat %.

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References

1. AOAC, (2005). Official Methods of Analysis. Association of Official Analytical Chemists, Washington, DC, USA.
2. 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(2): 362-366.
3. Cheng, Y.H., Chen, S. and Weng, C. (2006). Investigation of Goats' Milk Adulteration with Cows' Milk by PCR. *Asian-Australian Journal of Animal Sciences*. 19(10): 1503-1507.
4. Darwish, S.F., Hanaa, A.A. and Amin, A.S. (2009). Evaluation of PCR Assay for Detection of Cow's Milk in Water Buffalo's Milk. *World Applied Sciences Journal*. *7*(4): 461-467.
5. 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*. 7(4):257-262.
6. Maskova, E. and Paulickova, I. (2006). PCR-based Detection of Cow's Milk in Goat and Sheep Cheeses Marketed in the Czech Republic. *Czech Journal of Food Sciences*. 24(3): 127-132.
7. Monnet, C. and Matijasic, B.B. (2012). Application of PCR-Based Methods to Dairy Products and to Non-Dairy Probiotic Products. In: Polymerase Chain Reaction (Ed. Patricia Hernandez-Rodriguez) InTech Publisher. pp. 11-50.
8. Osman, A.A., Mahmoud, A.M. and Soliman, A.S. (2014). Rapid Detection of Bovine-Specific Nucleic Acid Sequences in Cow Milk Using Polymerase Chain Reaction. *International Journal of Nutrition and Food Sciences*. 3(3):141-144.
9. Rajapaksha, W.R.A.K.J.S., Thilakaratne, I.D.S.I.P. Chandrasiri, A.D.N. and Niroshan, T.D. (2003). Development of PCR Assay for Identification of Buffalo Meat. *Asian-Australian Journal of Animal Sciences*. 16(7):1046-1048.
10. Sri Lanka Standard Institution (SLSI) (1988). Specification for Fermented Milk Products - Curd, Sri Lanka Standard Institution, Sri Lanka. 824: part 1.
11. Vishweshwar, S.K., Krishnaiah, N. and Sunder, P.R. (2005). Quality Control of Milk and Processing, Intermediate Vocational Course, 2nd Year, State Institution of Vocational Education, Government of Andhra Pradesh, India. 5 pp.
12. Weerasekara, W.M.S.I.M., Karunarathne, G.M.C.R. and Prathapasinghe, G.A. (2010). Assessment of Quality Parameters in Curd and Yoghurt of Small Scale Processors in North Central Province. *Wayamba Journal of Animal Science*. 26-29.
13. Zarei, M., Maktabi, S. Yousefv, A. and Tajbakhsh, S. (2016a). Fraud Identification of Undeclared Milk Species in Composition of Sheep Yogurt and Cheese Using Multiplex PCR Assay. *Journal of Food Quality and Hazards Control*. 3: 15-19.
14. Zarei, M., Maktabi, S. and Nasiri, M. (2016b). Fraud Identification of Cow's Milk in Buffalo's Milk and it's Products Using the Polymerase Chain Reaction. *Jundishapur Journal of Health Sciences*. 8(4): 1-5.

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