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Original Article



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Chemical Composition and Bacteriological Quality of Cow Raw Milk Collected From Daim Algarrai Area

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Abstract

This study was conducted to assess the factors influencing milk quality from dairy farmers in the Deam ALgarri aera in Sudan. A total of twenty row cow's milk samples were collected from dairy farmers. The physicochemical parameters, bacteriological quality, milk adulteration and drug residues were assessed. The presence and enumeration of microorganisms was done based on Total Plate Count (TPC), Total Coliform Count (TCC), Total Staphylococcal count (TSC), and detection of pathogenic E. coli (O157: H7). The mean value of aerobic bacteria in the milk samples showed the higher TPC, TCC followed by TSC which were 111608 CFU/ml (log 4.95), 8145CFU/ml (log 3.42) and 6793.25 CFU/ml (log 3.29) respectively. The result of the detection of Escherichia coli O157:H7 which is a major foodborne pathogen causing severe disease in humans worldwide, all milk samples appear free from this pathogenic bacterium. The results of the physical properties of cow milk showed normal flavor, taste and colors, and higher pH (7.12), and total water (156%), the exception was the freezing point (-0.444) and specific gravity while showed lower value (1.029) compared to SSMO limits but within FAO limits. The chemical properties of cow milk showed high amount of protein content (3.49%), lower solid not fat (SNF) which was (7.97%), Lactose (3.73%) and total solid (TS) (0.58%) when compared to both Sudanese Standard and Metrology Organization (SSMO) and Food and Agriculture Organization (FAO) standards. The result of presence or absence of antibiotic residue to the all milk samples was indicated absence antibiotic residues with (6 mm) and (7 mm) zone inhibitor against Bacillus subtilis. Isolation and identification of some pathogenic bacteria was performed according to standard methods, the results demonstrated the presence of food-borne microorganisms E. coli, klebsiela, sp, Pseudomonas sp, and Shiglla sp. with percentages of 31.1%, 29.7%, 38.4%, and 1% respectively, the presence of these bacteriological quality in the raw milk samples indicted an urgent need to implement good hygiene practices from farm to the consumer.

Keywords: Raw milk quality; Microbiological quality; Antimicrobial residue; Sudan.

1. Introduction

Milk is one of the widely consumed products and contains carbohydrates, proteins, lipids, other organic compounds and inorganic salts in water. It is a suitable medium for contamination by microorganisms and support the rapid growth and multiplication of bacteria [1].

Milk is an ideal environment for microbial growth and for this reason the separation of some pathogens is very important. So, analysis of milk regarding pathogenic microorganisms is a clear indicator of hygienic quality and this influences the dairy production. The milk is very easily contaminated if collected un-hygienically and handled carelessly leading to quick spoilage [2, 3] and is often contaminated by *Escherichia coli* under poor sanitary conditions which can affect public health. Bacterial contamination of raw milk can originate from different sources such as air, milking equipment, feed, soil, and grass [4].

Farm animals represent a major reservoir of pathogens that can be transferred to milk [5]. *Staphylococcus aureus, Salmonella* spp., *Listeria monocytogenes* and *Escherichia coli* O157:H7 are the most frequent potential pathogens associated with milk or dairy products in industrialized countries [6] and are the main microbiological hazards linked to raw milk [7] and raw cheese [8].

As widespread use of antibiotics has created potential residue problems in milk and milk products that are consumed by the general public. The consumption of raw milk holds a risk for the consumer, due to the addition of some antibiotics into feed additive as growth promoters and to the possible presence of human pathogenic microorganisms in the raw milk; the available information on quality of milk and some pathogens or dairy products made from raw milk is limited. Moreover, there is no systematic data available on the prevalence of human pathogenic microorganisms in raw milk at Daim ALgarrai area.

The main objective of this study was to assess the factors influencing milk quality from dairy farmers in the Daim ALgarria in Sudan.

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2. Materials and Methods

2.1. Study Aera

The study was carried out in Daim ALgarrai area which is located in the River Nile State and lies between latitude 16.47,36 °N - 16.50,19 °N and longitude 33.37,45 E and 33.39,37°E Fig 1. The study area was selected because the milk is the main source of food to all people living in the village.





2.2. Collection and Handling of Milk Samples

A total of 20 raw milk samples of the cows were collected directly, in label of sterilized 100 ml bottles from farmers' containers by using simple random sampling. The samples were immediately cooled and transported to the laboratory for analysis in cool boxes containing ice packs. Fig.2





2.3. Physiochemical analysis of Cow Raw Milk

Chemical and physical analyses (fat, protein, TS, SNF, lactose, salt and added water etc.) of twenty cow's raw milk samples were determined using Lactoscan milk analyzer (LCD display-4-Lines X16 characters, Milkotronic Ltd, Bulgaria). According to the standard [9].

2.4. Antibiotic Residue in Cow Raw Milk Samples by Disc Assay

An antibiotic residue in milk samples was determined by disc-plate method. Twenty milliliters of Mueller Hinton Agar were poured in the petri dish and allowed to cool and solidify. A sterile cotton buds was used to spread a thin even layer of the *Bacillus subtilis* spores onto a plate of MHA, Paper discs (6 mm) were soaked in the milk sample and placed on the agar. The plates were left at room temperature for 2 hours to allow for the diffusion of the milk samples into the media, the plates were incubated for 24 h at 37°C. The antibiotic activity of the milk samples was assessed as the clear inhibition zone around the disc and it was measured in mm.

2.5. Microbial Analysis of Cow Row Milk

For evaluation of milk quality, microbial contamination was assessed through estimating Total Plate Count (TPC), Coliform Plate Count (CPC), *Staphylococci* count and detection of pathogenic *E. coli* (O157: H7) in milk produced in farming systems of the study area. In this study, a few selected pathogens including *E. coli*, *Salmonella* spp., and *S. aureus* were targeted. for determination of total plate count, coliform count and staphylococcus counts general standard procedures of ISO 4833-2 [10] standard protocol was carried out.

2.6. Detection of Pathogenic E. coli (O157: H7)

To investigate the presence of pathogenic *E.coli* (O157: H7) in the milk samples, the BactiCard Kits and latex kits(REMEL Inc., 12076 Santa Fe Trail Dr. Lenxa, and KS66215 USA. (BactiCard *E.Coli*) and OXOID Limited. Wade Road, Basingstche, Hampshire, RG24 BPW UK. *E.Coli* 0157 Latex). Was used according to the manufacture's instruction. Detection was performed by veterinary research institute laboratories.

2.7. Identification of some Pathogenic Bacteria

The preliminary identification tests included Gram staining, microscopic examination, catalase and oxidase, Indole Test, Starch Hydrolysis, Catalase, Activity Production of acid and gas (CO_2) from glucose anaerobically and Mortality Test activity, were carried out to confirm the presence of microorganism in the milk samples which the organism grows. The methods described according to Cowan and Steel's manual for Identification of Medical Bacteria [11].

3. Results and Discussion

Milk is an important source of nutrients to human and animals, but due to its high-water activity and nutritional value it serves as an excellent medium for growth of most kinds of microorganisms under suitable conditions. The present study was conducted to assess microbial quality, physicochemical properties and adulteration of milk from small-scale farmers in Daim Algarrai and to characterize some pathogenic bacteria in the cow's milk.

The results from this study will be used to generate information showing the microbial quality of the raw cow's milk in the study area. Such information is useful to farmers when looking for more profitable markets for their milk and also informing the stake holders in the dairy sector.

3.1. Physio-Chemical Properties of Cow Raw Milk Samples

Physicochemical analyses have been done as percentages (%) of fat content, protein, total solid (TS), solid nonfat (SNF), lactose, salt, water content, pH, freezing point and specific gravity (SG). Physical and chemical parameters of all cow milk samples were almost correlated with the Sudanese Standard and Metrology Organization (SSMO), FAO and European Union quality standard for unprocessed whole milk standard.

The physical properties of cow's raw milk showed that; the flavor of all milk samples were normal aromatic as standard level and the taste slightly sweet without any deviation from standard, on the other hand the color of all samples showed yellowish white color except sample (S3) which was whitish in appearance. However, the Specific Gravity varied from 1.022 to 1.037 with average 1.029 low to the SSMO and within FAO. Table 1.

The results of pH were showed the average values 7.12 and which is higher to both SSMO and FAO standard. FAO [12] reported that the pH of fresh cow milk has a pH value range from 6.6 to 6.8.

The average values of the total water content of milk samples Fig 3, which were 156% these results showed higher to the SSMO, FAO and EU standard values. On the other hand, the freezing points of the milk samples were showed average values -.0444; these results showed higher to SSMO. Fig 4, the amount of water is controlled by the amount of lactose synthesized by the secretory cells of the mammary.

Physical properties parameters								
Samples	Color	Flavor	Taste	SG	Temp.	pН		
S1	Yellowish white	Normal	Slightly sweet	1.037	19.7	7.02		
S2	Yellowish white	Normal	Slightly sweet	1.036	12.0	7.88		
S3	Whitish	Normal	Slightly sweet	1.023	11.9	7.77		
S4	Yellowish white	Normal	Slightly sweet	1.030	12.4	7.90		
S5	Yellowish white	Normal	Slightly sweet	1.025	12.4	7.80		
S6	Yellowish white	Normal	Slightly sweet	1.026	14.0	7.92		
S7	Yellowish white	Normal	Slightly sweet	1.028	16.0	7.74		
S8	Yellowish white	Normal	Slightly sweet	1.025	12.0	7.72		
S9	Yellowish white	Normal	Slightly sweet	1.022	14.20	7.54		
S10	Yellowish white	Normal	Slightly sweet	1.025	13.7	7.57		
S11	Yellowish white	Normal	Slightly sweet	1.030	12.0	6.12		
S12	Yellowish white	Normal	Slightly sweet	1.030	16.7	6.71		
S13	Yellowish white	Normal	Slightly sweet	1.030	22.8	6.45		
S14	Yellowish white	Normal	Slightly sweet	1.028	23.7	6.53		
S15	Yellowish white	Normal	Slightly sweet	1.028	12.7	6.64		
S16	Yellowish white	Normal	Slightly sweet	1.023	23.3	6.54		
S17	Yellowish white	Normal	Slightly sweet	1.032	12.1	6.58		
S18	Yellowish white	Normal	Slightly sweet	1.032	20.7	6.61		
S19	Yellowish white	Normal	Slightly sweet	1.036	23.8	6.77		
S20	Yellowish white	Normal	Slightly sweet	1.033	11.9	6.50		
Average				1.029		7.12		

Table-1. Physical properties of collected Cow's raw milk samples

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Fig-3. Water Contents of the Twenty Cow's Raw Milk Samples





Fig-4. Freezing Points of the Twenty Cow's Raw Milk Samples

3.2. Chemical Analysis of Cow Milk Samples

Result obtained from chemical analysis of the milk samples was presented in table.2. The chemical properties of twenty cow milk samples revealed that the values of Fat content ranged from 3.44% to 4.96% with the average of 4.41%, these values within the range of SSMO standard level but higher to FAO standard as shown in table 2. According to European Union quality standard for unprocessed whole milk fat content should not be less than 3.5% Tamime [13]. Consequently, the average of the fat content of milk samples in this study is higher to the range of defined standard. This difference might be due to variability among the breeds of cows, within a bread and stage of lactation and feeding.

The results of protein were showed the average protein contents of milk samples in the current study which were 3.49%, these results were higher to the value of Sudanese standard level (SSMO) and similar to the FAO standard.

The results of the solid nonfat (SNF) content of milk samples showed the average value of 7.97%, this value of solid non fats lower to the ideals value of SSMO level. According to European Union quality standards for unprocessed whole milk, Solid-not fat content should not be less than 8.5% [13]. Accordingly, the pooled average SNF content 7.97% observed in the milk samples were less than the recommended standard, the difference observed in SNF content of milk could be due to difference in the feeding practices, season, milking methods and lactation period exerted.

The results of sugar (lactose) content of milk samples showed the average value of 3.73%. These results were lower to SSMO and FAO standard level together. However, the nutritional as well as economic value of milk is directly associated with its solids content. The higher solid content, it's better for nutritional value and greater the milk product yield [14], the average of total solids (TS) content among milk samples which were 0.58% of which the values range 11.23% to 14.24%. These results were the lowest to SSMO and FAO. The standard for total solids content of cow milk is not less than 12.5% (FAO/WHO, 2007). Therefore, the average total solids content of milk samples in the present study were the lowest of the recommended standard.

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Table-2. Chemical composition of cow's raw milk samples								
Samples	Fat	SNF	protein	Lactose	Salt	Total solid		
1	4.58	9.73	4.48	4.30	0.81	0.69		
2	4.54	9.53	4.39	4.22	0.79	0.68		
3	3.46	6.11	2.81	2.70	0.51	0.43		
4	4.61	8.14	3.74	3.60	0.68	0.57		
5	4.21	6.81	3.12	3.01	0.57	0.48		
6	7.76	7.69	3.48	3.40	0.64	0.53		
7	4.68	8.04	2.94	4.42	0.67	0.65		
8	4.50	7.25	2.65	3.99	0.61	0.59		
9	2.76	6.34	2.32	3.49	0.53	0.52		
10	4.19	7.29	2.67	4.01	0.61	0.59		
11	5.38	8.14	3.73	3.60	0.68	0.57		
12	3.52	7.92	3.65	3.50	0.66	0.56		
13	5.87	8.17	3.73	3.66	0.68	0.57		
14	3.20	7.31	3.37	3.23	0.61	0.52		
15	4.10	7.35	3.37	3.25	0.61	0.59		
16	3.48	8.36	3.86	3.70	0.69	0.59		
17	3.00	8.85	3.24	4.87	0.73	0.73		
18	5.03	8.52	3.91	3.77	0.71	0.60		
19	6.24	9.16	4.19	4.05	0.76	0.64		
20	4.26	8.72	4.01	3.86	0.72	0.62		
Average	4.41	7.97	3.49	3.73	0.67	0.58		

Key: SNF= solid nonfat

3.3. Antibiotic Residues in Cow's Raw Milk Samples

This test of the antibiotic residues was done to detect the presence of antibiotic residue in all milk samples, the results of inhibition zone ranged between (6 mm) and (7mm) zone of inhibitor for all milk samples against *Bacillus subtilis* compared to the standard drugs Penicillin G, Tetracycline and Amoxicillin which had inhibition zone of (22 mm), (23mm) and (32 mm) respectively, as shown in Fig 5, this results showed that all milk samples are free from antibiotic residue of drugs, and this is favorable finding because the presence of antibiotic residues in the raw milk are harmful to humans; resulting in the rap failure and development of antibiotics in the milk samples of most of the studied farms might be due to the fact that during winter when weather becomes cold, diseases such as pneumonia increased and farmers used antibiotics to treat animals, therefore antibiotics residue transferred into milk. Also, it might indicate the increase of awareness among the animal owners, which could be attributed to the increase veterinarian visits during animal treatment.

All milk and milk-based products sold to consumers in the EU will have originated from raw milk that meets these standards. In addition, the cows must be kept in hygienic conditions, be subject to good management practices and be free from disease.



Fig-5. Antibiotic residues in cow's raw milk samples

A- Milk samples against Bacillus subtilis

B- Drug Penicillin G, Tetracycline and Amoxicillin against Bacillus subtilis

3.4. Determination of Total Plat Count (TPC), Total Coliform Count (TCC) and Total Staphylococcal Count (TSC)

A total of twenty cow's raw milk samples from Daim ALgarrai area were cultured for Total Plat Count (TPC), Total Coliform Count (TCC) and Total Staphylococcal count. Table 3 showed that the total plate count has ranged from 5.85×10^4 to 35.85×10^3 CFU/ml with the average values of 111608 CFU/ml (log 4.95). These results were higher than the maximum recommended level (Sudanese Cow Raw milk standard), on other hand, these results were agreed with Kurwijila, *et al.* [15] However, these results disagreed with Siham, *et al.* [16], which was found the average of TPC in raw milk samples collected from Omdurman and Khartoum North were 9.29 and 8.23 log10cfu/ml. This implied that raw cow milk from Daim ALgarrai area had poor microbiological quality. The high Presence of total plate load in raw milk indicates contamination possibly from lactating cows, milking equipment's, storage containers, unsatisfactory hygiene/sanitation practiced at farm level, unsuitable storage condition, unclean udder and/or teats, poor quality of water used for cleanliness and dirty hands of milkers. Generally, it further indicates the degree level of hygiene practices in the whole milk production process and reflects the time elapsed since milking and/or processing at ambient temperature [17, 18].

For total coliform count the laboratory investigation confirms presence of coliform in cow milk samples. Table 3 showed that the TCC ranged from 0 to 11.75×10^3 CFU/ml with average values of 8145CFU/ml (log 3.42), this result is higher than the maximum recommended level [19]. However, these results were agreed with previously published study in Sylhet Agricultural University area in Bangladesh [20] who found that the presence of high number of total viable count and total coliform count in the raw milk of samples. Also, these results were disagreed with Siham, *et al.* [16] which was reported the average TCC of the raw milk samples from Omdurman and Khartoum North is ranged between 7.11 log₁₀ to 6.55 log₁₀ respectively. Presence of pathogenic bacteria or coliforms is not acceptable and presence of coliform is often used as a parameter of proper sanitary condition in different countries. The presence of high numbers of coliforms in milk, which is mainly associated with unclean udder and teats arise from a variety of sources such as manure, soil, food personnel and even water and thus associated with unclean udder and teats, provides an index of hygienic standard used in the production of milk [21]. When the number of total coliforms is increased, it may lead to food poisonings [22]. Sporadic high coliform counts may also be a consequence of unrecognized coliform mastitis, mostly caused by *E. coli* Torkar and Teger [4]. Detection of *E. coli* in milk often reflects fecal contamination and is the known causative agent of diarrhea and other food born related illnesses through the ingestion of contaminated foodstuffs [23].

The results of *Staphylococcus aureus* count showed that the total of Staphylococcal count range between 0 to 13.65x10³CFU/ml with the average value 6793.25 CFU/ml (log 3.29) as shown in, and table 3. These results showed the higher count *Staphylococcus aureus* in the milk samples when compared to the to EU and USA standard table, of Staphylococcal in row milk. However, these results disagree with Siham, *et al.* [16] which was found that the average of *Staphylococcus aureus* in row milk samples collected from Omdurman and Khartoum North is 6.61 log10. The obtained results in the present study indicates a possible risk to Staphylococcal poisoning in milk in the study area, as mentioned in some literature review that *Staphylococcus aureus* count in milk above 1.0x105 CFU/ml is enough for the occurrence of milk staphylococcal poisoning [24]. *S. aureus* is considered as the third most important cause of disease in the world among the reported food borne illnesses due to its capability to produce a wide range of heat stable enterotoxins [25]. *S. aureus* can gain access to milk either by direct excretion from udders with clinical or subclinical staphylococcal mastitis or by contamination from the environment during handling and processing of raw milk [25].

NO of	Total Plat Count. TPC		Total Coliform	Count. TCC	Total Staphylococcal Count. TSC		
Samples	CFU/ml	Log	CFU/ml	CFU/ml Log		Log	
1	8.75 x 10^4	4.94	7.75×10^3	3.89	2400	3.83	
2	21.16×10^4	5.34	2.6×10^3	3.41	3500	3.54	
3	$11.25 \text{ x } 10^4$	5.05	0	0	13650	4.14	
4	5.85 $\times 10^4$	4.77	0	0	7750	3.89	
5	12.75×10^4	5.11	9.6×10^3	3.98	11275	4.05	
6	$11.25 \text{ x } 10^4$	5.05	2.7×10^4	4.43	10350	4.02	
7	35.85×10^3	4.55	2.1×10^4	4.32	16000	4.20	
8	7.6 x 10^4	4.88	5.75×10^3	3.76	0	0	
9	$11.1 \text{ x } 10^4$	5.05	2.3×10^4	4.36	10400	4.02	
10	22.5×10^4	5.35	$2x10^{3}$	3.30	12550	4.10	
11	25.0 x 10 ⁴	5.40	7.75×10^3	3.89	5070	3.71	
12	9.15×10^4	4.96	1.6×10^3	3.20	2800	3.45	
13	$17.1 \text{ x } 10^3$	4.23	9.75×10^3	3.99	6600	3.82	
14	25 x 10 ⁴	5.40	8.35×10^3	3.92	0	0	
15	11.25×10^4	5.05	11.25×10^3	4.05	12250	4.09	
16	$7.4 \text{ x } 10^4$	4.87	1.21×10^3	3.08	11050	4.04	
17	31×10^3	4.49	11.75×10^3	4.07	0	0	
18	2.91 x 10 ⁴	4.46	5.05×10^3	3.70	3930	3.95	
19	10.25×10^4	5.01	$2.x10^{3}$	3.30	2455	3.39	
20	$11.25 \text{ x } 10^4$	5.05	5.5×10^2	3.74	3835	3.58	
Average	111608	4.95	8145	3.42	6793.25	3.29	

Table-3. Total Plate Count, Total Coliform Count and Total Staphylococcal Count. of cow raw milk

3.5. Identification of Some Pathogenic Bacteria

Isolation and identification of major bacterial species were carried out by biochemical tests according to Cowan and Steels [11] for identification of medical bacteria. These biochemical tests included, Motility, Indole, Urease, citrate hydrolysis, glucose sucrose and lactose table 4. The highest source of microbial contamination of the milk samples was the *Pseudomonas* spp, *E. Coli, klebsiella, and Shigllasp*). The percentages of these pathogenic bacteria were 38.4%, 31.1%, 29.7%, and 1% respectively, the presence of those bacteria in milk suggested the contamination of the milk samples. Moreover, the higher incidence of isolated bacteria was found to be *Pseudomonas sp*. followed by *E. coli*. This might be due to the improper hygiene and sanitation, poor cleaning or poor healthy animals and unsatisfactory hygiene/sanitation practices.

The apparent dominance of these pathogenic bacteria in this study might be due to the fact that dairy farm owners did not use detergents and disinfectants to wash cows' udder, which could have significantly reduced the level of coliform and some non-coliform bacteria. Pathogenic bacteria in milk have been a matter of public health concern since the early days of the dairy industry. Many diseases such as tuberculosis, brucellosis, diphtheria, scarlet fever, Q-fever, and gastroenteritis are transmissible via milk products. The potential threat of pathogenic bacteria has been minimized and the numbers of outbreaks involving milk and milk products have steadily declined mainly due to modern milk production practices which emphasize sanitary measures, improved udder health, herd inspections, proper cooling, careful handling and storage of raw milk, and almost universal use of pasteurization [26]. However, a recent flurry of well-publicized outbreaks of salmonellosis [27, 28], *listeriosis* [28, 29], *campylobacteriosis* [29, 30], and *yersiniosis* [31] has refocused our attention on milk borne pathogens, especially the newly emerging pathogens, such as *Listeria rnonocytogenes*, *Campylobacter jejuni*, *Yersinia enterocolitica*, and enteropathogenic *Escherichia coli*.

Isolates	Gram and	Catalase	Citrate	Urease	Indole	Motility	Acid form		Gas	Organisms name	
	shape	Test	Test	Test	Test	Test	Glu	Suc	Lac		
Isolate1	- rod	+ve	-	-	+	+	+	-	+	+	E. Coli
Isolate2	- rod	+ve	+	+	-	-	+	+	+	+	Klebsiela spp.
Isolate3	- rod	+ve	+	-ve	-	-	-	-	-	-	Pseudomonas spp.
Isolate4	- rod	+ve	-	-	-	-	-	-	-	+	Shiglla spp.
Isolate5	+ cocci	+	-	-	-	-	nil	nil	Nil	nil	Stapylococcus
											arures

Table-4. Identification of isolated bacteria

Key: Glu= Glucose, Suc= sucrose, Lac= Lactose, -ve= negative, +ve= positive

4. Conclusion

- The current study of the physical properties of cow milk showed normal flavor, taste and colors, and higher pH, freezing point and total water, the exception was the specific gravity while showed lower value compared to SSMO limits but within FAO limits.
- The chemical properties of cow milk showed high amount of protein content, lower SNF, Lactose and total solid when compared to both SSMO and FAO standards, also showed normal fat to SSMO.
- The result indicated absence of the antibiotic residues in all milk samples.
- The study showed higher Total plates account, Coliform account than SSMO standard, and higher *staphylococcal* account to the EU and USA standards.
- All milk samples appear free from pathogenic *E.coli* (O157:H7).
- The result demonstrated the presence of food-borne microorganisms E. *coli, klebsiela* spp., *Pseudomonas* spp. and *Shiglla* spp. with percentage 31.1%, 29.7%, 38.4%, and 1% respectively.

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