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Evaluation of *Escherichia Coli* and *Salmonella spp.* in Yoghurt Processing in Khartoum State – Sudan

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Abstract

The aim of this study was to investigate the total viable bacterial load in the different stages of yoghurt processing, and to isolate and identify *Salmonella spp*. and *Escherichia coli* during the period from November 2015 to October 2017 in yoghurt production unit in Khartoum- Sudan. Sixty samples were collected at different six stages of the processing (Whole milk, pasteurized milk, cold pasteurized milk, milk +starter, after incubation and Cold Yoghurt)where the isolation and identification of the two types of bacteria (*Salmonella spp.* and *Escherichia Coli*) were investigated. The results revealed that the Total Viable Count (T. V. C.) indicated the highest level of contamination in Whole Milk stage (Mean (Log.CFU/ml) = 6.93±0.20 in which the positive samples for *Salmonella spp.* 2(3.84)% and *Escherichia coli* . 10(19.16)% while the lower contamination level was at hot pasteurized milk stage (Mean (Log-CFU/ml)=6.34±0.07 in which the positive samples for *Salmonella spp*. 2(3.6) %, and *Escherichia coli* . 3(5.4)%.The statistical analysis of the result revealed there was a significant difference at ($P \leq 0.05$) of total viable bacterial count in different processing stages of yoghurt production The study concluded that, contamination was reported in all stages of yoghurt production processes with *Escherichia Coli* and *Salmonella spp*.

Keywords: Yoghurt production ,Pasteurized milk, Contamination

Introduction

There is evidence of fermented milk products being produced as food for at least 8000 years. The earliest yogurt was probably spontaneously fermented by wild bacteria living on the goat skin carried by nomadic people, (Yildiz 2010). Yoghurt and related yoghurt like dairy beverages are probably the first functional foods to be researched by the scientific community .Yoghurt is a very nutritious food and its continued consumption in the western world owes much to the development of its health food image(Leyer , 1995). Consumption of yoghurt is highest in countries around the Mediterranean, Asia, and central Europe,(Bylund 1995). Today many

different counties claim yogurt as their own invention, yet there is no clear evidence as to where it was first discovered, and it may have been .Independently discovered several times. Sudan is a large country with different climatic conditions and considered as one of the largest countries in animal resources . According to the international classification it is the 1st in the number of camel 3.54 million heads ,the forth in the number of goats 43.14 million heads, the sixth in the number of cattle 40 million heads and the seventh in the number of sheep 49.05 million heads,(SSMO 2007). The art of making ZABADI (yoghurt) came to Sudan from Egypt, must likely during the time of the Anglo-Egyptian rule (1898-1956).it was prepared by households, in its preparation cow's milk is boiled, cooled and inoculated by back-shopping from previous lot, it is then incubated in worm carrier where it sours and refrigerated and consumed with sugar as a desert or to eat wheat bread with .Sometimes it is fed to babies and often turned into sauce for *Aceda*,(Mortada, 2012). Dirar (1992) divided the fermented dairy products of Sud an into two major groups, the truly indigenous which include *roub*, *gariss* and *mish* , and the quasi-indigenous which include *Zabadi* and *Jibna beida*

Food-borne diseases caused by non-typhoid salmonella an important public health problem worldwide, nearly 1-4 million cases of salmonellosis in human occur each year in the United States (David *et al*.,2001). The ability of *Escherichia Coli*. to survive in high –acid food as in case of yoghurt is of public health significance *Escherichia Coli* a natural inhabitant of the tracts of humans & worm-blooded animals, is used as an indicator bacterium because it acquires antimicrobial resistance faster than other conventional bacteria (Miranda *et al*.,2007). HACCP as the system of choice for ensuring food safety and is becoming enshrined in national legislation proactive application in food industry will facilitate compliance with developing legislation and demonstrates a diligent approach to food safety ,(Jervis 2002). The HACCP procedure is generally targeted at food safety management (pathogenic microorganisms and their toxins) but as an approaching the context of border quality management. HACCP system which is science based and systematic identified specific hazards and measures for their control to ensure the safety of food, (Roberts 2001).

The objectives of this research work were to indentify microbial contamination point for establishing the critical control points(CCP) in yoghurt manufacturing processes and to evaluate the bacteriological level contamination of yoghurt with Salmonella spp. and Escherichia coli during manufacturing processes.

Materials and Methods

Sampling:

Total of 60 samples were collected in test tubes from 6 stages of yoghurt production process, from yoghurt production unit in Khartoum. Sudan. These samples were divided into 2 groups, firstly group of 30 samples, and then (30)samples repeated for the same stages. Samples were taken at different stages of yoghurt production process . These samples for microbiological analysis were taken aseptically and kept between 1-5 c˚, samples for chemical analysis analyzed immediately(Hoolasi,2005). Samples were collected from whole milk stage, pasteurized milk stage, cold pasteurized milk stage, milk+starter stage, after incubation stage , and cold yoghurt stage were taken aseptically and kept, then transferred to laboratory for microbiological and chemical tests to determine the general health condition of

yoghurt, and to detect the critical points in the process .

Total Viable Bacterial Count

The (T.V.B.C.) was conducted by making of a 10- fold serial dilutions of each sample (Mile and Misera,1938).Each dilution was then cultured by the pouring plate method using the standard plate count agar medium and cultured plates were then incubated at 37c for 24 hours .After that the number of all colonies was counted for each dilution and the mean count was determined.

Isolation of *Salmonella spp*. and *Escherichia coli*:-

The standard procedures for isolation and identification of *Salmonella species*, *Escherichia coli*, were conducted by using the surface plate method and the respective media (Barrow and Felthman 1993).The collected samples cultured in plates incubated at 37C° for 24 hours. *Escherichia Coli* Isolated in MacConKeys Agar Media(gives colorless, not fermenting lactose), or pink color for that fermenting lactose. *Salmonella spp*.were Isolated in Dexyochoclate Citrate Agar gives pale yellow color, not fermenting lactose.

The data were analyzed with statistical package for Social Science (SPSS), version software (SSPS Inc, and Chicago IL, USA). All bacterial counts were converted to log 10CFU/ml (g) for analysis and ANOVA was performed. Statistical significance was set at a p-value of $p<0.05$.

Statistical analysis

The generated data were subjected to analysis of variance. The difference between means was separated by LSD test.

Results

The Total Viable Count for yoghurt at different stages of productions from the first stage whole milk up to last stage of production yoghurt at packing, are shown in table(1) .Identification of *Salmonella spp*. and *Escherichia coli* by biochemical tests are shown in Table(2).

Table 1: Total viable bacterial count ($X10^6$) (cfu/ml) at the different stages of yoghurt processing

Stage	Bacterial count	minimum	Maximum	significance
Whole milk	7.825 ± 2.45^b	2.15	10.55	
Pasteurized milk	2.133 ± 0.26^a	1.70	2.35	
Cold pasteurized milk	7.405 ± 0.94^b	6.45	9.50	**
Starter addition	7.030 ± 0.91^b	5.75	8.55	
Incubation	7.720 ± 0.89^b	6.40	9.50	
Cold yoghurt	7.430 ± 0.95^b	6.25	9.45	

P-value ≤ 0.05 **

a,b: Means within the same column followed by different superscripts are significantly ($P<0.05$) different

As shown in table (1) Figure (1)whole milk Point showed contamination (mean (log10 cfu/ml) 7.825±2.45 . Hot pasteurized milk Point showed contamination (mean (log10 cfu/ml) 2.133±0.26 . Cold pasteurized milk Point showed contamination (mean (log10 cfu/ml) 7.405±0.94 .Milk + starter Point showed contamination (mean (log10 cfu/ml) 7.030±0.91. After incubation 7.720±0.89. Point showed contamination (mean (log10 cfu/ml). Cold yoghurt Point showed contamination (mean (log10 cfu/ml) 7.430±0.95.

Table 2: Biochemical Tests:

Primary chemical tests

Type of bacteria	**Oxidase Test**	**Catalase Test**	**OF Test**	**Sugar Test**	**Motility Test**
***Escherichia** coli*	-	+	F	+	Motile
Salmonella	-	+	F	+	

Secondary chemical tests

Type of bacteria	Indole (Kovac-s) Test	Urease Test	**Citrate Test**	**KIA(kilger Iron Agar)tests**			
				Slope	H2s	Butt	Gas
Escherichia coli	+	-	-	Yellow	yellow	-	+
Salmonella	-	-	+	Red	yellow	+	+

OF=Oxidation &Fermentation

The results in Table (3) showed that the number of isolated bacteria(*Salmonella spp*. and *Escherichia coli*) in different points in yoghurt processing as follows :in Whole milk, The number of isolated bacteria in 12 samples, 2(3.84)% samples were positive for Salmonella and 10(19.16) for Escherichia coli. In Pasteurized milk, in 5 samples, 2(3.6)% samples were positive for Salmonella and 3(5.4)% for *Escherichia coli* .In Cold pasteurized milk , in 10samples, 4(7.6)% samples were positive for *Salmonella* and 6(11.4)% for *Escherichia coli.* In Milk +starter, in 8 samples, 3(5.63)% samples were positive for *Salmonella* and 5(9.37)% for *Escherichia coli*. In After incubation in 6 samples,3(5.63)% samples were positive for *Salmonella* and 3(5.4)% for *Escherichia coli.* In Cold yoghurt, in 9 samples 3(6)% samples were positive for *Salmonella spp.* and 6(12)% *for Escherichia coli.*

Table 3 : Number and Percentage of *Salmonella spp*. and E-coli isolated from different stages of yoghurt processing

No	Stage	*Salmonella Spp.%*	*Escherichia coli%*	Total %
1	Whole milk	2(3.84)%	10(19.16)	12(23)%
2	Pasteurized milk	2(3.6)%	3(5.4)%	5(9)%
3	Cold pasteurized milk	4(7.6)%	6(11.4)%	10(19)%
4	Milk+starter	3(5.63)%	5(9.37)%	8(15)%
5	After incubation	3(5.63)%	5(9.37)%	8(15)%
6	Cold yoghurt	3(6)%	6(12)%	9(18)%
	Total	17(32.3)%	35(66.7)%	52(99)

Isolation and identification of bacteria at different operation process under investigation showed that , whole milk stage as high level of contamination and the T.V.C . 6.93±0.20 with *Salmonella spp*. was 23.84% and *Escherichia coli* 19.16% ($p \leq 0.05$).While the lower level of contamination in hot pasteurized milk and the T.V.C. with *Salmonella spp*. was 3.6% and *Escherichia coli* 5.4% as in table 3 .

Discussion

In this study bacterial contamination was obtained in all stages of yoghurt process Table(1)which was in agreement with Montville, (2005) who stated that fermented dairy products are often not manufactured under sterile conditions or with sterile milk (unpasteurized) and this can allow non-starter Lactic Acid Bacteria (LAB) as well as spoilage or pathogenic bacteria to gain access to the fermenting food system. In general, within the *Escherichia* genus, pathogenic *Escherichia coli* organisms are significantly coli forms is one of the standard tests required by the International Dairy Federation (Mossel et al. 1995), the ability of Escherichia coliO157 :H7 to survive in high-acid food is of public health significance. Also the results agree with Johonson(2013) who stated that bacteria associated with dairy fermentations can grow over a wide temperature range from 4 to 5 C°.Mesophilic bacteria have an optimum growth range of 25 -35 C°., while Thermophilic species have an optimum range of 37 -45 C°.

The present research agrees with Yang et al. (2012) who stated that bacteria are naturally present and are used extensively across all areas of dairy and food fermentation either as natural micro- flora or as starter culture added under controlled conditions.

Morgan *et al* (1993) reported that number of potential problems could be identified at dairies : the milk might be inadequately pasteurized, or contaminated after pasteurization either because of inadequate cleaning of systems or by farm yard matter. In the present results the Total Viable Count (T.V.C.) showed the lower contamination stage is after pasteurization hot milk, and this agrees with FAO(2006) which reported that the heat treatment using sterilization methods completely inactivates enzymes and destroyed the most heat resistant micro organisms

D'aost (1989) who stated that standard methods of pasteurization both via pasteurization and high-temperature, short time pasteurization, are very effective in destroying *Salmonella spp*. and this agrees with the present findings.

Crow *et al*. (2001) agreed with this study and stated that pasteurization inactivates pathogenic bacteria ,but also the results in significant reduction or in activation of naturally occurring micro-flora population.

On conclusions reported that contamination was detected in all yoghurt production process. *Escherichia Coli* and *Salmonella spp.* were detected and isolated from 6 stages of yoghurt production process, the lower contamination stage was after pasteurization hot milk, the highest contamination stage was in whole milk

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تقييم البكتريا الاشريكية القولونية وانواع السالمونيلا في تصنيع الزبادي في ولاية الخرطوم - السودان

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المستخلص

هدفت هذه الدراسة تقصي العد الكلى للبكتريا في مراحل تصنيع الزبادي المختلفة وعزل ومعرفة بكتيريا السالمونيلا والاشريكية القولونية في الفترة من نوفمبر 2015 وحتي اكتوبر 2017 فى وحدة تصنيع الزبادي بولاية الخرطوم- السودان. جمعت 60 عينة من ست مراحل مختلفة للتصنيع (الحليب الخام , الحليب المبستر الدافئ ،الحليب المبستر البارد، الحليب مع البادئ ,الحليب بعد التخمير والمنتج الزبادي البارد)وتم عزل ومعرفة نوعين من البكتيريا وهما بكتيريا السالمونيلا وبكتيريا الإشريكية القولونية في ست مراحل خلال عملية تصنيع الزبادي حيث أظهرت النتائج ارتفاع عالى لمستوي العد البكتيري بمتوسط وانحراف معيارى(6.93±0.05) فى الحليب الخام وكانت نسبةالعينات الايجابية للسالمونيلا2 (3,84)% وللاشريكية القولونية10(19,16)% و المتوسط والانحراف المعياري هو(0,05 ±6.94) وأقل انخفاض للتلوث كان في مرحلة البسترة الدافئ بمتوسط وانحراف معيارى(6.34±0.0) حيث ان نسبةالعينات الايجابية للسالمونيلا 2 (3,6) % و للاشريكية القولونية 3(5,4)% . أظهر التحليل الإحصائي للنتائج أن هنالك فروق معنوية ($P\leq0.05$)بين مراحل تصنيع الزبادي المختلفة . توصلت الدراسة الى ان التلوث قد تم تسجيله فى كل المراحل التصنيعية لانتاج الزبادى.