ORIGINAL ARTICLE

PHYSIOCHEMICAL AND MICROBIOLOGICAL QUALITY ASSESSMENT OF COMMERCIALLY AVAILABLE PROCESSED AND UN-PROCESSED YOGURT, AVAILABLE IN MARKETS OF PESHAWAR

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ABSTRACT

Objectives: To assess the physiochemical and microbiological quality of commercially available processed and un-processed yogurt, available in markets of Peshawar and to compare it with WHO standards.

Material and Methods: This was a cross sectional study done from february 2018 to August 2018 in district Peshawar. A sample size of 150 was taken using WHO calculator. Multistage cluster sampling technique was applied. Samples of yogurt were collected and physicochemical, microbiological parameters were analyzed using SPSS version 22. T test was applied. P value ≤0.05 was considered significant.

Results: Total sample was 150.Comparing the properties of both yogurts no significant difference in mean values for all physicochemical properties with (P >0.05) except for titratable acidity (P = 0.014) and similarly no significant results for all microbiological properties were observed.

Conclusion: There was no statistically significant difference in mean values except for titratable acidity. Moreover, physicochemical parameters i.e. Ash content and titrtable acidity and total viable count, mold count were not up to WHO defined standards

Keywords: Physicochemical, microbiological, yogurt, processed, un-processed.

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INTRODUCTION

Yogurt is fermented milk product produced by lactic acid fermentation (through bacteriological action of Streptococcus salivarius, Lactobacillus thermophilus, Lactobacillu s delbrueckii. Bulgaricus)[1]. Yogurt acidification is an oldest method of preserving milk through lactic acid coagulation. Yogurt was introduced in American diet in early 1920s. In 1980s, yogurt became product of choice for dieters and part of lunch for young women[2]. Yogurt is an essential component of food consumption patterns, worldwide. Several experimental and observational studies are utilized for understanding beneficial health effects of yogurt[3].

Nutritional profile of yogurt is similar as that of milk. Yogurt is a rich source of calcium, proteins, phosphorus, vitamin B12, riboflavin, Thiamin, magnesium, zinc,folate and niacin. Yogurt is a nutrient dense food in relation to energy and fat content. Daily utilization of yogurt improves quality of life and helps in achieving nutritional recommendations[4].

Yogurt is an essential fermented milk product that provides digested lactose. Two viable strains of bacteria are utilized for fermentation (Streptococcus thermophiles and Lactobacillus bulgaricus)[5]. Yogurt is associated with essential nutrients (protein, potassium, vitamin B 12, calcium, vitamin B2 and used as vehicle for fortification process. Yogurt is one of ancient products that is named as Dahi, Zabadi, mast, roba in India,

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Egypt, Iran and Sudan respectively. It was reported that milk products become human nutrient part in 10,000 to 5000 B.C3. In India, milk products were associated with 6000 B.C referring to several health benefits. Founder of Mughal Empire, The Genghis Khan was reputed for providing yogurt to his army as symbol of bravery in worriers. King Francoise introduced dairy products in Western Europe while in 20th Century, scientist explained several health benefits

Globally, 40-50% individuals daily consume yogurt for quality of life improvement. An allergy to cow's milk, lack of accessibility and lactose intolerance are major barriers for consumption of dairy products. European Union Nations reported that daily dairy intake was 266g/day in 16 Unions. However, Finland and Denmark had daily calcium intake with dairy products approximately 1000mg/day[7]. An estimated 90-95% of female and 75-90% males were recommended 3 servings per day in United States. In Brazil, 99% of adults are calcium Deficit. Moreover, children in Brazil had 500 to 600 mg calcium per day in 99% of cases due to low yogurt intake[8].

An estimated 35-45% population utilize yogurt in daily diet plan. Males are more likely to use yogurt in diet as compare to females in Pakistan (25% vs 15% respectively). Literature reported that every 5 children/1000 had yogurt as significant component of diet in rural areas of Pakistan as compare to urban areas[9].

Yogurt is manufactured by many methods which results into a lot of differences among the quality of products. In Pakistani local markets, yogurts are usually manufactured in polluted/unhealthy conditions and are processed underestimating the SOPs of products, which is hazardous for health. Furthermore, un-processed yogurts are sold openly in shops in inappropriate temperature without covering the pot. Despite this fact, there is still a high demand for quality, stability, taste and shelf life of the yogurt from consumers. Therefore there is a much need of research for qualitative assessment of yogurt to aware the common masses of the society. The rationale of this study is to do the qualitative assessment of physicochemical and microbiological properties of available yogurt in Peshawar market. It will helps us to draw the true picture of physicochemical and microbiological parameters of quality of yogurt available to improves the overall health and quality of life of the general people of community.

MATERIAL AND METHODS

A cross-sectional (descriptive) study was conducted from February 2018 to August 2018, samples of yogurt collected from the markets of district Peshawar, KP, Pakistan. Sample size was calculated using following formula n = is the desired sample size for the study,d = is the margin of error, set at 5% (0.05) for the purpose of this study, P = 0.1,q =p-1=0.9,z = is the standard normal deviation generally set at 1.96 which relates to the 95% confidence level.

A total of 150 samples of processed and unprocessed yogurt was collected from the four towns of district Peshawar through multi-stage probability sampling technique. In first stage, Peshawar was divided into four towns, and two towns were selected randomly. In the second stage, out of total twenty two (22) union councils, five (5) each was selected from each town through simple random sampling technique. And finally from each union council, five (5) super stores and fourteen (14) small shops were selected randomly. From each super store, one sample of processed yogurt was selected and similarly from each union council five (5) samples of unprocessed yogurt was selected to have 150 sample size, as given in flow chart given below.

Data collection for the study was started after synopsis approval was granted by Graduate Committee, Advance Study Review Board (ASRB) and Ethical Board. The Yogurt samples was categorized in two categories; P Group (Processed yogurt) and UP Group (Unprocessed Yogurt). Ten (10) samples of processed and one hundred & forty (140) samples of unprocessed yogurt was collected from the local markets under standard technique for collection of food samples under proper hygienic conditions. All the samples were coded & labeled to keep privacy and avoid bias. Samples were then brought to laboratory of Khyber medical college in a very controlled hygienic condition by placing it in a wide mouthed large ice-pot having the temp of 4-5°C. The weight of each sample was 200g packet in small size sterile plastic pots. The laboratory analysis was carried out in the Department of Public Health & Community Medicine, Khyber Medical College, Peshawar.

All the samples were analyzed in the Public health laboratory Khyber Medical College Peshawar. The collected yogurt samples were prepared and analyzed for different Physicochemical & Microbiological parameters. "Association of Official Analytical Chemists method is used to measure the physicochemical properties.PH meter is used to measure directly the PH of yogurt. Titrable acidity is calculated when 15 ml of the yogurt is titrated with 0.1M NaOH so that a product of 8.2 PH is attained, reaching to the phenolphthalein end

point. Total solids and moisture content measurement is done through drying sample of yogurt by oven at 105 C for almost 3 hours. Reading is noted at a constant weight. Ash: According to the AOAC Ash content in the dry yogurt samples can be measured at 550 degree Celsius. The inorganic residue left in a percentage of the total weight of yogurt incinerated was then the ash content of the yogurt sample.Fat content is calculated by the modified ''Mojonnier ether extraction method''. The viscosity of yogurt according to AOAC is measured using an apparatus called Viscometer containing a glass tube and a normalized ball along with a chronometer at 20 °C.The temperature of the yogurt samples were measured by dipping a glass thermometer into the yogurt sample and the corresponding reading was noted in Celsius.Specific gravity was measured using a device called lacto-densitometer and was expressed in unit of g/ml.Microbiological quality of the yogurt was measured by the culture method.

A Method known as ''Pour plate method''' was used for the determination of total viable bacterial count. In this method 1 ml of sample was taken in the appropriate dilution in a sterile Petri dishes and was incubated at temperature of 32 ± 1°C for time of 48 ± 3 hr. And finally Total viable bacteria were counted under the microscope and was expressed in number. MacConkey agar method was used to calculate the coliform count in yogurt. For this purpose, the plates will incubated at temperature of 37°C for time of 48 hr. Total coliform bacteria was counted under the microscope and was expressed in number.

A specific agar ''Mannitol salt'' was used for the determination of of Staphylococcus aureus. 1 ml from each sample decimal dilution was sprinkled on the surface of pre-solidified mannitol salt agar medium and incubated at temperature of 37°C for time of 48 hr.

For determination of lactobacilli bacteria count in yogurt A decimal dilutions of the sample were streaked on solidified sterile MRS (DeMan, Rogosa and Sharpe) medium, and plates were incubated at temperature of 37°C for time of 48 ± 3 hrs. Lactobacilli bacteria were counted under the microscope and were expressed in number.

The total count of yeasts and molds was determined using yeast extract agar. The plates were incubated at 25°C for 5 days. Yeast and mold was counted under the microscope and was expressed in number.

Data was analyzed using statistical package for social sciences (S.P.S.S) version 23 for windows. All results were presented in the form of tables and graphs.

RESULTS

Two towns of Peshawar district were selected randomly out of four towns. One was Town 1 and 2nd was Town 2. For Town 1 the total number of samples planned were 75 (70 Unprocessed and 5 processed yogurt samples) similarly, For Town 2 the total number of samples planned were 75 (70 Unprocessed and 5 processed yogurt samples) resulting a total of 140 (93%) unprocessed and 10 (7%) processed yogurt samples of yogurt from both towns of district Peshawar. The samples collected after given consent were 150 (100%).

With regards to physicochemical properties the mean PH for the samples was 5.1375 ± 0.3366, Ash content with mean of 4.2772± 1.046. Mean specific gravity of total samples was 1035.9 ± 3.433. Fat content was with mean of 4.8 ± 0.954. Mean of total solid was 16.053 ± 1.937. Mean Titratable acidity was found to be 1.1467 ± 0.1210. The content of Moisture was 4.6621 ± 1.3015. Mean Viscosity and temperature of the samples was 1276 ± 187 and 5.66 ± 1.46.

While, for microbiological properties of the yogurt samples the mean total viable count was 5.84± 1.71. Mean Coliform count was with numbers of 2.313 ± 3.01. The Staphylococcus Aureus Count was 1.569± 2.616 and Yeast count was 23.03± 27.01. Moreover, Mean Mould count was 5.22± 2.09. Physicochemical properties comparison between processed and Un-processed yogurt

An independent sample t-test compared the physicochemical parameters for the total samples of yogurt taken for this study among the types of yogurt (Process (P)/Un-process (UP)). P-value of less than 0.05 was taken significantly. The findings in the table 4.3 revealed that there is no statistical difference in mean values of processed and Un-processed yogurt among all physiochemical properties i.e. pH, Ash, Sp. Gravity, Fat, Total solids, Moisture, Viscosity and Temperature. However, one parameter i.e.for Titratable acidity was significant among both groups of yogurts (Process (P)/ Un-process (UP)) and mean value of titratable acidity was greater in Un-processed yogurt (1.1532 ± .07826) compared to processed (1.0560 ± .37197) with ($t = -2.495$; $p = 0.014$). As can be seen in Table 4.3for the others physiochemical parameters there is no significance difference in mean values among processed and unprocessed yogurt.

It is seen that mean value of PH for processed yogurt is (4.9850 ± 0.52834) and for Un-processed yogurt (5.1484 ± 0.31875) with (t = -1.489; p = 0.139). Regarding ASH content, for processed yogurt is mean value is (4.4150 ± 1.24496) and for Un-processed yogurt (4.2674 ± 1.03573) with (t = .430; p = 0.668). Concerning the Specific Gravity, for processed yogurt Sp: gravity mean value is (1036.2000 ± 3.42540) and for Un-processed yogurt (1035.9071 ± 3.44514) with (t = 0.260; p = 0.795).Related to the Fat content, its mean value for processed yogurt is (4.8800 ± .62858) and for Un-processed yogurt (4.7964 ± .97548) with (t = 0.267; p = 0.790).

Moreover, that mean value of Total solids for processed yogurt is (17.0000 ± 1.49071) and for Un-processed yogurt (15.9857 ± 1.95262) with (t = 1.607; p = 0.110).With regard to the Moisture content, its mean value for processed yogurt is (5.0470 ± 1.40415) and for Un-processed yogurt (4.6346 ± 1.29495) with (t = -1.511; p = 0.335).

Finally,the mean values of viscosity and temperature among processed yogurt found is (1190.0000 ± 196.92074) and for Un-processed yogurt (1282.1429 ± 185.54522) with (t = 0.968; p = 0.133) and mean temperature in processed yogurt is (5.8000 ± 1.54919) and in Un-processed yogurt (5.6571 ± 1.46799) with (t = 0.296; p = 0.767).

Microbiological properties comparison between processed and Unprocessed yogurt. The statistics of table 2 revealed that there is no statistical difference in mean values of processed and Un-processed yogurt among all microbiological properties i.e. Total Viable Count, Coliform count, Staph. Aureus count, Yeast count and Mould count.It is seen that mean value of Total Viable Count for processed yogurt is (5.7361 ± 2.06556) and for Un-processed yogurt (5.8536 ± 1.69293) with (t = -.209; p = 0.835). Regarding Coliform count, for processed yogurt its mean value is (3.0652 ± 3.23389) and for Un-processed yogurt (2.2598 ± 2.99924) with (t = 0.816; p = 0.416).Concerning the Staph;Aureus count, for processed yogurt Staph. Aureus count mean value is (1.7757 ± 2.86094) and for Un-processed yogurt (1.5547 ± 2.60820) with (t = 0.257; p = 0.797). Related to the Yeast count, its mean value for processed yogurt is (20.2000± 28.82437) and for Un-processed yogurt (23.2357 ± 27.07905) with (t = -.341; p = 0.734).Moreover, that mean value of Mould count for processed yogurt is (5.6000 ± 2.87518) and for Un-processed yogurt (5.1929 ± 2.03529) with (t = 0.593; p = 0.554). Association of Physicochemical properties with WHO reference range using chi-square:

The table 3 below delineate that the results for PH were not statistically significant with p-value (.080) and majority of processed 80.0% were within the normal range similarly among unprocessed 94.3% were in reference range of WHO. The Ash content of processed yogurt was not in range and 94.3% of unprocessed yogurt were not in normal range. Among both processed and unprocessed yogurt almost 40% of yogurt were having acceptable range for specific gravity. All the sample of both processed and yogurt were having the fats content in normal range .For titratable acidity the results were significant with p value of 0.000 and majority of the sample 90% processed and 100% unprocessed yogurt were not lying in the normal range. Moreover the moisture content were high in almost 60% of processed yogurt and 52% of unprocessed. The temperature was maintained for both groups of sample and were in suitable temperature. Association of Microbiological properties with WHO reference range using chi-square:

The table 4 shows the results that majority of (90%) of processed yogurt and 92% of unprocessed yogurt were having highest bacterial content of total viable count similarly the mould count was also high in both groups. However the coliform count, Staph. Aureus Count, and yeast count were in the normal in majority

$$n = \frac{z^2(pq)}{d^2}$$

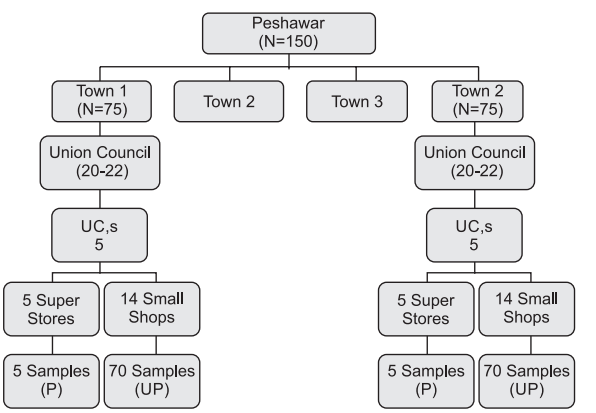

Fig 1: Sampling Frame for data collection

Table 1: Physicochemical properties comparison between in processed and Unprocessed yogurt by Independent sample t-test.

	Process N= 10 Mean ± S.D	Unprocessed N= 140 Mean± S.D	P-value	T	95% Confidence Interval of the Difference	
					Lower	Upper
PH	4.9850 ± .52834	5.1484 ± .31875	0.139	-1.489	-.38029	.05343
Ash	4.4150 ± 1.24496	4.2674 ± 1.03573	0.668	.430	-.53131	.82659
Sp. Gravity	1036.2000 ± 3.42540	1035.9071 ± 3.44514	0.795	.260	-1.93482	2.52053
Fat	4.8800 ± .62858	4.7964 ± .97548	0.790	.267	-.53609	.70323
Total solids	17.0000 ± 1.49071	15.9857 ± 1.95262	0.110	1.607	-.23262	2.26119
Titratable acidity	1.0560 ± .37197	1.1532 ± .07826	0.014	-2.495	-.17420	-.02023
Moisture	5.0470 ± 1.40415	4.6346 ± 1.29495	0.335	.968	-.42973	1.25445
Viscosity	1190.0000 ± 196.92074	1282.1429 ± 185.54522	0.133	-1.511	-212.62083	28.33512
Temperature	5.8000± 1.54919	5.6571± 1.46799	0.767	.296	-.80997	1.09568

Table 2: Microbiological properties comparison between processed and Unprocessed yogurt by Independent sample t-test.

	Process N= 10 Mean ± S.D	Unprocessed N= 140 Mean± S.D	P-value	T	95% Confidence Interval of the Difference	
					Lower	Upper
Total Viable Count	5.7361 ± 2.06556	5.8536 ± 1.69293	.835	-.209	-1.22869	.99371
Coliform count	3.0652 ± 3.23389	2.2598 ± 2.99924	.416	.816	-1.14421	2.75497
Staph. Aureus count	1.7757 ± 2.86094	1.5547 ± 2.60820	.797	.257	-1.47644	1.91851
Yeast count	20.2000 ± 28.82437	23.2357 ± 27.07905	.734	-.341	-20.62219	14.55076
Mould count	5.6000 ± 2.87518	5.1929 ± 2.03529	.554	.593	-.94863	1.76291

Table 3: Association of Physicochemical properties with reference range using chi-square.

Variables N= 150		Within WHO Reference Rang		Not within WHO Reference Rang		P-value
		Frequency	%	Frequency	%	
pH	Process	8	80	2	20	.080
	Unprocessed	132	94.3	8	5.7	
Ash	Process	0	0	10	100	0.437
	Unprocessed	8	5.7	132	94.3	
Sp. Gravity	Process	4	40	6	60	0.860
	Unprocessed	60	42.9	80	57.1	
Fat	Process	10	100	0	0	-
	Unprocessed	140	100	0	0	
Titerable acidity	Process	1	10	9	90	0.000
	Unprocessed	0	0	140	100	
Moisture	Process	4	40	6	60	.631
	Unprocessed	67	47.9	73	52.1	
Temperature	Process	10	100	0	0	-
	Unprocessed	140	100	0	0	

*Significant p-value (chi square test applied)

Table 4: Association of Microbiological properties with reference range using chi-square.

Variables N= 150		Within WHO Reference Rang		Not within WHO Reference Rang		P-value
		Frequency	%	Frequency	%	
Total Viable Count	Process	1	10	9	90	0.738
	Unprocessed	10	7.1	130	92.9	
Coliform count	Process	5	50	5	50	0.391
	Unprocessed	89	63.6	51	36.4	
Staph. Aureus Count	Process	7	70	3	30	0.805
	Unprocessed	103	73.6	37	26.4	
Yeast count	Process	8	80	2	20	0.181
	Unprocessed	82	58.6	58	41.4	
Mould count	Process	2	20	8	80	0.470
	Unprocessed	17	12.1	123	87.3	

of samples with insignificant p values i.e. 0.391, 0.805, and 0.181 respectively.

DISCUSSIONS

As per our knowledge, this was first effort to measure patient physiochemical and microbiological parameters of yogurt samples in district Peshawar. Quality assessment of yogurt is an emerging trend in public health quality assessment. One the basis of our study result we have different observation found between processed and unprocessed yogurt regarding, physicochemical properties and bacterial load. In present study, total 150 Yogurt samples were included in study. There were 10(7%) processed and 140(93%) Un-processed samples. Data regarding physicochemical properties of fresh and UHT milk are shown in table 4.3 Imtiaz et al reported that in quality assessment of yogurt processed and unprocessed yogurt had significance importance. They reported that processed yogurt are more likely to be contained as compare to unprocessed yogurt ($p<0.05$)[10] Among all the 150 samples analyzed In present study, among process yogurt (N=10), mean pH was 4.9850± 0.52. while un-processed yogurt (N=140), mean pH was 5.1484±0.31875. there were no significant difference with mean PH of both groups (P= 0.139). These findings were not same to the study of Kosikowski et al in which he found that pH of yogurt was significantly higher in un process yogurt as compare to process yogurt ($p=0.05$)[11,12]

With reference to Ash content in this study, it was revealed that samples taken from process yogurt have mean ash 4.415± 1.2449. On the other hand un-processed yogurt was having mean ash was 4.2674± 1.035 with no significance difference (P=0.668). these findings were not in line with a study conducted by Kuchroo et al. in which he reported that ash contamination was higher in process yogurt as compare to un process yogurt ($p=0.01$)[13,14]. For process yogurt (N=10), the mean specific gravity was found to be 1036.20± 1.3.425 and for un-processed yogurt, mean specific gravity was 1035.90±3.44514 with (P=0.795). These results were contradictory to study of Liong et al, in which he concluded that process yogurt is more likely to have high specific gravity as compare to un process yogurt ($p=0.00$)[15,16] with relevance to the Fats content in present study, in process yogurt mean fat was 4.880± 0.628 and in un-processed yogurt, mean fat was 4.7964±0.97548 (P=0.790). The results were in accordance with the studies by Otieno D et al. and Ouwehand A et al that there was no statistically significant difference in process and unprocessed yogurt. ($p>0.05$)[17,18] with regard to the total solid content in our study, for process yogurt, mean total solids were 17.00± 1.49. However in unprocessed yogurt, mean total solids was less than processed yogurt 15.985± 1.952 but not significant (P=0.110). A similar study done by Rani et al reported that total solid content was high in process yogurt as compare to un process yogurt ($p=0.01$)[19,20].

Mean titratable acidity was 1.0560 ± 0.371 for processed yogurt and for unprocessed yogurt it was higher, 1.1532± 0.07and found to be statistically significant with (P=0.01). A study by SAS reported that titratable acidity had significant positive correlation with process yogurt quality ($r=0.8$)[21,22]. According to the independent sample t-test the mean difference of moisture content in process and unprocessed yogurt was not significant. In process yogurt mean was 5.0470± 1.404 while un-process yogurt, mean moisture was 4.6346±1.294 with (P=0.968).these findings are not linear with the findings of in a study reported that un process yogurt had significant higher moisture content as compare to process yogurt ($p=0.00$)[23].

Mean Viscosity for the process yogurt, was 1190± 1.96SD and for unprocessed yogurt mean viscosity was 1282.14± 185.54 however with no significant p value (P=0.133). On the other hand a study by Vinderola et al reported that viscosity is significantly associated with process yogurt quality as compare to un process yogurt (p=0.00)[24].

There is no statistical difference found in the temperature between both groups in our study .In process yogurt mean temperature was 5.80± 1.54. But for unprocessed yogurt, mean temperature was 5.65± 1.46 having P=0.296 .These findings were consistent with the findings of Vinderola et al in which they reported that there is no significant difference in temperature of process and un process yogurt (p>0.05)[25].

Regarding the microbiological properties of yogurt and its comparison in both groups ie processed and unprocessed yogurt were not significant for all of the 5 parameters in this study. In present study, among all samples taken from process yogurt mean total viable count was 5.736± 2.06. and for all sample taken from unprocessed yogurt, mean total viable count were slightly higher ie 5.8536± 1.69 but not significant (P=0.835). The variation seen in our study are linear to the study of Yang et al that total viable count was insignificantly differ in both process and un process group (p>0.05)[26].

The Coliform count in process yogurt was 3.065±3.23SD. but lower in un-processed yogurt, with mean coliform count 2.2598± 2.999 with insignificant difference in both groups (P=0.416). These findings contradict the results of Yazici et al that coliform count was significantly higher in un process yogurt as compare to process yogurt (p=0.05)[27].

No significant difference was found in both groups of yogurt regarding Staph. Aureus Count but it was higher in process yogurt, with mean 3.065±3.23 and mean count for unprocessed was 2.2598± 2.99 having (P=0.416). However, Yazici et al in their study that coliform count was significantly higher in un process yogurt as compare to process yogurt (p=0.05)[28].

For process yogurt Yeast count in present study, was 20.20±28.82 and mold count was 5.600 ± 2.87. While for unprocessed yogurt, mean yeast count was 23.23± 27.07and mold count was 5.19 ± 2.03. P-value for both parameters was not significant i.e.(P= 0.734) and (P=0.554) respectively.

CONCLUSIONS

The unprocessed yogurt were found to be more viscous as compared to processed yogurt. Specific gravity and temperature of both groups were almost same with very marginal difference. Coliform count, Staph. Aureaus count and mould count were less in unprocessed yogurt The titrable acidity was significantly difference in processed and unprocessed yogurt..

Recommendation for further research

The researcher suggests that some further studies should be conducted in this area for with the aim re-examine those indicators and variables in the recent study which were having a significant relationship with greater microbial load and to identify the gaps. Further studies should incorporate other variables such as the source of the milk, and their effect and association with the physicochemical and microbiological properties of yogurt which couldn't be evaluated in this study.To get a complete understanding of the associations with these parameters more variables should be included.

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Following authors have made substantial contributions to the manuscript as under:

Junaid N: Developed study, manuscript writing.

Bilal S: Methodology and laboratory analysis.

Mustafa A: Statistical analysis.

Ayub R: Referencing and critical analysis.

Authors agree to be accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved.