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A STUDY ON ADULTERANTS OF MILK OF SELECTED COASTAL DISTRICTS OF ANDHRA PRADESH

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ABSTRACT

Milk is a complete or whole food and biologically effective nutrient medium. Milk is perishable food that supports growth of wide variety of microorganisms that cause the spoilage of milk. Milk acts as transport vehicle for transmission of contaminants and adulterants that affect the quality of milk in terms of adulterants such as urea, starch, neutralizers etc. These agents enter milk through contaminated cattle feedstuff or can be introduced into milk intentionally. Therefore, the present research study aimed to study adulterants from milk supply chain of 5 coastal districts of Andhra Pradesh, India, including Visakhapatnam, Vizianagaram, Srikakulam, East Godavari and West Godavari districts. The milk samples were collected randomly and directly from milk producers (farmers), bulk chilling centers, vendors and pasteurized milk samples from various dairy outlets. The results were expressed in mean ± standard deviation. The results were analyzed by ANOVA. The milk samples analysed for adulterants among various districts have shown the significant p-value for Urea, Starch, Detergent, NaCl, Sugar, Skimmed milk powder, Glucose and Water. Significant p - values of analysis of various milk samples across five districts indicate that milk quality is compromised and affected. The values obtained confirm the same and the study concluded as milk samples of poor quality which should be carefully monitored to minimize the health risks.

Introduction

An adulterant is a chemical substance mixed with other substances to make them of poor quality. These substances should not be contained in foods, beverages and fuels for legal or other reasons. The addition of adulterants is called adulteration. The word is appropriate only when the additions are unwanted by the recipient. Otherwise the expression would be food additive. Adulterants used as illicit drugs are known as cutting agents. The addition of toxic adulterants to food and other products that are consumed by humans results in poisoning (Singuluri and Sukumaran, 2014).

Milk is adulterated and its quality is affected and as well as other dairy products. These adulterants are low value ingredients that reduce the quality of milk and milk products. A national survey conducted in India has revealed that 70% of milk sold and consumed is adulterated by low value contaminants such as detergent, skim milk powder, water, urea, neutralizers, starch, sucrose, glucose, formaldehyde, ammonium sulphate and hydrogen peroxide etc. The Food Safety Standards Authority of India (FSSAI) conducted a national survey during the year 2011 and found that the most common adulterants detected in milk are water and detergent.

In the year 2012 68% of milk samples were found to be contaminants of adulterants of which 31% of milk samples were from rural areas. Among these adulterated milk samples 16.7% samples were packed or branded milk samples and the rest of the samples were sold loose from dairies. In urban areas 68.9% of milk samples were detected with adulterants like water, detergent, urea and skim milk powder. About 88% of milk in Uttarakhand was detected as adulterated. In Gujarat, 89% of milk was reported to be adulterated with glucose, sodium chloride, sucrose, ammonium sulphate at 30%, 46%, 50%, 96% and 100% respectively (Jivraj Makadiya and Astha Pandey, 2015). The milk samples collected from 7 different regions of Amaravathi were found to be good in quality though some adulterants were detected in one or two milk samples, they were found to be more than the standard values prescribed by Food Quality Authority of India (Hande Ashwini, 2015). The presence of high or low amounts of adulterants in milk is public health concern and fixes the poor quality of the milk (Ananya Debnath *et al*., 2015). In Hyderabad a study revealed that urea, neutralizers and salts were found in 60%, 26% and 82% of milk samples respectively along with formalin, detergents and hydrogen peroxide at 32%, 44% and 32% of milk samples respectively (Singaluri and Sukumaran, 2014).

Water is an adulterant added to the milk to increase the volume but it also decreases the nutritive value of the milk. Detergents are agents added to the milk to emulsify and dissolve oil in the water and give a frothy solution and characteristic milky white colour. Urea is mixed with the milk to increase the solid not fat (SNF). The urea contributes to the white color of the milk and increases its consistency. Starch is added to the milk

to increase its density and to prevent water detection from milk. Hydrogen peroxide is added to milk to preserve it for long time with freshness. Carbonates and bicarbonates added in the milk cause disruption in hormone function and reproduction. Sucrose is added to the milk to increase density. Sodium chloride, ammonium sulphate and neutralizers like caustic soda, sodium bicarbonate and carbonate are added to milk to increase the density and to neutralize the acidity of milk (Singuluri and Sukumaran, 2014). Other adulterants like benzoic acid, boric acid, salicylic acid, vanaspathi, formaldehyde and skimmed milk powder are frequently added to the milk for various purposes and to make consumer to believe that the milk is of good quality (Mohit Kamthania *et al*., 2014). A study in Punjab concluded that milk adulteration was mainly caused due to a gap between demand and supply and unhygienic practices of milking, handling, transport and post pasteurization contamination (Ritu Tangri and Anshu, 2014).

Adulteration of milk may be intentional or unintentional. Sometimes adulteration may be incidental contamination caused due to ignorance, negligence or poor practices. The adulteration of milk is otherwise known as economic adulteration that decreases the quality of milk which may be reflected on milk based products. From the literature it is evident that no studies have been reported from south of India and hence present study was undertaken to test the adulteration of milk both in the selected commercial and vendors milk supplies.

Methodology

Detection of adulterants in milk samples:

A standard milk adulteration kit manufactured by HIMEDIA laboratories, Mumbai, India was used. The milk samples were tested for the following adulterants – urea, starch, neutralizers ($NaHCO_3$, Na_2CO_3, $Na(OH)_2$, $Ca(OH)_2$, detergents, sodium chloride and skim milk powder (smp) etc. A standard milk adulteration kit manufactured by HIMEDIA laboratories, Mumbai, India was used. The milk samples were tested for the following adulterants – urea, starch, neutralizers ($NaHCO_3$, Na_2CO_3, $Na(OH)_2$, $Ca(OH)_2$), detergents, sodium chloride, skim milk powder, sugar, glucose and water (Awan *et.al.*, 2014).

Urea detection test

To 2 ml of milk in a sample test tube 2 ml of urea reagent -1 was added and mixed. Appearance of distinct yellow color indicated the presence of urea in milk.

Starch detection test

To 3 ml of milk sample in test tube little amount of water was added and boiled for a few minutes and cooled. To this milk sample 3 drops of starch reagent-1 (ST-1) was added and mixed. Appearance of blue color indicates the presence of starch in the milk.

Neutralizers detection method

About 5 ml of milk sample was taken in test tube and 4 drops of neutralizers reagent -1 (N-1) was added and mixed well. Appearance of red color

(or deep rose color) indicates the presence of neutralizers in milk.

Detection of detergents

About 5 ml of milk sample was taken in a test tube and 5 drops of detergent-1 reagent (DT-1) was added and mixed well. Appearance of dark purple color indicates the presence of detergent.

Detection of sugar

About 5 ml of milk sample was taken in test tube to this few drops of sugar reagent -1 (S-1) and 4 drops of sugar reagent -2 (S-2) were added and mixed. The contents were placed in boiling water bath for 2 minutes. Appearance of red color indicates the presence of the sugar in milk.

Detection of Sodium chloride

To 2 ml of milk in test tube 2 drops of sodium chloride reagent-1 and 2 ml of sodium chloride reagent-2 were added and contents were mixed. Appearance of an yellow precipitate was indicates presence of salt in the milk

Detection of skim milk powder

To the milk sample nitric acid was added and appearance of violet color indicates the presence of skim milk powder (SMP). Absence of SMP indicates yellow colored milk.

Statistical analysis:

The data obtained for various milk samples collected from various districts was analyzed by two way ANOVA and P-value was obtained by using SPSS software (version 20) (Awal Shihabul *et al*., 2016).

Results and Discussion

Detection of adulterants in raw and chilled milk samples:

The mean values of Adulterants in positive raw milk samples obtained from various districts were given in Table 1. In Visakhapatnam, highest mean value was reported for Urea (50±0) and lowest mean value was reported for Skimmed Milk Powder or SMP (4±1). In Srikakulam highest mean value was reported for Urea (50±0) and lowest mean value for Starch (4±1.7). In Vizianagaram highest mean value was reported for Urea (46±1.7) and lowest mean value was reported for Starch (4±2). The Adulterants like detergents, sugar, SMP and glucose were not detected in raw milk samples of Vizianagaram. In East Godavari highest mean value was reported for Urea (46±3.1) and lowest mean value was reported for NaCl (43±7.0). Starch, detergent, sugar, SMP and glucose were not detected in milk samples of both East and West Godavari districts. In West Godavari highest mean value was reported for Urea (48±0) and lowest mean value for NaCl (40±10.6). The comparison of mean values for each Adulterant among districts was carried out to obtain p-value. The p-value was found to be significant for Urea, Starch, Detergent, NaCl, Sugar, SMP, Glucose and Water and non-significant for Neutralizers. The mean values of adulterants in raw milk samples were shown in figure 1.

Table 1: Mean values of adulterants in positive raw milk samples in various regions

Name of the adulterant	Total number of samples	Visakhapatnam (sd mean+/- positive sample)	Srikakulam (sd mean+/- positive sample)	Vizianagaram (% of positive sample)	East Godavari (mean+/- positive sample)	West Godavari (mean+/- sd positive sample)	p-Value
Urea	50	50±0	50±0	46±1.73	46±3.05	48±0	>0.031*
Starch	50	40±4	4±1.73	4±2	ND	ND	>0.001*
Neutralizers	50	40±10	30±2	30±0	46±2.9	40±5.3	0.031*
Detergents	50	20±3.6	45±1	ND	ND	ND	>0.000*
NaCl	50	49±1.15	10±3.46	43±5.13	43±7.03	40±10.58	>0.000*
SUGAR	50	20±4	50±0	ND	ND	ND	>0.000*
SMP	50	4±1	45±1	ND	ND	ND	>0.000*
GLUCOSE	50	20±8	20±8	ND	ND	ND	0.001*
Water	50	20±6	ND	20±8	43±3.05	47±4.6	>0.000*

*- Significant; **ND**- not detected

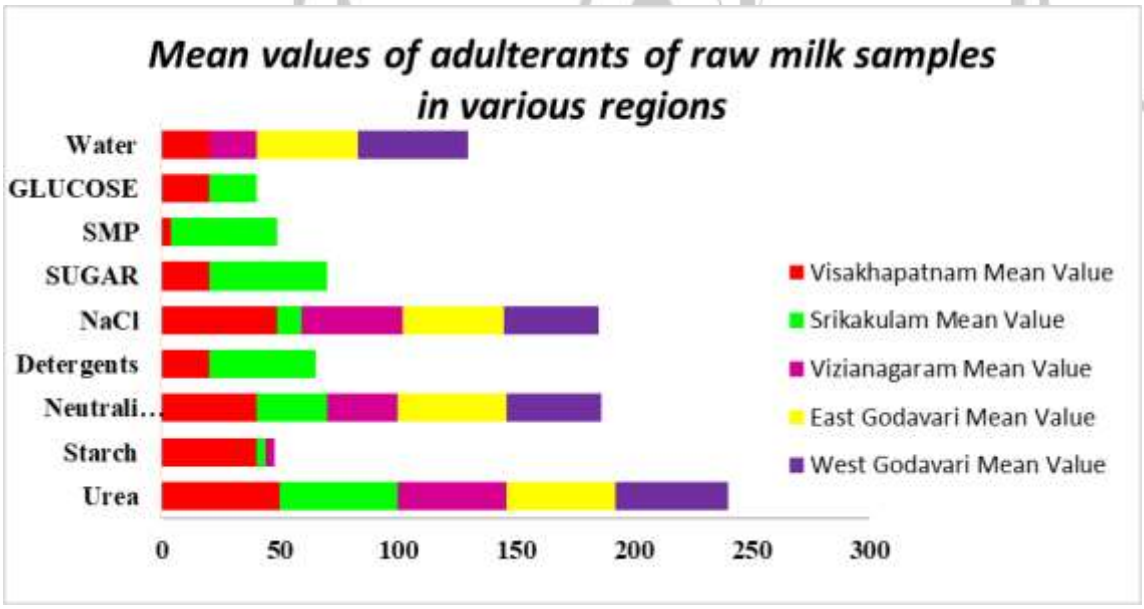

Figure 1: Mean values of adulterants of raw milk samples in selected districts

The mean values of Adulterants in positive chilled milk samples in various districts were given in Table 2. In Visakhapatnam, Srikakulam, Vizianagaram, East and West Godavari districts highest mean value was observed for Urea. Water was

reported to have lowest mean value in Visakhapatnam, Srikakulam and East Godavari. Lowest mean value was observed for detergents in Vizianagaram (4±2.6). Lowest mean value in West Godavari was observed for NaCl (2±2). Comparison of mean values of Adulterants among five districts was carried out to obtain p-value. Significant p-values were observed for neutralizers, detergents, NaCl, sugar, glucose and water and non-significant for Urea and Starch. The mean values of adulterants in chilled milk samples were shown in figure 2.

Table 2: Mean values of adulterants in positive chilled milk samples of selected regions

Name of the adulterant	Total number of samples	Visakhapatnam (mean +/-sd positive sample)	Srikakulam (mean+/- sd positive sample)	Vizianagaram (mean+/-sd positive sample)	EastGodavari (% of positive sample)	WestGodavari (% of positive sample)	p-Value
Urea	50	50±0	46±3.05	50±0	48±2	48±0	0.125 ^{NS}
Starch	50	ND	ND	4±1.7	4±2.6	4±3.6	0.065 ^{NS}
Neutralizers	50	10±5	13±7	20±5.2	40±5	40±5.3	>0.000*
Detergents	50	ND	4±3.4	4±2.64	4±2	10±3	0.015 *
NACL	50	45±5	9±1.7	20±10	20±5	2±2	>0.000*
Sugar	50	ND	ND	ND	ND	20±9.5	0.001*
Glucose	50	ND	ND	ND	8±4	ND	0.001*
Water	50	4±2	2±0	ND	2±1.73	2±1	0.04*

*- Significant; NS-nonsignificant; ND – Not detected

The mean values of Adulterants in both raw and chilled milk samples from all five districts were compared and p-values were given in Table 3. In Visakhapatnam significant p-values were observed for Urea, Starch, Neutralizers, Detergents, Sugar, SMP, Glucose and Water. The p-value was non-significant for NaCl. In Srikakulam, significant P-values were obtained for Detergents, SMP and Glucose. The p-values were non-significant for Urea, Starch, Neutralizers and NaCl. The p-values Sugar and Water were not obtained. In Vizianagaram significant p-values were observed for NaCl and Water. The p-values were non-significant for Urea, Starch, Neutralizers and Detergents. The p- values were not reported for Sugar, SMP and Glucose. In East Godavari significant p-values were obtained for Detergents, NaCl, Glucose and Water. The p- values were non-significant for Urea, Starch and Neutralizers and not reported for Sugar and SMP. In West Godavari p-values were significant for Detergents, NaCl, Sugar and Water. The p-values were non-significant for Starch and Neutralizers and not obtained for Urea, SMP and Glucose.

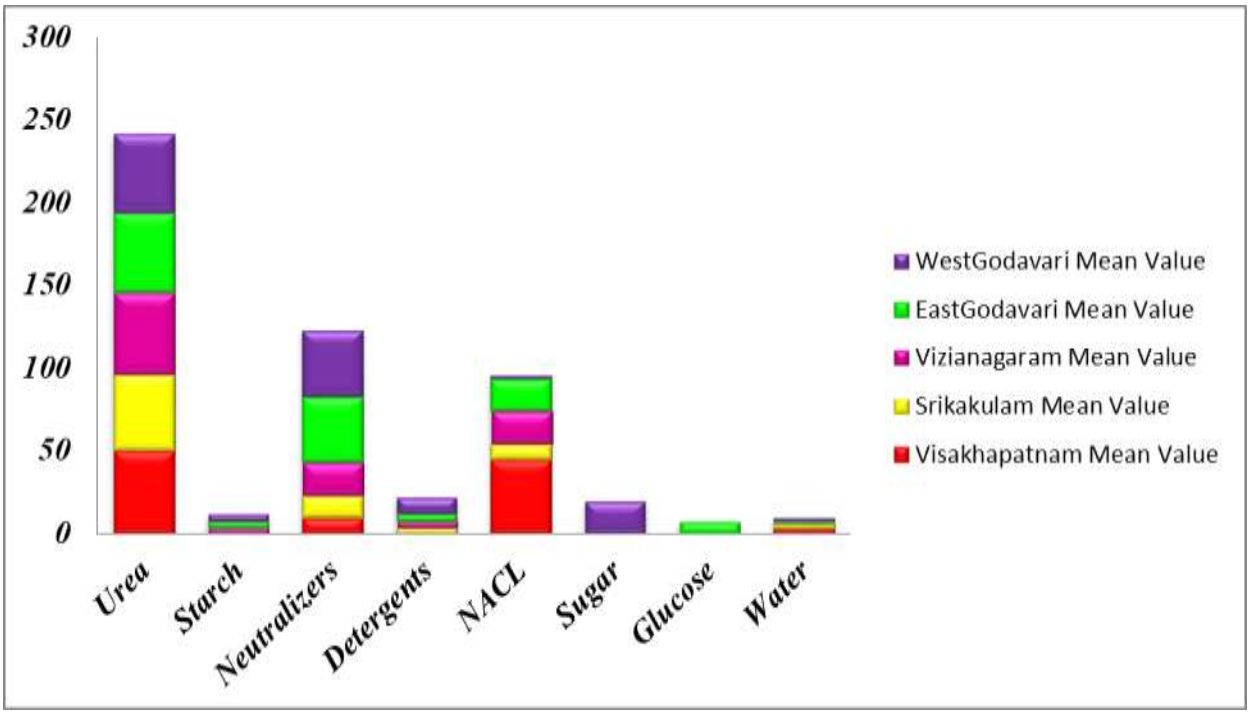

Figure 2: Mean values of adulterants of chilled milk samples in various regions

Table 3: Comparison of adulterants in positive raw and chilled milk samples

Name of the region	Urea	Starch	Neutralizers	Detergents	NaCl	Sugar	SMP	Glucose	Water
Vishakaptnam	0*	0.003*	0.020*	0.011*	0.217	0.01*	0.002*	0.015*	0.01*
Srikakulam	0.191^{NS}	0.057^{NS}	1.0^{NS}	>0.0001*	0.678	-	>0.001*	0.015*	-
Vizianagaram	0.057^{NS}	1.0^{NS}	0.082^{NS}	0.120^{NS}	0.023*	-	-	-	0.012*
EastGodavari	0.561^{NS}	0.120^{NS}	0.116^{NS}	0.026*	0.009*	-	-	0.026*	>0.00*
WestGodavari	-	0.195^{NS}	1.00^{NS}	0.041*	0.004*	0.022*	-	-	0.002*

***-** Significant; NS-nonsignificant;

The mean values of adulterants in pasteurized milk samples were shown in Table 4. Starch was not detected in all types of pasteurized milk samples. Neutralizers were detected in all samples. Detergents were not detected in homogenized toned milk. NaCl and Sugar were not detected in any of the samples. SMP was detected in all samples. Glucose and water were not

detected in any of the pasteurized milk samples. The mean values of adulterants in pasteurized milk samples were shown in figure 3. The adulterants present in raw and chilled milk samples respectively were more or less similar to the results presented by Chanda *et al*., (2012). A research study was carried out to analyze the adulteration of raw milk and other samples like tetra pack milk for presence of adulterant like starch and was detected in samples more than the standard limits. (Hassabo Adam, 2009). A study on the hygienic status of milk in educational institutions and public places of Faisalabad, Pakistan was carried out by physical examination. The study of chemical composition, hygienic status and adulterants of milk samples was reported that the quality of milk samples was not up to the standards (Faraz *et al*., 2013).

Table 4: Mean values of adulterants in positive pasteurized milk samples

Name of adulterant	Total number of samples	TM	HTM	DTM	FCM	STD
Neutralizers	50	3.666±0.57	4.6±0.57	6.3±1.52	2.6±0.57	4.3±1.15
Detergents	50	0.66±0.57	ND	0.6±0.3	0.6±0.33	0.66±0.57
NaCl	50	ND	ND	ND	ND	ND
Sugar	50	ND	ND	ND	ND	ND
SMP	50	15±5	26.6±2.8	25±5	9.3±1.15	6±1.73
Glucose	50	ND	ND	ND	ND	ND
Water	50	ND	ND	ND	ND	ND

***-** Significant; ND –not detected; TM- Toned milk HTM- Homogenized Toned milk DTM- Double toned milk FCM-Full cream milk STD- standardized milk

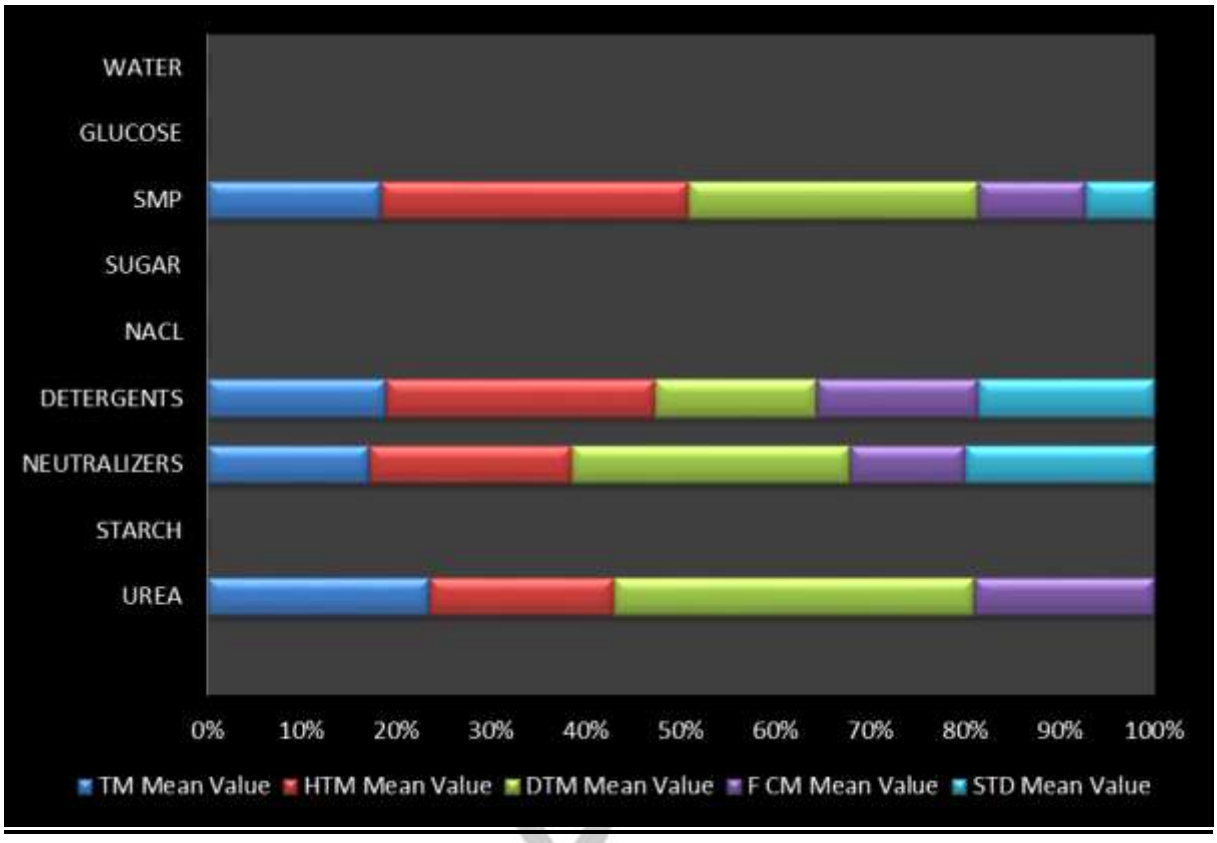

Figure 3: Mean values of adulterants of pasteurized milk samples in various regions

Analyses of Vendor's milk samples

The vendor's milk samples were analyzed for presence of specific pathogens, Aflatoxin M1 and antibiotic residues. Standard plate count was also carried out. The number of samples collected was 50 and the mean values of various analyses were given in table 5. These mean values indicate the poor quality of Vendor's milk samples. The Mean values of specific pathogens, standard plate count, aflatoxinM1 and antibiotic residues in vendor's milk samples were shown in figure 4. The mean values of pesticide residues, heavy metal residues and adulterants in vendor's milk samples were given in table 6 and figure 5.

Table 5: Mean values of specific pathogens, standard plate count, aflatoxinM1 and antibiotic residues in vendor's milk samples

Specific pathogens	Total number of cells	Mean	S.D
E.coli	50	44	1.7
Staphylococci		45	0.57
Salmonella		25	2.8
Shigella		15	3.5
Listeria		4	0.57
Standard plate count		47	12.16
Aflatoxin M1		33	4.58
		Antibiotics	
Penicillin -G		4	0.57
Tetra cycline		6	1.22
Oxy tetra cycline		6	1.42

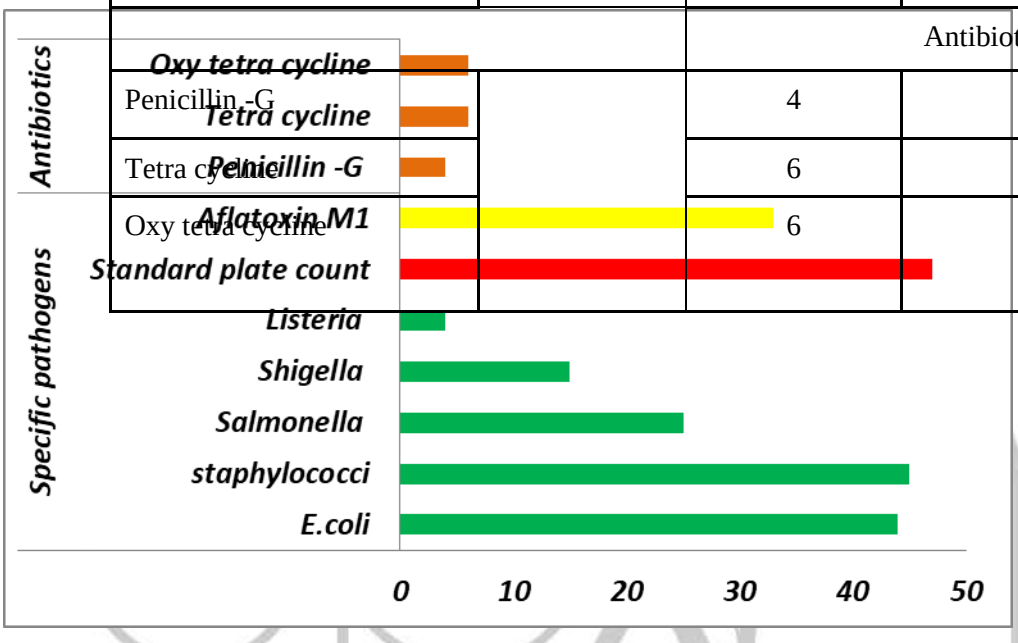

Figure 4: Mean values of specific pathogens, standard plate count, aflatoxinM1 and antibiotic residues in vendor's milk samples

Table 6: Mean value of pesticide residues, heavy metal residues and adulterants in vendors milk samples

Pesticide residues	Mean	S.D.
Lindane	40	3.6
Endosulfan	50	4.61
Chlorane	40	1.52
Heptachlor	43	8.11
Methoxy chlorane	40	7.93
Heavy metal residues		
Lead	40	4.58
Arsenic	30	6.083
Zinc	36	4.9

Mercury	2	0.97
Adulterants		
Urea	46	2.12
Starch	23	1.76
Neutralizer	43	6
Detergents	4	0.61
NaCl	33	1
Sugar	43	8.66
Glucose	40	6.5
Water	35	1.73

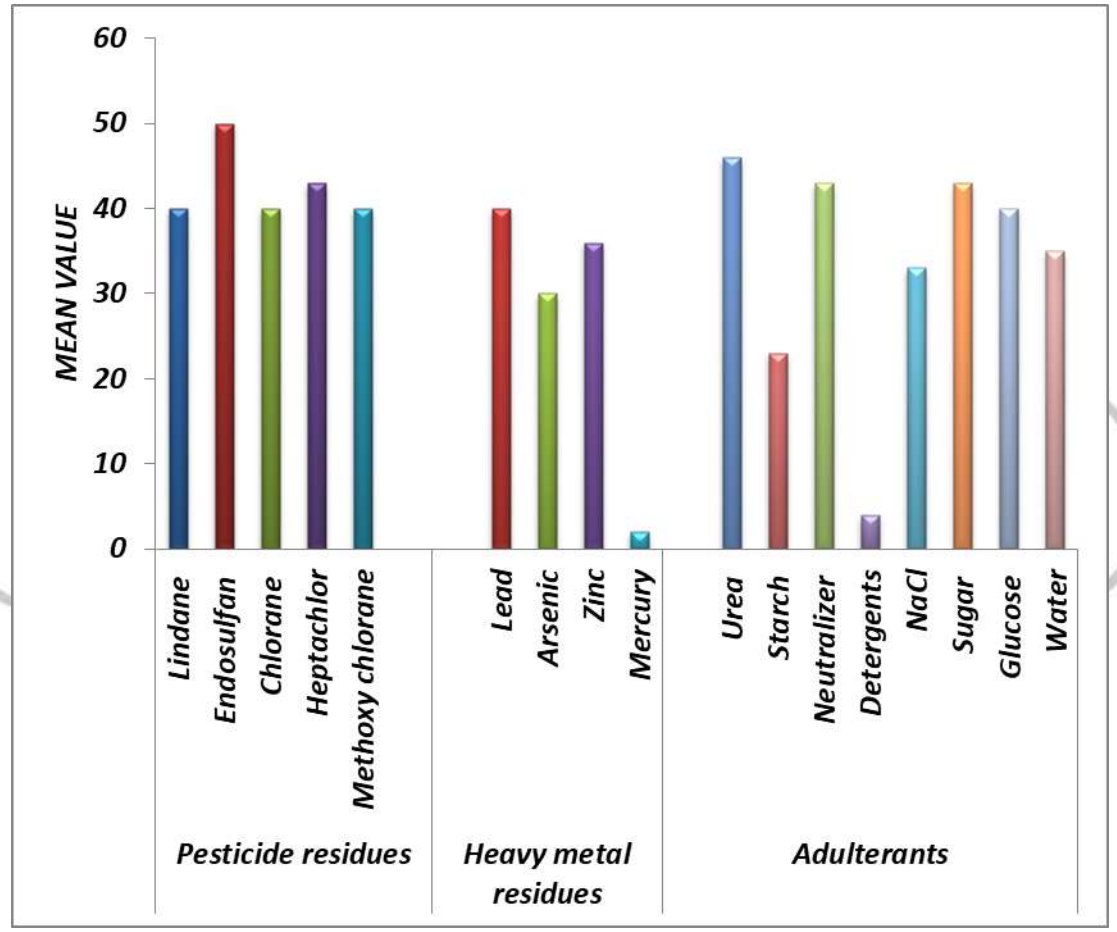

Figure 5: Mean values of pesticide residues, heavy metal residues and adulterants in vendor's milk samples

Table 7: Standard Values established by FSSAI for various parameters

Name of the parameters	FSSAI Standard – 2011
Arsenic	0.1 ppm/w
Lead	0.2 ppm/w
Zinc	50 (not less than 25.0 ppm/w)

Mercury	1.0 ppm/w
Aflotoxin M1	0.5 µg/kg
Antibiotic Residues	
Tetra Cycline	0.1 ppm/mg/kg
Oxy Tetra Cycline	0.1 ppm/mg/kg
Penicillin – G	0.1 ppm/mg/kg
Pathogens	
SPC/APC	30000 CFU/ML(10-3*30 CFU/ML)
E. coli	Absent/0.1 ml
Staphylococcus aureus	Absent
Salmonella	Absent/25ml/gm
Shigella	Absent/25ml/gm
Listeria spp	Absent/25ml/gm
Pesticide residues	Tolerance limit mg/kg ppm
methoxchlor	Not specified
Endosulfan	*Not Specified
Hepatochlor	0.15
Lindane	0.01

*** Source: Food safety and standard (Contaminants, Toxins and Residues) Regulation - 2011**

The standard values of pathogens, aflatoxin M1, antibiotic residues, pesticide residues and heavy metal residues were given in table 7. Basing on comparison of values of current study with FSSAI standard values, the milk samples in all districts collected were can be described as poor quality.

Conclusion

Adulterants were detected in milk samples of five districts and significant p-values were observed for neutralizers, detergents, NaCl, sugar, glucose and water. The p-values were found to be non-significant for Urea and Starch. Presence of adulterants in milk affects the quality of milk. The comparison of mean values and p - values of positive raw, chilled and pasteurized milk samples of current study with the standard values indicate that the milk samples collected from 5 coastal districts of Andhra Pradesh can be described as poor quality.

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