**Bacteriological Evaluation of Yoghurt in Misurata City, Libya**

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**Abstract**

Laban raieb is one of the important fermented milk consumed by different ages in Middle east and throughout the world and characterized by high nutritive value, maintenance of normal intestinal microflora. The aim of this study to evaluate the bacterial content of yoghurt in Misurats's markets, 20 samples have been collected for plain, fruit, and flavored yoghurt. These samples were transported to the lab to perform microbial examinations. First of all, we measured the pH in the yoghurt samples which were 4.58 **±** 0.27 in plain yoghurt, 4.61**±** 0.23 in fruit yoghurt and 4.61**±** 0.22 in flavored yoghurt.we investigated the presence of coliform species in each type of the yoghurt samples which were *Enterobacter cloacae, Citrobacter freundii*, *Enterobacter agglomerans*, *Klebsiella panticola, E. coli* and *Enterobacter aerogenes* in the plain yoghurt samples, While *Klebsiella panticola*, *Klebsiella oxytoca, Klebsiella pneumonia, E. coli* and *Enterobacter areogenes* present in the flavored yoghurt samples. However, in the fruit yoghurt samples we isolated *Klebsiella oxytoca, Klebsiella pneumonia*, and *E. coli* only. Besides coliform, we were able to isolate *Staphylococcus aureus* and *Staphylococci epidermidis* of all the yoghurt samples.

**Keywords**: Plain yoghurt, Flavored yoghurt, Fruit yoghurt

**Introduction**

A large variety of fermented food products are produced and consumed around the world. Fermentation serves to preserve raw foods and increases the diversity of available food products **[27, 32].** Cereals, oil seeds, milk, fish, meat and vegetables are raw foods that are fermented world-wide **[19, 23].** As part of the human diet, fermented foods can play an important role in maintaining a healthy intestinal tract and increase the acceptability of dairy products to lactose intolerant individuals **[8, 9].**

Natural or plain yoghurt is the traditional type of fermented milk with a sharp acidic taste, while fruit yoghurt is made by the addition of fruits and sweeting agents to natural yoghurt [29].

Fruit yoghurt usually have stabilizers incorporated to reduce whey separation during distribution many of the stabilizers are complex carbohydrates which providing “a bulking agent” so stimulating intestinal peristalsis and avoiding some of the risk of colonic malfunction. It also absorb some of the potentially toxic chemicals that may be formed in the large intestine as a result of bacterial action. This unavailable carbohydrates acting to further delay the diffusion of sugar to the intestinal wall that could help lactose intolerant patients and those prone to post prandial hyperglycemia **[30, 36].**

# **MATERIALS AND METHODS**

## **Collection of samples:**

A total of 60 random samples plain, flavored and fruit yoghurt (20, 20 and 20 respectively) collected from different localities in Misurata city, from 10-1-2017 to 4-3-2017.

### **Preparation of samples:**

On arrival to the laboratory each sample was perfectly mixed before being divided into two sub-samples. The first one used for determination of pH, while the second was examined bacteriologically.

## **Determination of pH**: *Standard method* [5].

Was used to determine pH by using pH meter (Jenway- model:3505, Made in UK).

**Microbiological examination:**

**Preparation of serial dilution** [5].

After thoroughly mixing of a sample, weigh 1 ml. were transferred into sterile, wide mouthed container, containing 9 ml of sterile water, shake well until a homogeneous dispersion of 1:10 dilution obtained, withdraw appropriate amounts of this dilution for plating or further decimal dilution.

**Total bacterial count:** [20].

1. Using aseptic technique, transfer 1 ml of sample to a 9 ml sterile distilled water to made 10-1 dilution.
2. Immediately after the 10-1 dilution, it has been shaken, uncap it and aseptically transfer 1 ml to a second 9ml sterile distilled water. Since this is a 10-2 dilution, and else to produce a 10-14 dilution.
3. Then Shook the 10-1 diluted sample again and aseptically transfer 1.0 ml to one petri plate and 1 ml to another petri plate. All the samples were done in similar way.
4. Removed one agar pour tube from the 48 to 50° C water bath and aseptically pour the agar into it. The agar and sample are immediately mixed gently moving the plate in a figure-eight motion or a circular motion while it rests on the tabletop.
5. After the pour plates have cooled and the agar has hardened, they are inverted and incubated at 37° C for 24 hours.
6. Calculated the number of bacteria (C.F.U) per milliliter of sample by dividing the number of colonies by the dilution factor multiplied by the amount of specimen added to liquefied agar.

**Enumeration and isolation of Coliforms** (MPN\ml): [5].

A series of 3 fermentation tubes containing 9 ml. MacConkey’s broth (Oxoid, 1990) with inverted Durham’s tube were inoculated with 1 ml for each of the previously prepared decimal dilutions as well as from the original sample after thorough mixing.

Inoculated and control tubes were incubated at 37°C \ 24 hours.

**API 20E kits for identification of Enterobacteriaceae (Biomerieux, France)**

**1. Preparation of the strip:**

The incubation box (tray and lid) was prepared by distributing 5 ml of distilled water into the honey-combed wells of the tray to create a humid atmosphere.

The strain reference was recorded on the elongated flap.

The strip was removed from individual packaging and placed in the incubation box.

1. **Preparation of the inoculums**

The organism was cultivated onto nutrient agar 18-24 hours at 37°C.

About four to five colonies were transferred to the API 20E.

The turbidity was then adjusted to match a McFarland 0.5 barium sulphate standard (1.5x108 CFU).

1. **Inoculation of the strip**

The micro tube were filled with the inoculated API 20E medium by using a pipette, then the capules of ADH, LDC, ODC and URE were filled with mineral oil to ensure anaerobic condition, after that the capules of CIT, VP and GEL were completely filled by suspension and placed in, then Closed the incubation box and incubate at 37oC for 18-24 hours.

1. **Reading and interpretation**

Read the strip after 18-24 hours at 37oC, the following reagents were added as the follows:

VP: 1 drop **VP1** and 1 drop **VP2** reagents

TDA: 1 drop **TDA** reagent

IND: 1 drop Kovacs reagent **\James** reagent

**Enumeration and isolation of Staphylococci**: [5]

0.1 ml from the previously prepared decimal dilutions of the examined samples was transferred and evenly spread on the dry surface of Mannitol salt agar (Oxoid, 1998) medium plates using a sterile bent glass rod. Inoculated plates were incubated at 37°C for 24 hours and Staphylococci count were calculated and recorded.

**Identification of Staphylococcus by Biochemical reactions:** [10].

1. Catalase test:

A loopful of the tested culture was suspended in a drop 3% Hydrogen peroxide (H2O2) on a slide. Evolution of bubbles within one minute was recorded as positive.

2. Coagulase tube test:

In a sterile tube, 0.1 ml of 24 hours nutrient Cultures were transferred to 0.3 ml of plasma. The tubes were incubated at 37°C for 4 hours before being examined for clot formation. Extent of coagulase reaction (1-4+) was recorded. Tubes were left at room temperature for an additional 20 hours and then re-examined for clot formation. The extent of coagulation of the plasma was reported after 4 and 24 hours.

**Statistical analysis: Done by ANOVA one way and t- test**

**Results and Discussion**

The results in table (1) and figure (1) show that the pH in examined plain yoghurt samples was ranged from 4.2 to 5.1 with a mean value of 4.58 ± 0.27 and in examined fruit yoghurt samples was ranged from 4.2 to 5.1 with a mean value of 4.61± 0.23.

Table (1): Statistical analytical results of pH in examined yoghurt samples.

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
| Product | No. | Minimum | Maximum | Mean ± S.D | p. value |
| Plain | 20 | 4.2 | 5.1 | 4.58 ± 0.27 | 0.902 |
| Fruit | 20 | 4.2 | 5.1 | 4.61± 0.23 |
| Flavored | 20 | 4.3 | 5.0 | 4.61± 0.22 |

While in examined flavored yoghurt samples was in a range from 4.3 to 5.0 with a mean value of 4.61± 0.22. Nearly similar data were obtained by [22, 24].

**Figure (1): pH in examined yoghurt samples**

A careful inspection of table (2) reveals that according to frequency distribution of examined yoghurt samples based on pH. The highest frequency distribution of examined plain yoghurt samples (25%) lies within the range of 4.31- 4.4, while the highest frequenc0y distribution of examined fruit yoghurt samples (20%) lies within the range of 4.31- 4.4 and 4.41- 4.5, while the highest frequency distribution of examined flavored yoghurt samples (25%) lies within the range of 4.51- 4.6.

**Table (2): Frequency distribution of examined yoghurt samples based on their pH**

|  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- |
| Intervals | Plain Yoghurt | | Fruit yoghurt | | Flavored yoghurt | |
| No. | % | No. | % | No. | % |
| 4.21-4.3 | 4 | 20% | 2 | 10% | 3 | 15% |
| 4.31-4.4 | 5 | 25% | 4 | 20% | 2 | 10% |
| 4.41-4.5 | 1 | 5% | 4 | 20% | 2 | 10% |
| 4.51-4.6 | 3 | 15% | 2 | 10% | 5 | 25% |
| 4.61-4.7 | 2 | 10% | 2 | 10% | 3 | 15% |
| 4.71-4.8 | 1 | 5% | 3 | 15% | 1 | 5% |
| 4.81-4.9 | 1 | 5% | 1 | 5% | 3 | 15% |
| 4.91-5.0 | 2 | 10% | 1 | 5% | 1 | 5% |
| 5.01-5.1 | 1 | 5% | 1 | 5% | 0 | 0% |
| Total | 20 | 100% | 20 | 100% | 20 | 100% |

The results in table (3) and figure (2) reveal that the total bacterial count in examined plain yoghurt samples was ranged from 1.32X102 to 1.30X106 with a mean value of 9.63X104 ± 2.88X105 and from 1.27X102 to 1.97 X106 with a mean value of 1.67X105 ± 4.69X105 for examined fruit samples.

Table (3): Statistical analytical results of total bacterial count\ml in examined yoghurt samples.

|  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- |
| Examined yoghurt samples | No. of exam. samples | Positive samples | | Count\ml | | Mean ± S.D | p. Value |
| No. | % | Min. | Max. |
| Plain | 20 | 20 | 100% | 1.32X102 | 1.30X106 | 9.63X104 ± 2.88X105 | 0.888 |
| Fruit | 20 | 20 | 100% | 1.27X102 | 1.97 X106 | 1.67X105 ± 4.69X105 |
| Flavored | 20 | 20 | 100% | 1.19X102 | 5.8X106 | 3.9X105 ± 1.3X105 |

While in examined flavored yoghurt samples was ranged from 1.19X102 to 5.8X106 with a mean value of 3.9X105 ± 1.3X105. These results were comparable with [12]. Results confirm no differences in the 3 types of yoghurt samples which may due to the use of the same starter for the products.

**Figure (2): Total bacterial count\ml in examined yoghurt samples**

**Figure (3, 4, 5)** show positive relation between pH and total bacterial count in the three products.

**Figure (3): Relation between pH & T.B.C in plain yoghurt**

**Figure (4): Relation between pH & T.B.C in Fruit yoghurt**

**Figure (5): Relation between pH & T.B.C in Flavored yoghurt**

The results in table (4) displays that nearly about (35%) of examined plain yoghurt samples based on their total bacterial count lies within the range of 1.0X102-1.0X103 and the majority (40%) of examined fruit yoghurt samples lies within the range 1.01X104-1.0X105 and nearly the half of examined flavored yoghurt (45%) lies within the range 1.0X102-1.0X103.

Table (4):Frequency distribution of examined yoghurt samples based on their total bacterial count.

|  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- |
| Intervals | Plain yoghurt samples | | Fruit yoghurt samples | | Flavored yoghurt samples | |
| No. | % | No. | % | No. | % |
| 1.0X102-1.0X103 | 7 | 35% | 6 | 30% | 9 | 45% |
| 1.01X103-1.0X104 | 6 | 30% | 3 | 15% | 6 | 30% |
| 1.01X104-1.0X105 | 4 | 20% | 8 | 40% | 2 | 10% |
| 1.01X105-1.0X106 | 2 | 10% | 2 | 10% | 1 | 5% |
| 1.01X106-1.0X107 | 1 | 5% | 1 | 5% | 2 | 10% |

The results in table (5) shows that total coliform count (MPN\ml) of examined plain yoghurt was only 20% of samples were contaminated and ranged from 2.30x103 to 4.0X105 with a mean value of 1.09X105 **±** 1.93X105.

**Table (5): Statistical analytical results of Coliform count\ml in examined yoghurt samples.**

|  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- |
| Examined yoghurt samples | No. of examined Samples | Positive samples | | Count\ml | | Mean ± S.D | p. value |
| No. | % | Min. | Max. |
| Plain | 20 | 4 | 20% | 2.30x103 | 4.0X105 | 1.09X105 ± 1.93X105 | 0.104 |
| Fruit | 20 | 2 | 10% | 2.10X104 | 1.40X105 | 8.05X104 ± 8.41X104 |
| Flavored | 20 | 2 | 10% | 1.10X103 | 2.30X104 | 1.21X104 ± 1.54X104 |

while in fruit yoghurt 10% of samples were positive and ranged from 2.10X104 to 1.40X105 with a mean value 8.05X104 **±** 8.41 X104, While in flavored yoghurt also 10% of samples were contaminated and ranged from 1.10X103 to 2.30X104 with a mean value 1.21X104 **±** 1.54X104. These findings substantiate those reported by **[11, 25, 33].** Lower values were obtained by **[1, 7, 35].** While higher counts were declared by **[3, 13, 15, 18, 28]. [26]** Concluded that 97% of examined yoghurt samples were free from coliform bacteria, while **[31]** reported that Enterobacrteiaceae failed to be detected in examined yoghurt samples.

The results listed in table (6) reveals that the highest frequency distribution based on their coliform counts of examined plain yoghurt samples (50%) lies within the range of 1.01X104-1.0X105, in examined fruit yoghurt samples (50%) lies within the range 1.01X104-1.0X105 and 1.01X105-1.0X106 also in examined flavored yoghurt samples (50% ) lies within the range 1.0X103-1.0X104 and 1.01X104-1.0X105.

**Table (6): Frequency distribution of examined Yoghurt samples based on their coliform counts.**

|  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- |
| Interval | Plain yoghurt | | Fruit yoghurt | | Flavored yoghurt | |
| No. | % | No. | % | No. | % |
| 1.0X103-1.0X104 | 1 | 25% | 0 | 0% | 1 | 50% |
| 1.01X104-1.0X105 | 2 | 50% | 1 | 50% | 1 | 50% |
| 1.01X105-1.0X106 | 1 | 25% | 1 | 50% | 0 | 0% |
| Total | 4 | 100% | 2 | 100% | 2 | 100% |

The results reported in table (7) and figure (6) present that *Enterobacter cloacae, Citrobacter freundii*, *Enterobacter agglomerans*, *Klebsiella panticola, E. coli* and *Enterobacter aerogenes* were isolated from the examined plain yoghurt samples in the percentage of 37.5%, 12.5%, 12.5%, 12.5%, 12.5% and 12.5% respectively.

**Table (7): Incidence of isolated Coliforms in examined yoghurt samples.**

|  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- |
| Isolates | Yoghurt samples | | | | | |
| Plain yoghurt | | Fruit yoghurt | | Flavored yoghurt | |
| Positive | % | Positive | % | Positive | % |
| *Citrobacter freundii* | 1 | 12.5% | 0 | 0% | 0 | 0% |
| *Enterobacter agglomerans* | 1 | 12.5% | 0 | 0% | 0 | 0% |
| *Enterobacter cloacae* | 3 | 37.5% | 0 | 0% | 0 | 0% |
| *Klebsiella panticola* | 1 | 12.5% | 0 | 0% | 1 | 20% |
| *Klebsiella oxytoca* | 0 | 0% | 1 | 25% | 1 | 20% |
| *Klebsiella pneumonia* | 0 | 0% | 1 | 25% | 1 | 20% |
| *E. coli* | 1 | 12.5% | 2 | 50% | 1 | 20% |
| *Enterobacter areogenes* | 1 | 12.5% | 0 | 0% | 1 | 20% |
| Total | 8 | 100% | 4 | 100% | 5 | 100% |

While in fruit yoghurt only *Klebsiella oxytoca*, *Klebsiella pneumoniae* and *E. coli* were isolated by the percentage of 25, 25 and 50% respectively. While in flavored yoghurt *Klebsiella panticola*, *Klebsiella oxytoca, Klebsiella pneumonia, E. coli* and *Enterobacter areogenes* were isolated by the percentage 20, 20, 20, 20 and 20% consecutively. The results obtained are nearly similar to that reported by **[2, 7, 14, 17, 37].** On other hand, **[37]** said*Citrobacter* spp. was not found in any of the analyzed samples**.**

**Figure (6): Incidence of isolated Coliforms in examined yoghurt samples**

Higher levels of Coliforms (106 or more) believed to be necessary for food borne illness to occur [39]. Certain numbers of *Citrobacter* have been suspected to cause enteric infection [38]. *Citrobacter freundii* has been found among urinary and other pyogenic infections in humans [40]. Some strains of *Klebsiella* and *Enterobacter* species had been implicated in acute and chronic diarrhea [41]. The results tabulated in table (8) revealed that (15%) of examined plain yoghurt samples were contaminated by *Staphylococci*, the level of contamination was ranged from 1.13X102 to 8.30X103 with a mean value of 2.92X103 ± 4.65X103, also (15%) of examined fruit yoghurt samples were contaminated by *Staphylococci*, the level of contamination was ranged from 7.30X102 to 4.30X103 with a mean value of 2.00X103 ±1.99X103 and the same percentage (15%) of examined flavored yoghurt samples were contaminated by *Staphylococci*, the level of contamination was ranged from 5.20X102 to 6.70X103 with a mean value of 2.59X103 ± 3.55X103.

**Table (8): Statistical analytical results of Staphylococci count\ml in examined yoghurt samples.**

|  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- |
| Examined yoghurt samples | No. of examined samples | Positive samples | | Staph count\ml | | Mean ± S.D | p. Value |
| No. | % | Min. | Max. |
| Plain | 20 | 3 | 15% | 1.13X102 | 8.30X103 | 2.92X103 ± 4.65X103 | 0.954 |
| Fruit | 20 | 3 | 15% | 7.30X102 | 4.30X103 | 2.00X103 ± 1.99X103 |
| Flavored | 20 | 3 | 15% | 5.20X102 | 6.70X103 | 2.59X103 ± 3.55X103 |

The results reported in table (9) reveals that 66.66% of examined plain, fruit and flavored yoghurt samples lies in the range of 1.0X102-1.0X103. Relatively similar results were obtained by **[1, 4, 6, 16, 17, 21]**, while higher values were reported by **[34].**

**Table (9): Frequency distribution of examined yoghurt samples based on their Staphylococci count.**

|  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- |
| Intervals | Yoghurt samples | | | | | |
| Plain yoghurt | | Fruit yoghurt | | Flavored yoghurt | |
| No. | % | No. | % | No. | % |
| 1.0X102-1.0X103 | 2 | 66.6% | 2 | 66.6% | 2 | 66.6% |
| 1.01X103-1.0X104 | 1 | 33.3% | 1 | 33.3% | 1 | 33.3% |
| Total | 3 | 100% | 3 | 100% | 3 | 100% |

Table (10) and figure (7) show that the isolated Staphylococci, *Staphylococcus aureus* was detected in 40% of positive examined plain yoghurt samples while 60% of contaminated examined samples were by *Staphylococci epidermidis*.

**Table (10): Incidence of isolated Staphylococci in examined yoghurt samples.**

|  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- |
| Isolated strain | Yoghurt samples | | | | | |
| Plain yoghurt | | Fruit yoghurt | | Flavored yoghurt | |
| Positive samples | % | Positive samples | % | Positive samples | % |
| *Staphylococcus aureus* | 2 | 40% | 1 | 25% | 1 | 33.3% |
| *Staphylococcus epidermides* | 3 | 60% | 3 | 75% | 2 | 66.6% |
| Total | 5 | 100% | 4 | 100% | 3 | 100% |

But in case of fruit yoghurt only 75% were contaminated by *Staphylcoccus epidermidis* while *staphylococcus aureus* detected in 25% of positive samples, while in examined flavored yoghurt samples 33.3% and 66.6% of samples were contaminated by *Staphylococcus aureus* and *Staphylococcus epidermides*.

**Figure (7): Incidence of isolated Staphylococci in examined yoghurt samples**

*Staph. aureus* may be found in the eyes, throat and the intestinal tract. Therefore, nasal carriers and individuals whose hands and arms were infected with boils and carbuncles are dangerous sources of food poisoning [43]. *Staphylococcus aureus* is by far the most important human pathogen among the Staphylococci. Under certain circumstances, *Staph. aureus* may cause a variety of infectious diseases, ranging from relatively benign skin infectious diseases, to life threating systemic illness. Enterotoxin producing Staphylococci are the leading cause of food borne illness throughout the world. *Staph. aureus* possess a public health hazard due to production of thermostable enterotoxin that is responsible for food poisoning.

Leucocidin, Enterotoxin (A to E) and toxic shock syndrome toxin, TSST, all were produced by Staphylococcus aureus, and Enterotoxins are heat stable molecules that are responsible for the clinical feature of Staphylococcal food poisoning. Ingestion of preformed enterotoxins in food, results in vomiting and diarrhea within 2 to 8 hours, sometimes followed by collapse [42]. Although *Staph. aureus*, the coagulase positive, is the most dangerous, but nowadays, coagulase negative Staphylococci have been recognized as important agents of human disease which include nosocomial and community-acquired urinary infections, bacteremia in compromised hosts, osteomyelitis and post-surgical infections.

Table (11) reported that just 3 plain yoghurt samples were unacceptable with the total bacterial count and Staphylococci, 4 plain yoghurt samples were unaccepted with coliform, while in examined fruit yoghurt samples there were 3 samples unaccepted with total bacterial count and Staphylococci and only 2 samples unacceptable with coliform in compare with Libyan standard.

And in flavored yoghurt samples most of the samples were going with the Libyan standard (total bacteria count, staphylococci, coliform and yeast) which were (17- 17 -18) respectively accepted with the Libyan standard.

Table (11): Comparison between Libyan standard criteria and examined yoghurt samples.

|  |  |  |  |  |  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
| Flavored samples | | | | Fruit samples | | | | Plain samples | | | | Standard |  |
| Acceptable | | Unacceptable | | Acceptable | | Unacceptable | | Acceptable | | Unacceptable | |
| % | No | % | No. | % | No. | % | No. | % | No. | % | No |
| 85 | 17 | 15 | 3 | 85 | 17 | 15 | 3 | 85 | 17 | 15 | 3 | <105 | Total Bacterial count |
| 85 | 17 | 15 | 3 | 85 | 17 | 15 | 3 | 85 | 17 | 15 | 3 | Free | Staphylococci |
| 90 | 18 | 10 | 2 | 90 | 18 | 10 | 2 | 80 | 16 | 20 | 4 | Free | Coliforms |

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