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Identification of E.coli. In Sudanese White Soft Cheese

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Abstract: Study of dairy products is of great interest to the daily consumer. This research studied the total count of bacteria and the identification of the Escherichia coli (E.coli) bacteria of ready for sale and consumption white cheese.

Three samples of locally produced cheese were collected from market in Khartoum at different dates and stored in polypropylene (PP) packaging at temperature of 4ºC. The different samples were then subjected to laboratory tests to determine the total bacterial colonies and hence the total count of bacteria and E.coli in white cheese.

The quality of white cheese including chemical and microbial characteristics which are affected by several factors were studied. The average bacterial colonies for cheese showed differences at two different dilutions ($10^{-4}$and 10^{-5}) of the Agar medium used. The average numbers of the bacterial colonies for the three samples were 15.54, 11.74 and 9.79. The biochemical reaction of the isolated bacteria from the three samples of white cheese showed no E.coli presence, gram negative bacteria, however, was found to be present.

***Keyword**: White soft cheese, E.coli, Gram negative bacteria.*

1. INTRODUCTION

Cheese is known as a complete nutritious food productand excellent source of many key nutrients, suiTable formany ages. It is rich in protein and minerals such as calcium, there are different types of cheese: soft cheese, semi hard and hard cheese, the difference in these types is mainly due to water content or water activity and the methods and technology for cheese making [1]. Previous studies mentioned that the cheese traditionally produced in the Sudan is a white-brined variety known locally as Gibna Bayda “white cheese” and it is widely consumed by people of all socioeconomic classes; most of it is made in houses and some private farms.

Recently, due to increase of population, the cheese industry has become a common sight without supervision or quality. Sudanese white cheese is delivered to the market immediately after processing, under inadequate conditions, poor handling technique, inappropriate packaging materials and lack of adequate storage facilities, however, it seems that essential dairy products including cheese must be safe, accepTable and meet consumer's satisfaction [2],[3],[4]. As a result, cheese production must be protected from pathogenic and spoilage microorganisms, as well as from decaying both on the sites of production and consumption [5]. Different factors influence the quality of white cheese and therefore its nutritive value, these factors include: composition of food materials, the nature of the compounds, the type of packaging system and the preservative added [6]. Proper packaging method is very important for chemical, physical and microbial quality of white cheese.

Microorganisms present in Dairy products (fermented milk, cheese) belong to three groups. Those responsible for transmission of food borne diseases (pathogens); others that may cause defects in dairy products and the ones that produce desirable flavor and physical characteristics. Dairy products are the major vehicle for transmission of human diseases such as Brucellosis, Salmonellosis and Tuberculosis. Unless milk used for cheese processing is pasteurized or otherwise treated to destroy pathogens, pathogenic or toxin producing organisms present in Raw milk could be found in cheese. These organisms may find their way into cheese as a result of environmental contamination during processing and packaging. The objective of this study is to determine the effects of shelf-life aging and packaging on the quality of Sudanese white cheese and to identify the presence of bacteria using standard chemical and microbial parameter testing methods.

Numerous literatures has been published on the origin of cheese and its classifications, reader may refer to [7],[8],[9], [10] and [11] where in excess of 1000 cheese variety has been classified. Classification based on the moisture content has also been discussed in literature [12], [13], [14], [15] and [16]. Furthermore, classification based on coagulant used can also be found in literature, the reader may refer to [17], [15], [18], [19], [20], [21] and [22].

2. Materials and Methods

The quality of white cheese including chemical and microbial characteristics that are affected by several factors were studied. In this study the cheese samples were examined for total bacteria and E.coli. All selective media were prepared according to the manufactures’ manual.

2.1 Microbiological Analysis

2.2.1 Bacterial Count (Viable Count)

Plate Count Method

First prepare series of dilutions from the sample using 0.1% peptone water as diluents. This is done through a preparation of a 9 ml amount of diluents solution into sterile test tubes with. This is mixed well with a sample. A 1.0 ml is then delivered into the first diluents tube about one centimeter above the level of the liquid. From tube one a 1.0 ml is transferred to the next tube to make the second dilution ratio. This is repeated until all the required number of dilutionis obtained. The dilution ratios used for the purpose of this study are shown in Table 1.

Table 1. Dilution Ratio

Tube Number	Dilution Ratio
1	0.1
2	0.01
3	0.001
4	0.0001
5	0.00001

Drop Count Method

In this method small drops of the material are placed on agar plates, colonies are counted in the inoculated areas after incubation. This is first prepared in 20 dropper pipettes (the pipette deliver 0.05 ml 20 drops per ml). At least five drops of each dilution of sample from a height of not more than 2 cm is delivered to a dry plate (to avoid splashing) where they are left to dry and incubated at 37ºC for 24 hours. Plates showing discrete colonies in drops areas are selected; preferably each one should give less than 40 colonies per count, if the drops are uncounTable (more than 40 colonies) then the colony is counted per ml.

Nutrient Agar

The nutrient agar includes Beef extract, Yeast extract, and Sodium chloride. (Isotoniesolution) from: 1.5 g / dl, Peptone water phosphate buffered, source of vitamin, carbohydrate and protein and agar (polysaccharide agarose and agaropectin).

The ingredients are completely dissolved in the distilled water using a glass rod and adjusted to a pH of 7.1 using pH meter.5ml amount is dispensed into test tubes or 3ml amount into Bijou bottles. This is sterilized by an autoclave at 121ºC for 15 minutes. The tube or the bottles are placed in a slanting position so that the agar will solidify at an angle and incubated overnight to confirm its sterility before use.

Pouring Media in Petri Dish

The nutrient agar is melt in the water bath and cooled to about 45^0C. For a given Petri dish, the mouth of culture tube is flamed approximately 12 ml of the medium is poured into each Petri dish. The Petri dish is covered and maintained on a level surface, until the medium solidify (fig. 2). This is incubated at 37ºC for 18-24 hours to confirm sterility.

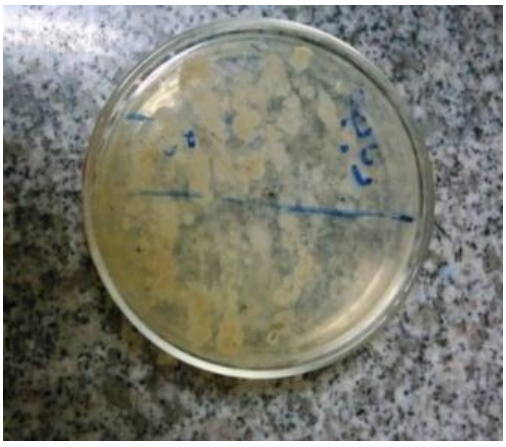

Fig.2. Media Studied in Petri dish

Culturing of Bacteria

For the streaking method employed (fig 3), the inoculums are smeared over area (A) (Fig 3) to give well inoculums. The well inoculums are spread in three parallel lines on to the fresh surface (B) of the medium and then repeated with a sterile loop for areas (C) and (D) as shown in Fig (3.15). The Petri dish is then incubated at 37ºC for 18 — 24 hours.

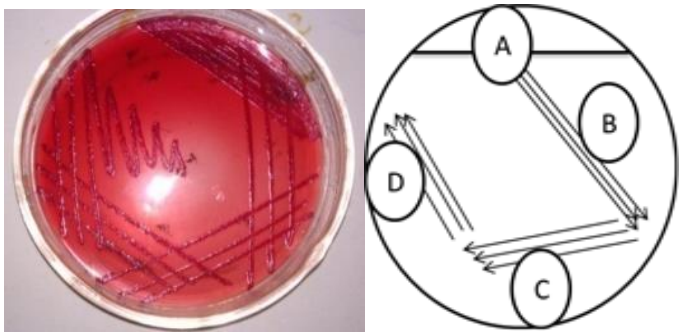

Fig.3. Plating out Method

Bacterial Smears or Films

In the preparation of bacterial smear film (Fig 4), one drop of normal saline is placed on the centre of the clean and dry slide. A small portion from the solid culture (strain A) is added using sterile loop and mix with the normal saline drop to make a fine suspension, spread evenly at the same time in an area 1.5 — 2.0 mm in diameter. The smear (film) is then left to air dry and fixed by passing the slide over flame (the smear uppermost) three times.

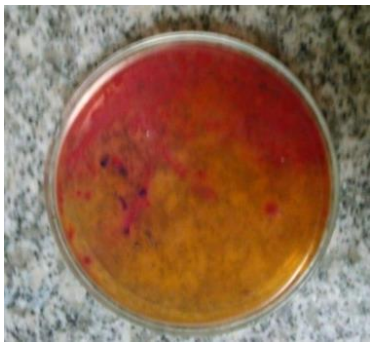

Fig.4. Bacterial Smears

2.2 Characterization Tests

2.2.1 Gram's Stain

A thin smear is placed on clean slide and left to air dry. A gentle heat is used to fix the smear. The fixed smear is then covered with crystal violet and leftfor 30-60 seconds and washed off with clean water. The Lugol's iodine is then added with clean water for 30sec and washed off with clean water. The smear is then decolorized rapidly with ethanol for few seconds before been washed with clean water. Saffranin is then added and left for further two minutes and then washed off with clean water. The smear is then dried and examined under the microscope using oil immersion objective. Gram positive bacteria should appear as dark violet while gram negative organisms as red or pink (fig.5).

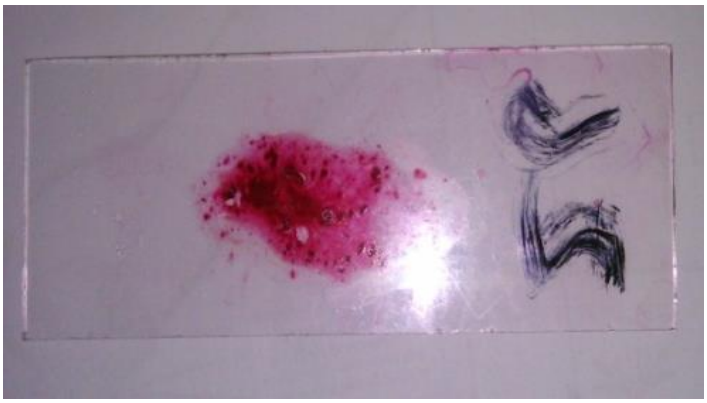

Fig.5. Gram negative Red Bacteria**.**

2.3 Biochemical Test
2.3.1 Primary Tests

Oxidase Test

A piece of filter paper is placed in a clean Petri dish, two 2 drops of oxidase reagent is then added. A wooden stick is then used to transfer a colony from strain A and smear it on the filter paper. Results should be read immediately.

Catalase Test

Hydrogen peroxide (H_2O_2) 3%, in two test tubes (2ml each). A small portion of culture from bacterial strain A is transferred to the first test tube. Immediately after immersion of strain in hydrogen peroxide, a release of air bubbles will indicate positive result.

Oxidation/Fermentation test (O/F)

O/F medium in two tubes were inoculated with the organism by straight wire (fig 6). Then in one of the tubes a layer of sterile paraffin oil was added 3cm above the medium level. Then both tubes were incubated at 37ºC and examined daily for up to one week [23]. The result is read as follows:

1. Oxidative if the tube without oil changed to pink color.
2. Fermentative if both tubes changed to pink color.
3. Negative result was indicated by no color changes in both tubes.

Fig .6. Oxidation/Fermentation Test (O/F)

Glucose Test

The medium was prepared by adding 1% glucose to peptone water with Andred's indicator in Bigu vials with inverted Durham tube (fig 7). This is inoculated with the tested organism and incubated at 37ºC and examined daily. Acid production was indicated by appearance of pinkish color, while gas production was indicated by presence of empty space in the inverted Durham's tube [24].

Fig.7. Glucose Test

Motility Test

Broth cultures of the organisms were incubated at the optimum growth temperature 37ºC and examined by hanging drop method. Motile organisms were indicated by their movement in different directions [24].

2.3.2 Secondary Tests
Indol Test

The organism was inoculated in peptone water or nutrient broth and incubated at 37ºC for 48h. 0.5 ml of kovacs reagent was added to the medium and shaken well and examined after 1min. Development of red color in the reagent layer indicated indol production (fig 8) [24].

Citrate Test

Simmons citrate medium was streaked over the surface of the slope and examined daily for up to 7 days. Blue color (fig 9) development indicated positive result [24].

Urease Activity Test

Tube of urease medium (fig 10) was inoculated with the test organism and incubated at 37ºC and examined daily for up to 7 days. Development of red color indicated urea hydrolysis [24].

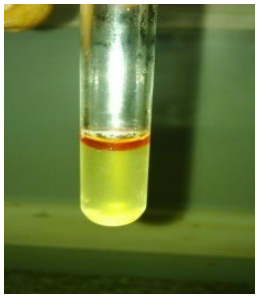

Fig .8. Indol Test

Fig .9. Citrate Test

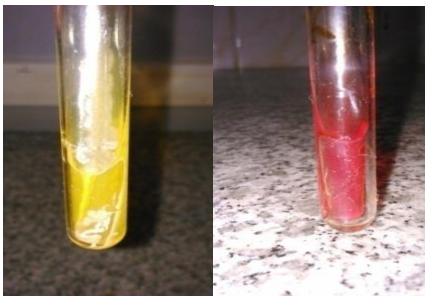

Fig.10. Urease activity Test

Kliger Iron Agar (KIA)

Streaking stabbing in the butt stab media and zigzag in slope media in the same tube and incubated overnight in incubator. The result will show the stab media butt and slope media or slant and production of Gas an H_2S.

3. Result and Discussion

3.1. Total Bacteria Count

Table 2 shows the total bacteria counts for three samples under study. The bacterial colonies have spread with a clear effect of the storage period on the growth of bacterial colonies. Fig 13 indicates that the numbers of colonies decreases with time of storage. The variation in the total number of bacteria in cheese may be related to heat treatment or to the parameters influenced by the environment in which the microorganisms proliferate. The growth of micro-organisms in cheese is believed to be controlled by a number of factors including salt content, water, pH, presence of organic acid and nitrate. In addition to redox potential and ripening temperature. All studied types of cheese can be considered as safe for consumption and harmless to consumer.

Table 2. Effect of storage period, and type of packaging on total bacterial viable count of Sudanese white soft cheese

	Bacterial Colonies per 5 gram of sample					
	Sample 1, Dilutions		**Sample 2, Dilutions**		**Sample 3, Dilutions**	
Storage period(days)	**10^{-4}**	**10^{-5}**	**10^{-4}**	**10^{-5}**	**10^{-4}**	**10^{-5}**
0.0	13	5	17	10	11	6
8	30	14	14	8	8	4
16	17	7	9	5	10	6
24	28	14	13	7	17	8
32	24	11	12	8	8	5
40	16	7	19	11	11	6
48	30	14	20	10	18	9
56	12	8	15	8	20	11
64	24	10	9	6	16	8
72	22	12	22	11	12	7
80	16	9	19	10	9	5
88	12	8	12	7	12	8
Average	**15.13**		**11.75**		**9.79**	

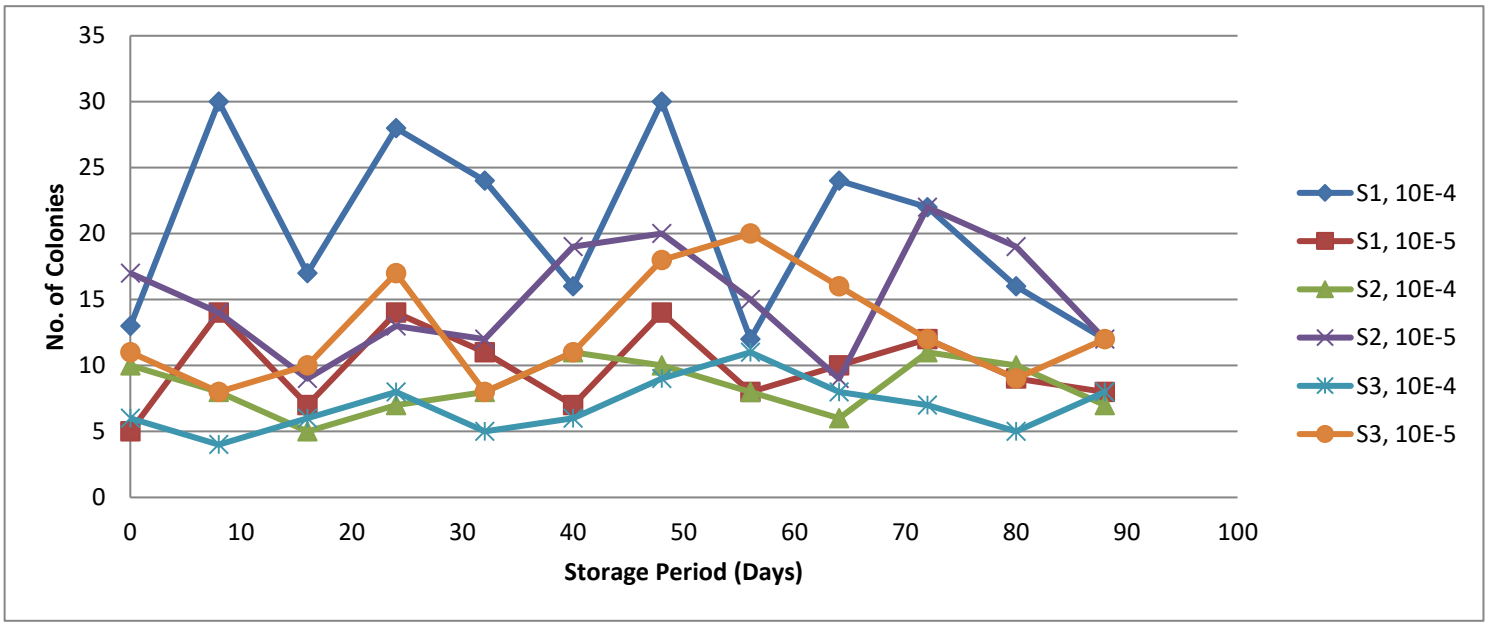

Fig 13 bacterial colony counted v.s. storage period.

Table 6. Biochemical Test Results.

StoragePeriod (days)	**I**	**II**	**III**	**IV**	**V**	**VI**	**VII**	**VIII**	**IX**	**X**	**XI**	**XII**
0.0	+	-	F	+	-	-	+	-	Y	Y	-	+
8	+	-	FF	+	-	-	+	-	Y	Y	-	+
16	+	-	F	+	-	-	+	-	Y	Y	-	+
24	+	-	F	+	-	-	+	-	Y	Y	-	+
32	+	-	F	+	-	-	+	-	Y	Y	-	+
40	+	-	F	+	-	-	+	-	Y	Y	-	+
48	+	-	F	+	-	-	+	-	Y	Y	-	+
56	+	-	F	+	-	-	+	-	Y	Y	-	+
64	+	-	F	+	-	-	+	-	Y	Y	-	+
72	+	-	F	+	-	-	+	-	Y	Y	-	+
80	+	-	F	+	-	-	+	-	Y	Y	-	+
88	+	-	F	+	-	-	+	-	Y	Y	-	+

Key: *I = Catalase, II = Oxidase, III = Glucose, IV= Oxidation/Fermentation (O/F), V = Motility, VI= Indol, VII = Citrate, VIII= Urease, IX = Butt, X= Slope, XII= H_2S, XIII = Gas.*

3.2. Bacteria Biochemical Reaction

Gram stain test showed a presence of gram negative bacilli. Table 6 shows identical results for the biochemical reaction of the three samples isolated bacteria. In all points of storage period studied, the three samples of cheese resulted in positive Catalase reading and negative Oxidase reading. The Glucose test indicated a fermentation, Oxidation/ Fermentation (O/F) showed a positive result with no Motility on the motility test. For the secondary tests, the Indol test showed a negative result with a positive reading on the Citrate test. A negative result of the Urease was recorded. KIA Butt colour was found to be Yellow with a Slope reading colour of Yellow. The H_2S gas was negatively recorded with positive reading of other Gas test. This indicates that E.coli bacteria is not present.

4. CONCLUSIONS

Cheese in general is widely consumed by the population of the Sudan. The handling and channels of marketing vary and this may contribute to the contamination of the cheese with bacteria and its spoilage. The method of preservation and packing differ from one sales point to another. It is of vital importance to study the total number of bacteria in the locally produced cheese. This will enable to determine the suitability of these cheeses for consumption. The current research studied all of these aspects and some recommendations have been made.

This study reported the total count of bacteria and identification of Escherichia coli (E.coli) in white soft Cheese which is produced locally. This achieved through the study on three samples of cheese of different production date.

Samples were packed in polypropylene (PP) at 4 ºC. Laboratory tests showed that, the average bacterial colonies in cheese at varied dilution of the Agar medium (10-4 and 10-5) were 15.54, 11.74, and 9.79. The variation in the total number of bacteria in cheeses is associated with many factors, such as moisture, added salt, fat content of the milk, pH, organic acids and nitrates and ripening temperature. The biochemical reaction of the isolated bacteria from all three samples of white soft cheese conducted in all storage period gave a result of Primary test: (Catalase: +ve, Oxidase: -ve, Glucose: F, Oxidation/ Fermentation (O/F): +ve, Motility: Non motility) and secondary test:(Indol: -ve, Citrate: +ve, Urease: -ve, KIA:(Butt: Yellow, Slope: Yellow, H_2S: -ve, Gas: +ve)). These results mean that no E.coli is present while a gram negative bacteria is present.

5. Recommendations

By comparing the result obtained for the total bacteria count in all studied samples of cheese with Sudanese Standards [25] given for the number of bacteria in cheese, they are considered safe for consumption and harmless to consumers provided that the following points are observed:

1- The consumers must be sure that the cheese is kept and preserved in a hygienic environment by different sales point owners.

2- The consumers must reject any cheese showing abnormality in its characteristics e.g. change color, drought, swelling or acid.

3- The consumers must observe the production and expiry date of cheese to determine its suitability and to avoid any possible health hazards.

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