Pre-Identification of Lactic Acid Bacteria Isolated During Fermentation Process of Egyptian Kishk

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ABSTRACT

The cereal-based fermented food Egyptian Kishk constitutes a major source of high quality dietary nutrients for Upper Egyptians and is popular among all social strata. Lactic acid bacteria were one of the main flora which is responsible for fermentation. To-date, kishk is still traditional making and the diversity of LAB in Kishk has not been fully investigated and improving the Kishk throughout using selected cultures for its processing has never been established. In this work, LAB have been isolated from samples collected during traditional making the Kishk. The obtained results showed that the dominant lactic acid bacteria isolates were obligatory heterofermentative and Enterococci spp. The homofermentative LAB were found in the first steps of the fermentation. Homofermentative and facultative heterofermentative of lactobacilli were found in the first fermentation process steps and then- almost disappeared after that in Kishk. On the other hand obligatory hetero fermentative and Enterococci spp. were detected in all steps of kishk manufacturing while Lactococcus was a minor isolate during fermentation process. The majority of isolates are y- haemolytic except one isolate of *Enterococci* spp. which was β haemolytic. There are significant differences (P≤ 0.01) in organoleptic characteristics (colour, taste, smell, texture, appearance) and overall acceptability among all isolates used to ferment the milk. Fifteen isolates that received the highest levels of overall organoleptic properties are selected as starter culture in improving the Kishk making.

Key words: Lactic acid bacteria, Laban Zeer and Kishk

INTRODUCTION

Kishk is typically prepared by mixing Laban Zeer (butter milk separated from freshly drawn milk and left to sour in an unglazed earthenware container: the "Zeer") with coarsely ground parboiled wheat. The locally grown mature freshly harvested whole wheat is boiled in a metal barrel on an open fire until soft then sun dried for 24 hours. The following day, the dried grains are moistened with water (about 20 %), cracked and husked by winnowing, and then sized as coarse and fine. It is recommended that coarse burghol be used for kishk making. The concentrated Laban Zeer that is fermented for at least 40 days is mixed with the moistened coarsely ground parboiled wheat in a large earthenware magour at ratio of 2:1 to 4:1, to produce a

heavy paste called "Hama" Ahmed and Hassan-Wassef (2010). The milk cereal mixture is then allowed to ferment again for about 24 hours after which it is kneaded with the addition of more of the fermented salted milk diluted with a little water to reach the required consistency. Alternatively, the fermentation of the Hama allowed continuing for a further 24 hours. The resultant mass is thoroughly mixed incorporating the aromatic spices before cutting into unformed chunks (of about 3 cm in diameter) or shaped into small balls of about 2 cm in diameter, dried for 7–8 days in the hot shade or sun. (Abd El- Salam, 2011)

The microbiological analysis of Kishk is quite complex. Differences in the traditional methods employed during the manufacture of Kishk can influence the compositional, nutritional properties and microbiological characteristics of the product (Tamime and O'Connor, 1995). Lactic acid bacteria and yeasts constituted the main microbial flora during fermentation process of Egyptian Kishk (Demerdash, 1960). The chemical and microbial changes during preparation of wheat yoghurt, Kishk, have been studied (Demerdash, 1960; Shaker *et al.*, 1980; Hamad and Fields, 1982; Damir *et al.*, 1992). From the documented literatures, it seems that very little work has been attempted to identify the fermenting organisms from Laban Zeer to Kishk in this background.

The present study aims at the isolation and preidentification of lactic acid bacteria during actual Kishk manufacturing and studies their hemolytic activity, in addition to screening the isolates for flavor acceptability in fermented milk. The obtained results will be used to select appreciate strains to be as starter culture in producing second generation of kishk.

MATERIALS AND METHODS

1. Sample collection:

Sixty nine Laban Zeer samples, seven samples from each of (dough and Hama) and ten samples of Kishk were collected in sterile plastic bags from Bani -Mazar, Samlot, Abu-korkas, Mallawi and Der- Maus (Cites of El-Minya Governorate, Egypt during July, 2011 and 2012). The samples were transferred in ice box and then stored. Laban Zeer samples were evaluated for flavor and texture acceptability by 22 consumer panelists (all

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of them were familiar with Laban Zeer). Twenty five samples which accepted in flavor and texture were selected to isolate the lactic acid bacteria. Other seven samples of Laban Zeer, Dough, Hama and Kishk which collected during the actual manufacture process were also used for isolation the lactic acid bacteria.

2. Isolation of lactic acid bacteria (LAB) strains:

Three grams of each sample were cultured aseptically into 30 ml of sterilized reconstituted skimmed milk and were incubated until coagulation at 30 °C; 37 °C and 42 °C. Coagulated samples were then streaked on M17 (Biolife Italy), ST and SF agar media. These samples streaked also on MRS and Rogosa agar (Difco, USA) which were incubated under anaerobic condition using the gas pak system (GENER box anae indicator Biomerieux) at 30 °C, 37 °C and 42 °C for 48hr. The cultures were streaked on suitable media for their purification. The purified strains were stored at -20 °C in sterile reconstituted skim milk (12.5 % W/V) supplemented with 15 % glycerol (El-Soda *et al.*, 2003)

3. Pre Identification tests:

The isolates were roughly classified at genus level according to the Criteria of Sharpe (1979) and Axelsson (1993). Tests applied on all isolates were: Gram positive and catalase negative, consequently they were tested for growth at 10 °C for 10 days, 45 °C for 48hr, and CO₂ production from glucose. For cocci isolates, growth on SF broth medium and in the presence of 6.5 % NaCl were also considered.

4. Hemolytic activity:

Hemolytic activity was evaluated using blood agar base 2 (Oxoide) plates containing 5 % (v/v) human blood (EL-Shatby hospital, Alexandria) and incubated at 30, 37 and 42 °C for 48 hr. α - and β - hemolytic reactions were recorded by observation of greenish colonies and a clear zone around the colonies respectively. The assay was performed in duplicate. *Streptococcus pyogenes* MGAS 15252 serotype M 59 (obtained from department of Food Technology, Arid Land Cultivation and Development Institute, City of Scientific Research and Technology Applications) was used as β –hemolytic control strain (Maragkoudakis *et al.*, 2006).

5. Flavor potential for products having specific flavours:

Fermented milk prepared by adding 3% of active single tested strain at optimum temperature until milk coagulated at pH 4.6. The fermented milk was kept at 4 °C for 18h. Final products were evaluated for organoleptic characteristics (color, taste ,smell, texture ,appearance) and overall acceptability by 15 panelists included post graduate- students and staff members at

Faculty of Agriculture, Alexandria university; using ten point hedonic scale ranging from excellent (score = 10) to very poor (score = 0) as extremes. Also the panelists were asked to list any flavour defects (Abdel Rahman *et al.*, 2009).

6. Statistical analysis:

The experimental design was a completely randomized design with 15 replications in the experiment. F-test, and analysis of variance of treatments difference was performed according to (Steel and Torrie, 1984). Statistical analysis was done by, ANOVA, F-test, and L.S.D procedures available within the SAS software package (version 9.13 2008).

RESULTS AND DISCUSSION

1. Pre-identification tests of isolated lactic acid bacteria:

Pre-identification results of obtained isolates during Egyptian Kishk manufacturing are illustrated in Table (1). Three hundred and eight isolates were phenotypically identified, all of the isolates were Gram positive and catalase negative. The results showed that Enterococcus spp. isolates were found in high proportion (94.1, 93.7, 94 and 95.8%) for cocci in Laban Zeer, Dough, Hama and Kishk respectively. The highly number of *Enterococcus* spp. was in agreement with the results that obtained by El Soda et al.(2003). They found a relatively high number of Enterococcus spp in Egyptian dairy products (Ras, Domiatti and Kareish cheese, mish, cream, butter, Zabady and Laban Lactococcus spp. was detected in minor proportion (5.8, 6.3, 6 and 4.2 %) in Laban Zeer, Dough, Hama and Kishk respectively for cocci. As likewise reported by Abd El-Malek and Demerdash (1970), the presence of Lactococcus lactis and Lactococcus cremoris in Laban Zeer.

Lactobacillus isolates were classified into 3 groups (Kandler and Weiss, 1986). The domination of obligatory heterofermentative lactobacilli isolates (group C) (56.9, 54.8, 70.9 and 79.1 %) were observed in samples followed by facultative heterofermentative lactobacilli isolates (group B) (23.8, 38.7, 25.8 and 16.6 %), followed by obligatory homofermentative lactobacilli isolates (group A) (19.2, 6.5, 3.3 and 8.3 %) in Laban Zeer, Dough, Hama and Kishk respectively for lactobacilli.

This study presented the domination of heterofermentative isolates while Demerash (1960) has reported the homofermentative LAB were constituted the main microbial flora (about 84 % of the total microflora) during the fermentation process of Egyptian Kishk. This diversity of lactic acid bacteria is relative and depends primarily on the source of fermented

buttermilk which was used in Kishk manufacturing kind (Abou-Donia, 1984).

2. Following Lactic acid bacteria during Egyptian kishk manufacturing steps:

Table (2) shows the isolated lactic acid bacteria from collected samples during the actual Kishk manufacture steps (Laban Zeer, Dough, Hama and Kishk). The results in (Table 2 and Figure 1) shows that Enterococcus was found in all steps of all samples during actual manufacturing. This result was different in what was reported by Atia and Khattab (1985) who detected Enterococcus faecium in only one out of eight samples of tested Kishk, this difference might be attributed to the kind of sour milk used in Kishk preparation as well as the temperature and time of fermentation and drying. The chemical compositions have also a role on the microbial diversity of tested samples. On the other hand Lactococcus was found in one sample (sample 11) during the first steps of manufacture (Laban Zeer, Dough and Hama). The presence of this organism in the first steps due to the low salt content (3.98, 3.68 and 1.98 %) respectively compared with the last step (Kishk 4.74 %). Generally, group C was the dominant isolates in all steps of all samples except (sample 64), as it was found in Laban Zeer and then was not detected after that. This result referred to the low numbers of group C at Laban Zeer samples when compared to Dough, Hama and kishk samples. In all samples, almost group B was found in Laban Zeer and Dough, sometimes found in Hama. Group A was a minor isolates which was isolated from only one sample (sample 63) at the first steps. Similar results were reported by El-Sadek et al., (1958).

3. Hemolytic activity:

Hemolytic activity is a trait associated with virulence in some food-associated microorganisms, such as enterococci (Jett et al., 1994 and Franz et al., 1999). In this study, all isolates referring to Enterococcus did not have any hemolytic activity (γ-hemolytic) except only one strain (125RZ) that was β - hemolytic (Figure 2). While no hemolytic activity was observed for the five isolates of *Lactococcus*. These results are in agreement with (Maragkoudakis et al., 2009) who reported that there is no hemolytic activity for lactococci of dairy origin. None of the examined Lactobacillus isolates exhibited β-hemolytic activity when grown in human blood agar base 2. Most of lactobacilli isolates (214 isolates) were γ -hemolytic (i.e. no hemolytic), while two isolates exhibited α-hemolytic (85RZ and 59RZ). These results agreed with those reported by Maragkoudakis et al. (2006).

4. Off-flavour test:

The isolates which had γ –hemolytic activity were evaluated for organoleptic characteristics (color, taste, smell, texture, appearance) and overall acceptability by panelists. The results showed that there were significant differences (P \leq 0.01) among all isolates in color, taste, smell, texture, appearance and overall (Table 3). The percentage of selected samples (using different isolates) according to the overall organoleptic characteristics scores were done using the ratio between the accepted overall (overall code 2) to all tested isolates. The overall acceptability for tested isolates were (16.66, 13.20 and 0.75 %) of group A, group B and group C respectively. While, the overall acceptability for tested isolates of *Enterococci* and *Lactococci* were (1.17 % and 20 %) respectively.

Table 1. Pre-identification of isolated LAB during Egyptian Kishk manufacturing

| Genus | Laban Zeer (32samples) | Dough (7 samples) | Hama (7 samples) | Kishk (10samples) | Total ^a |
|--|------------------------|----------------------|---------------------|----------------------|--------------------|
| Enterococcus spp. | 32 (94.1 %) | 15 (93.7 %) | 16 (94 %) | 23 (95.8 %) | 86(94.5 %) |
| Lactococcus spp. | 2 (5.8 %) | 1(6.3 %) | 1 (6 %) | 1 (4.2 %) | 5 (5.5 %) |
| Total cocci | 34 | 16 | 17 | 24 | 91 |
| homofermentative lactobacill (group A) | 25 (19.2 %) | 2 (6.5 %) | 1(3.3 %) | 2 (8.3 %) | 30(13.8 %) |
| Facultative heterofermentative lactobacilli(group B) | 31(23.8 %) | 12(38.7 %) | 8 (25.8 %) | 4 (16.6 %) | 55(25.34%) |
| obligatory heterofermentative lactobacilli(group C) | 74 (56.9 %) | 17 (54.8 %) | 22(70.9 %) | 19 (79.1 %) | 132(60.8%) |
| Total lactobacilli | 130 | 31 | 31 | 25 | 217 |
| Total ^b | 164 | 47 | 48 | 49 | 308 |

a refers to total isolates of each genus during the manufacture of Kishk.

^b refers to total of all isolates from each source (Laban Zeer, Dough, Hama and Kishk).

Table 2. Pre-identification tests of Lactic acid bacteria isolates

| | | | | Pre – ident | ification test | | | = |
|----------|----------|----------|--------------|--------------|----------------------------|--------------|-----------------|---------------------|
| Samples | Isolates | Catalase | Growth at | Growth at | CO ₂ production | SF growth | Growth at 6.5 % | Pre- identification |
| Camala (| - | | 10 °C | 45 °C | | | NaCl | |
| Sample 6 | | | 1 | | | | | F4 |
| | 44RZ | - | + | + | - | + | + | Enterococcus spp |
| 6L | 45RZ | - | + | + | - | + | + | Enterococcus spp. |
| OL | 46RZ | - | + | - | - | | | G.B |
| | 47RZ | - | - | + | + | | | G.C |
| | 48RZ | - | - | + | + | | | G.C |
| | 58RZ | - | + | + | - | + | + | Enterococcus spp |
| | 59RZ | - | + | - | - | | | G.B |
| 6D | 60RZ | - | + | + | - | | | G.B |
| | 61RZ | - | - | + | + | | | G.C |
| | 62RZ | - | - | + | + | | | G.C |
| | 63RZ | - | - | + | + | | | G.C |
| | 49RZ | | + | + | - | + | + | Enterococcus spp |
| | 50RZ | | + | + | - | + | + | Enterococcus spp |
| | 51RZ | - | + | + | - | + | + | Enterococcus spp |
| 6H | 52RZ | - | + | + | + | | | G.C |
| | 53RZ | - | + | + | + | | | G.C |
| | 54RZ | - | - | + | + | | | G.C |
| | 55RZ | - | - | + | + | | | G.C |
| | 56RZ | - | - | + | + | | | G.C |
| | 39ks | - | - | + | + | | | G.C |
| | 40ks | - | - | + | + | | | G.C |
| 6K | 41ks | - | + | + | - | + | + | Enterococcus spp |
| | 42ks | - | + | + | - | + | - | Enterococcus spp |
| | 43ks | - | + | + | + | | | G.C |
| | 44ks | - | + | + | - | + | + | Enterococcus spp |
| Sample 1 | 1 | | | | | | | |
| _ | 64RZ | - | + | + | - | + | + | Enterococcus spp |
| | 65RZ | - | - | + | + | | | G.C |
| | 66RZ | - | + | - | _ | - | - | Lactococcus spp. |
| 11L | 67RZ | - | - | + | + | | | G.C |
| | 68RZ | - | + | + | + | | | G.C |
| | 69RZ | - | - | + | + | | | G.C |
| | 70RZ | - | + | + | + | | | G.C |
| | 71RZ | - | - | + | + | | | G.C |
| | 37RZ | - | + | _ | _ | _ | _ | Lactococcus spp. |
| | 38RZ | - | + | + | _ | + | + | Enterococcus spp. |
| 11D | 39RZ | - | | + | + | • | • | G.C |
| | 40RZ | _ | _ | + | + | | | G.C |
| | 41RZ | <u>-</u> | <u> </u> | + | + | | | G.C |

| | | | | Pre – ident | ification test | | - | _ |
|-----------|----------|----------|--------|-------------|----------------|--------|----------|-------------------|
| Camples | isolates | Catalase | Growth | Growth | CO2 | SF | Growth | Pre- |
| Samples | isolates | | at | at | production | growth | at 6.5 % | identification |
| | | | 10 °C | 45 °C | | | NaCl | |
| 11D | 42RZ | - | - | + | + | | | G.C |
| | 43RZ | - | + | + | - | + | + | Enterococcus spp. |
| | 72RZ | - | + | + | - | - | - | Lactocccus spp. |
| | 73RZ | - | + | + | - | + | + | Enterococcus spp. |
| | 74RZ | - | + | + | - | + | + | Enterococcus spp. |
| 11H | 75RZ | - | + | + | + | | | G.C |
| | 76RZ | - | + | + | + | | | G.C |
| | 77RZ | - | + | + | + | | | G.C |
| | 78RZ | - | - | + | + | | | G.C |
| | 50KS | - | + | + | - | + | + | Enterococcus spp. |
| 11K | 51KS | - | + | + | - | + | + | Enterococcus spp. |
| | 52KS | - | + | + | - | + | + | Enterococcus spp. |
| Sample 29 | | | | | | | | |
| | 94RZ | - | - | + | + | | | G.C |
| 29L | 95RZ | - | + | + | - | + | - | Enterococcus spp. |
| | 97RZ | - | + | - | - | | | G.B |
| | 98RZ | - | + | + | - | + | + | Enterococcus spp. |
| | 99RZ | - | + | + | - | + | + | Enterococcus spp. |
| | 100RZ | - | + | + | - | + | + | Enterococcus spp. |
| | 101RZ | - | + | - | - | | | G.B |
| 29D | 102RZ | - | + | + | + | | | G.C |
| | 103RZ | - | - | + | + | | | G.C |
| | 104RZ | - | + | - | - | | | G.B |
| | 105RZ | - | - | + | + | | | G.C |
| | 106RZ | - | + | - | | | | G.B |
| | 107RZ | - | + | + | - | | | G.B |
| _ | 219RZ | - | + | + | - | + | + | Enterococcus spp. |
| | 220RZ | - | + | + | - | + | + | Enterococcus spp. |
| | 221RZ | - | + | + | - | | | G.B |
| 29H | 222RZ | - | + | - | | - | | G.B |
| | 223RZ | - | - | + | + | - | | G.C |
| | 224RZ | - | + | - | | | | G.B |
| | 225RZ | - | + | - | - | | | G.B |
| | 226RZ | - | - | + | + | _ | | G.C |
| _ | 64KS | - | + | + | - | + | + | Enterococcus spp. |
| 29K | 65KS | - | - | + | + | | | G.C |
| | 66KS | - | - | + | + | | | G.C |
| | 67KS | - | + | + | + | | | G.C |
| Sample 63 | | | | | | | | |
| 63L | 134RZ | | + | + | + | | | G.C |

| | | | | Pre – ident | ification test | | | _ |
|------------|----------|----------------|--------|--------------|----------------|--------|----------|---------------------|
| Samples | Isolates | Catalase | Growth | Growth | CO_2 | SF | Growth | Pre- identification |
| Samples | Isolates | | at | at | production | growth | at 6.5 % | |
| | | | 10 °C | 45 °C | | | NaCl | |
| | 135RZ | - | + | + | - | | | G.B |
| | 136RZ | - | + | + | - | + | + | Enterococcus spp. |
| 63L | 137RZ | | + | + | | + | + | Enterococcus spp. |
| | 138RZ | - | - | + | | | | G.A |
| | 139RZ | - | - | + | | | | G.A |
| | 140RZ | - | - | + | | | | G.A |
| | 243RZ | - | - | + | + | | | G.C |
| | 244RZ | - | + | + | - | | | G.B |
| 63D | 245RZ | - | + | + | - | + | + | Enterococcus spp. |
| | 246RZ | - | + | + | - | + | + | Enterococcus spp. |
| | 247RZ | - | - | + | - | | | G.A |
| | 248RZ | - | - | + | - | | | G.A |
| | 227RZ | - | + | + | - | + | + | Enterococcus spp. |
| | 228RZ | - | + | + | - | + | + | Enterococcus spp. |
| | 229RZ | - | + | + | - | | | G.B |
| 63H | 230RZ | - | - | + | + | | | G.C |
| | 231RZ | - | + | - | - | | - | G.B |
| | 232RZ | - | - | + | - | | | G.A |
| | 233RZ | - | + | + | - | + | + | Enterococcus spp. |
| | 234RZ | - | - | + | + | | | G.C |
| 63K | 71KS | - | + | + | - | + | + | Enterococcus spp. |
| | 72KS | - | - | + | + | | _ | G.C |
| Sample 64 | | | | | | | | |
| | 249RZ | - | + | + | - | + | + | Enterococcus spp. |
| | 250RZ | - | + | + | | + | + | Enterococcus spp. |
| 64L | 251RZ | - | + | - | + | | | G.C |
| | 252RZ | - | + | + | - | | - | G.B |
| | 253RZ | - | + | - | - | | | G.C |
| - | 254RZ | - | + | + | _ | + | + | Enterococcus spp. |
| | 255RZ | | + | + | | + | + | Enterococcus spp. |
| 64D | 256RZ | | + | + | | + | + | Enterococcus spp. |
| | 257RZ | - | + | _ | | | _ | G.B |
| | 258RZ | - | + | + | | _ | _ | G.B |
| | 259RZ | | + | - | | | | G.B |
| | 281RZ | | + | + | | | _ | G.B |
| 64H | 282RZ | | + | + | | - | | G.B |
| | 283RZ | - - | + | + | - | + | + | Enterococcus spp. |
| - | 68KS | - | + | + | - | + | + | Enterococcus spp. |
| 64K | | | | | | | | |
| J 111 | 69KS | - | + | + | - | + | + | Enterococcus spp. |
| Campal: 74 | 70KS | | + | + | | | | G.B |
| Sample 74 | | | | | _ | | | |
| 74L | 141RZ | | + | + | | + | + | Enterococcus spp. |

| | | Catalase | Growth | Growth | CO ₂ | SF | Growth | Pre- identification |
|-----------|----------|----------|--------------|-------------|-----------------|---------------|----------|---------------------|
| Samples | Isolates | Catalase | at | at | production | growth | at 6.5 % | 11c-identification |
| | | | 10 °C | 45 °C | production | growth | NaCl | |
| 74L | 142RZ | - | + | + | - | + | + | Enterococcus spp |
| | 143RZ | - | - | + | + | | | G.C |
| | 144RZ | - | + | + | + | | | G.C |
| | 145RZ | - | + | - | - | | | G.B |
| | 146RZ | - | - | + | + | | | G.C |
| | 147RZ | - | - | + | + | | | G.C |
| 74D | 236RZ | - | + | + | - | + | + | Enterococcus spp |
| | 237RZ | - | + | + | - | + | + | Enterococcus spp |
| | 238RZ | - | + | + | - | + | + | Enterococcus spp |
| | 239RZ | - | - | + | + | | | G.C |
| | 240RZ | - | + | - | - | | | G.B |
| | 241RZ | - | - | + | + | | | G.C |
| | 242RZ | - | - | + | + | | | G.C |
| 74H | 148RZ | - | - | + | - | + | + | Enterococcus spp |
| | 149RZ | - | + | + | + | | | G.C |
| | 150RZ | - | - | + | - | + | - | Enterococcus sp |
| | 151RZ | - | + | + | + | - | | G.C |
| | 152RZ | - | + | + | + | | | G.C |
| | 153RZ | - | + | + | + | | | G.C |
| | 58KS | - | + | + | - | + | + | Enterococcus spp |
| | 59KS | - | + | + | - | + | - | Enterococcus sp |
| 74K | 60KS | - | + | + | + | + | - | Enterococcus spp |
| | 61KS | - | + | + | + | | | G.C |
| | 62KS | - | + | + | + | | | G.C |
| | 63KS | - | - | + | + | | | G.C |
| Sample 76 | | | | | | | | • |
| | 260RZ | - | + | + | - | + | + | Enterococcus sp |
| | 261RZ | - | + | + | - | + | + | Enterococcus sp |
| | 262RZ | | + | + | - | + | + | Enterococcus sp |
| 76L | 263RZ | | + | | + | - | - | G.C |
| | 264RZ | | + | - | | | - | G.B |
| | 265RZ | | _ | + | + | _ | _ | G.C |
| | 266RZ | | + | | + | | | G.C |
| | 274RZ | | | + | + | | | G.C |
| | 275RZ | | | + | + | | - | G.C |
| | 276RZ | | - | + | + | | _ | G.C |
| 76D | 277RZ | | + | + | _ | + | + | |
| | | - | | | - | + | | Enterococcus spp |
| | 278RZ | | + | + | | | + | Enterococcus spp |
| | 279RZ | - | + | + | - | + | + | Enterococcus spp |
| | 280RZ | | + | + | | | _ | G.B |
| | 284RZ | | + | + | - | | | G.B ntinuous |

| | | | | Pre – identi | fication test | | | - |
|---------|----------|----------|--------|--------------|-----------------|--------|---------|----------------------------|
| | | Catalase | Growth | Growth | CO ₂ | SF | Growt | Pre- identification |
| Samples | Isolates | | at | at | production | growth | h | |
| | | | 10 °C | 45 °C | | | at 6.5% | |
| | | | | | | | NaCl | |
| 76H | 267RZ | | + | + | | + | + | Enterococcus spp. |
| | 268RZ | - | + | + | - | + | - | Enterococcus spp. |
| | 269RZ | - | + | - | + | | | G.C |
| | 270RZ | - | - | + | + | | | G.C |
| | 271RZ | - | - | + | + | | | G.C |
| | 272RZ | - | - | + | + | | | G.C |
| | 273RZ | - | - | + | + | | | G.C |
| 76K | 54KS | - | + | + | - | + | + | Enterococcus spp. |
| | 55KS | - | + | + | - | + | + | Enterococcus spp. |
| | 56KS | - | + | - | + | | | G.C |
| | 57KS | | + | - | + | | | G.C |

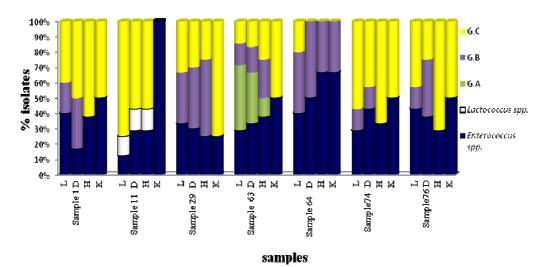


Fig. 1. Lactic acid bacteria diversity during Egyptian Kishk manufacturing (L) refers to Laban Zeer; (D) Dough; (H) Hama and (K) Kishk

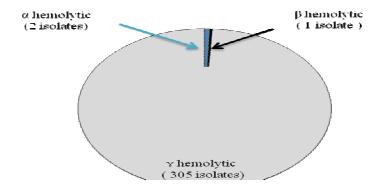


Fig. 2. Hemolytic activity of Lactic acid bacteria isolates

Group A isolates; The taste and odor of the curd were pleasant, The curds of fermented milk were smooth, compact and without syneresis when the isolates (23RZ, 86RZ, 162RZ, 182RZ and 207RZ) were used in fermentation, while the syneresis was noted with the both (232RZ and 27KS) isolates. Most isolates produced the bitterness defect with varying degrees in fermented milk, this is refer to high proteolytic activity of tested isolates. It is important to bear in mind that highly proteolytic strains are not always the most suitable for use as starter cultures, since excessive proteolysis can cause uncontrolled production of bitter peptides and other undesirable compounds, or even excessive casein hydrolysis resulting in a too-soft final

product (Buffa *et al.*, 2005). Results in Table 4 were referred to sensorial evaluation means of group A isolates.

Group B isolates; they were divided into four different tastes. Firstly, the taste was flat. Secondly, the curd had bitterness taste for example but not limited to (225RZ, 231RZ, 240RZand 244RZ). Thirdly, the curd was unpleasant taste like (106RZ, 160RZ AND 170 RZ). Finally, the curd was pleasant taste and good odor without any defects such as (101RZ, 157RZ, 159RZ, 172RZ, 190RZ, 281 RZ and 284RZ). It is observed in Tables (3 and 5) that there were significant differences ($p \ge 0.05$) between panelists in overall acceptance.

Table 4. Sensorial evaluation means of fermented milk by isolates (group A)

| Inclotos | | | | Mean | | | |
|----------|-----------|------------|-----------|--------------|----------------|-------------------|----------|
| Isolates | Color(10) | Taste (10) | Smell(10) | Texture (10) | Appearance(10) | Total (50) | Over all |
| 23RZ | 8.66 | 8.86 | 9 | 7.73 | 8.53 | 42.8 | 2 |
| 86RZ | 9.23 | 9 | 9 | 8.93 | 8.93 | 45.1 | 2 |
| 87RZ | 8.00 | 2.33 | 5.13 | 6.66 | 7.06 | 29.2 | 1 |
| 115RZ | 5.60 | 3.06 | 1.93 | 3.20 | 3.33 | 17.13 | 1 |
| 116RZ | 4.86 | 3 | 2 | 2.86 | 3 | 15.73 | 1 |
| 122RZ | 5.60 | 3.06 | 2.13 | 2.33 | 2.33 | 15.46 | 1 |
| 127RZ | 5.20 | 3.4 | 3.06 | 2.46 | 233 | 16.46 | 1 |
| 130RZ | 5.33 | 3.46 | 3.13 | 2.20 | 2.20 | 16.33 | 1 |
| 138RZ | 4.43 | 2.80 | 2.46 | 2.26 | 2.53 | 14.5 | 1 |
| 139RZ | 2.80 | 2.26 | 1.80 | 1.80 | 1.73 | 10.26 | 1 |
| 140RZ | 3.93 | 2.80 | 2.13 | 1.93 | 1.86 | 12.8 | 1 |
| 154RZ | 5.26 | 3.33 | 2.73 | 2.93 | 3 | 17.25 | 1 |
| 161RZ | 5.53 | 3.6 | 2.86 | 2.73 | 2.86 | 17.60 | 1 |
| 162RZ | 8.06 | 8.86 | 8.66 | 8.26 | 7.90 | 41.76 | 2 |
| 163RZ | 7.86 | 6.93 | 2.06 | 7.46 | 7.46 | 31.80 | 1 |
| 164RZ | 4.93 | 3.06 | 2.66 | 2.80 | 2.93 | 16.40 | 1 |
| 182RZ | 9.20 | 8.5 | 8.66 | 8.93 | 9.03 | 44.40 | 2 |
| 184RZ | 5.20 | 2.86 | 2.20 | 3.33 | 3.33 | 16.93 | 1 |
| 191RZ | 5.33 | 2.86 | 2.66 | 3 | 2.86 | 16.73 | 1 |
| 202RZ | 4.40 | 2.4 | 2.33 | 2.13 | 2.20 | 13.46 | 1 |
| 207RZ | 8.66 | 8.9 | 8.9 | 8.86 | 8.56 | 43.93 | 2 |
| 208RZ | 5.40 | 2.86 | 2.60 | 2.73 | 2.73 | 16.33 | 1 |
| 211RZ | 4.86 | 3.2 | 2.20 | 1.60 | 1.66 | 13.53 | 1 |
| 212RZ | 4.93 | 3.2 | 2.33 | 1.86 | 1.73 | 14.06 | 1 |
| 213RZ | 4.06 | 2.8 | 2.13 | 2 | 1.86 | 12.86 | 1 |
| 232RZ | 5.53 | 3.06 | 2.13 | 1.93 | 1.73 | 14.40 | 1 |
| 247RZ | 5.53 | 4.86 | 3.40 | 2.93 | 1.80 | 18.53 | 1 |
| 248RZ | 5.60 | 4.20 | 3.06 | 1.80 | 1.66 | 15.40 | 1 |
| 27KS | 5.20 | 3.40 | 2.40 | 1.86 | 1.80 | 14.66 | 1 |
| 29KS | 5.06 | 3.6 | 2.73 | 2.06 | 1.80 | 15.26 | 1 |
| Mean | 5.81 | 4.22 | 3.61 | 3.72 | 3.69 | 21.03 | 1.16 |

| $LSD_{0.01}$ | 0.84 | 0.76 | 0.62 | 0.71 | 0.66 | 2.26 | |
|--------------|------|------|------|------|------|------|--|
| LOD | 0.04 | 0.50 | 0.60 | 0.51 | 0.66 | 2.26 | |

Overall 1: unacceptable Overall 2: acceptable

Table 5. Sensorial evaluation means of fermented milk by isolates (group B)

| | 201201101 | , | | Mean | oy isolates (group | , | |
|----------------|-------------------|---|------------|--------------|--------------------|-------------------|------------|
| Isolates | Color (10) | Taste (10) | Smell (10) | Texture (10) | Appearance (10) | Total (50) | Over all |
| 17RZ | 7.40 | 2.93 | 4.06 | 1.33 | 1.86 | 17.60 | 1 |
| 27RZ | 7.9 | 5.13 | 5.13 | 6.8 | 6.86 | 31.86 | 1 |
| 28RZ | 7.9 | 5.13 | 5.13 | 6.8 | 6.86 | 31.86 | 1 |
| 46RZ | 8 | 7.06 | 6.60 | 5.13 | 6 | 32.8 | 1 |
| 97RZ | 8 | 3.53 | 3.26 | 5.20 | 5.40 | 25.4 | 1 |
| 101RZ | 9 | 8.1 | 7.40 | 8.6 | 8.60 | 41.7 | 2 |
| 104RZ | 8 | 1 | 1.46 | 4.53 | 4.53 | 19.53 | 1 |
| 106RZ | 8 | 1.13 | 1.46 | 4.46 | 4.53 | 19.60 | 1 |
| 107RZ | 8 | 5.96 | 5.53 | 6.53 | 6.60 | 32.63 | 1.06 |
| 120RZ | 5 | 3.06 | 2.20 | 2.13 | 1.93 | 14.33 | 1 |
| 131RZ | 5.46 | 3 | 2.40 | 2.93 | 2.93 | 16.73 | 1 |
| 132RZ | 3.20 | 1.53 | 1.26 | 0.53 | 1.40 | 7.93 | 1 |
| 133RZ | 5.73 | 3 | 2.40 | 2.80 | 2.66 | 16.60 | 1 |
| 135RZ | 7.66 | 5.43 | 5.73 | 6.7 | 6.46 | 32.03 | 1.26 |
| 145RZ | 4.53 | 3.2 | 2.86 | 2.80 | 2.66 | 16.0 | 1 |
| 156RZ | 7.40 | 3.20 | 3.40 | 5.13 | 5.33 | 24.46 | 1 |
| 157RZ | 8.86 | 8.1 | 8.46 | 7.73 | 7.80 | 41 | 2 |
| 158RZ | 7.60 | 1.93 | 2.73 | 4.53 | 5.26 | 22.26 | 1 |
| 159RZ | 8 | 7.8 | 7.53 | 6.6 | 7.36 | 37.36 | 2 |
| 160RZ | 4.53 | 3.06 | 2.86 | 2.60 | 2.60 | 15.66 | 1 |
| 170RZ | 5.20 | 2.80 | 2.00 | 3.46 | 3.46 | 16.93 | 1 |
| 171RZ | 4.53 | 1.80 | 1.73 | 1.46 | 1.73 | 11.26 | 1 |
| 172RZ | 9 | 8.9 | 8.86 | 8.9 | 8.66 | 44.36 | 2 |
| 175RZ | 4 | 0.53 | 0.66 | 1.06 | 1.26 | 7.53 | 1 |
| 176RZ | 7.86 | 5.06 | 5.26 | 6.33 | 6.46 | 31 | 1.06 |
| 190RZ | 8 | 7.06 | 7.83 | 8.8 | 8.86 | 40.56 | 2 |
| 192RZ | 5.53 | 3 | 2.26 | 2.66 | 2.73 | 16.20 | <u>2</u> 1 |
| 201RZ | 5.13 | 3.20 | 2.20 | 2.40 | 2.33 | 15.26 | 1 |
| 203RZ | 6.73 | 4.8 | 5.26 | 5.66 | 5.13 | 27.60 | 1 |
| 209RZ | 5.40 | 3.13 | 3.20 | 2.13 | 2.06 | 15.73 | 1 |
| 210RZ | 4.73 | 2.93 | 2.26 | 2.33 | 2.20 | 14.46 | 1 |
| 221RZ | 4.60 | 3.06 | 2.20 | 1.60 | 1.60 | 13.06 | 1 |
| 222RZ | 5.26 | 3.40 | 2.33 | 1.93 | 1.93 | 14.86 | 1 |
| 224RZ | 4.73 | 3 | 2.26 | 1.80 | 1.66 | 13.46 | 1 |
| 225RZ | 5.46 | 3.66 | 2.60 | 2.20 | 2 | 15.40 | 1 |
| 229RZ | 5.73 | 3.26 | 2.40 | 1.86 | 1.73 | 15.55 | 1 |
| 231RZ | 4.6 | 2.93 | 2.33 | 2.13 | 1.93 | 14 | 1 |
| 240RZ | 5.13 | 2.80 | 2.33 | 4.80 | 4.40 | 19.26 | 1 |
| 244RZ | 4.53 | 3.06 | 2.13 | 2.60 | 2.60 | 15.66 | 1 |
| 252RZ | 4.33 7 | 5.00 | 6.06 | 6.20 | 5.86 | 30.13 | 1.3 |
| 252RZ 253RZ | 5.26 | 3.26 | 2.73 | 2.93 | 2.93 | 17.13 | 1.3 |
| 257RZ | 7 | 5.4 | 6.20 | 6.40 | 6.33 | 31.33 | 1.3 |
| 257RZ 258RZ | 5.13 | 3.20 | 2.33 | 2.06 | 1.80 | 14.53 | 1.3 |
| 259RZ | 5.26 | 3.20 | 2.53 | 2.26 | 2.13 | 15.40 | 1 |
| Overall 1: r | 3.20 | 3.20 | | Z.20 | 2.13 | | |

Overall 1: non acceptable overall acceptability between panelists

Overall 2: acceptable Overall:2> overall 1>1; refer to different

Con. Table 5.

| | | | | Mean | | | |
|--------------|------------|-------------------|------------|------------|---------------|-------------------|----------|
| Isolates | Color (10) | Taste (10) | Smell (10) | Texture(10 | Appearance(10 | Total (50) | Over all |
| 264RZ | 7.46 | 4.56 | 5.80 | 5.80 | 6.06 | 29.70 | 1 |
| 280RZ | 8.20 | 4.73 | 4.66 | 4.80 | 4.60 | 27 | 1.13 |
| 281RZ | 7.53 | 7.6 | 8.36 | 7.26 | 8.06 | 38.90 | 2 |
| 282RZ | 7 | 4.50 | 6.23 | 5.66 | 5.80 | 29.20 | 1.2 |
| 284RZ | 7.60 | 7.9 | 7.33 | 7.26 | 7.93 | 38.1 | 2 |
| 31KS | 4.40 | 2.80 | 2.06 | 1.73 | 1.66 | 12.66 | 1 |
| 32KS | 5.26 | 3.06 | 2.13 | 2 | 2 | 14.46 | 1 |
| 45KS | 5.06 | 2.80 | 1.93 | 1.93 | 1.80 | 13.53 | 1 |
| 70KS | 4.66 | 3.26 | 2.40 | 1.80 | 1.80 | 13.93 | 1 |
| Mean | 6.29 | 4.02 | 3.83 | 4.08 | 4.13 | 22.37 | 1.17 |
| $LSD_{0.01}$ | 0.72 | 0.88 | 0.73 | 0.72 | 0.64 | 2.48 | - |

Overall 1: non acceptable

Overall 2: acceptable

Overall: 2> overall 1>1; refer to different overall acceptability between panelists

Table 6. Sensorial evaluation means of fermented milk by isolates (group C)

| | | | - | Mean | - | | |
|-----------------|-----------|-----------|------------|------------|---------------|-----------|----------|
| Isolates | Color(10) | Taste(10) | Smell (10) | Texture(10 | Appearance(10 | Total(50) | Over all |
| | 20101(10) | | . , |) |) | | Over an |
| 16RZ | 7.40 | 2.93 | 4.06 | 6.46 | 4.26 | 25.13 | 1 |
| 20RZ | 7.53 | 2.93 | 3.66 | 6.40 | 4.66 | 25.20 | 1 |
| 21RZ | 5.93 | 2.00 | 1.66 | 5.93 | 5.93 | 21.48 | 1 |
| 22RZ | 7.60 | 0.80 | 1.06 | 7.00 | 5.53 | 22.00 | 1 |
| 24RZ | 7.66 | 6 | 5.73 | 6.00 | 5.60 | 31.00 | 1 |
| 25RZ | 7.93 | 5.40 | 4.73 | 5.93 | 6.26 | 30.26 | 1.20 |
| 31RZ | 7.33 | 5.80 | 5.86 | 5.86 | 5.93 | 30.800 | 1.13 |
| 32RZ | 7.73 | 4.66 | 4.40 | 7.40 | 6.46 | 30.66 | 1 |
| 34RZ | 7.40 | 3.40 | 3.26 | 3.73 | 3.60 | 21.40 | 1 |
| 39RZ | 8.60 | 3.93 | 2.73 | 4.93 | 4.93 | 25.13 | 1 |
| 40RZ | 8 | 2.00 | 1.26 | 0.00 | 0.00 | 11.26 | 1 |
| 41RZ | 7.80 | 4.46 | 4.06 | 5.93 | 6.26 | 28.53 | 1 |
| 42RZ | 7.60 | 2.40 | 2.86 | 4.93 | 4.93 | 22.73 | 1 |
| 47RZ | 7.80 | 4.53 | 4.86 | 3.60 | 3.60 | 24.40 | 1.13 |
| 48RZ | 8 | 3.66 | 3.93 | 6.66 | 6.40 | 28.66 | 1 |
| 52RZ | 8 | 4.26 | 3.20 | 6.06 | 6.20 | 27.73 | 1 |
| 53RZ | 8 | 4.26 | 3.20 | 6.40 | 6.33 | 28.20 | 1 |
| 54RZ | 7.93 | 5.26 | 5.13 | 4.13 | 4.26 | 26.73 | 1 |
| 55RZ | 7.53 | 4.90 | 4.66 | 4.66 | 4.86 | 26.63 | 1 |
| 56RZ | 7.60 | 4.46 | 4.53 | 4.13 | 4.26 | 25.00 | 1 |
| 61RZ | 5.40 | 2.13 | 2.86 | 4.33 | 4.13 | 26.13 | 1 |
| 62RZ | 6.26 | 2.60 | 5.33 | 5.80 | 5.06 | 25.06 | 1 |
| 63RZ | 7.53 | 4.36 | 3.46 | 1.40 | 1.80 | 18.56 | 1 |
| 65RZ | 6.26 | 2.66 | 5.33 | 5.80 | 5.06 | 25.13 | 1 |
| 67RZ | 7.20 | 1.46 | 3.53 | 6.00 | 5.93 | 24.13 | 1 |
| 68RZ | 4.93 | 0.86 | 0.86 | 3.00 | 2.60 | 12.26 | 1 |
| 69RZ | 5.73 | 2.40 | 1.86 | 3.60 | 2.93 | 16.53 | 1 |
| 70RZ | 6.13 | 1.73 | 1.00 | 4.86 | 4.73 | 18.46 | 1 |
| | | | | | | | |

| 71RZ | 7.20 | 2.40 | 2.46 | 4.46 | 4.53 | 21.06 | 1 |
|------|------|------|------|------|------|-------|---|

Overall 1: non acceptable
Overall: 2> overall 1>1; refer to different overall acceptability between panelists

Con. Table 6.

| | | | | Mean | | | |
|----------|------------|-------|-------|---------|------------|-------|----------|
| Isolates | C 1 (10) | Taste | Smell | Texture | Appearance | Total | 0 11 |
| | Color (10) | (10) | (10) | (10) | (10) | (50) | Over all |
| 75RZ | 5.13 | 3.26 | 3.53 | 2.13 | 2.20 | 15.73 | 1 |
| 76RZ | 5.06 | 3.26 | 3.33 | 2.53 | 2.66 | 16.86 | 1 |
| 77RZ | 7.13 | 1.53 | 2.33 | 3.73 | 3.60 | 18.53 | 1 |
| 78RZ | 6.20 | 2.33 | 3.06 | 3.46 | 3.46 | 18.52 | 1 |
| 79RZ | 6.06 | 2.53 | 2.80 | 2.93 | 3.13 | 17.46 | 1 |
| 80RZ | 5.33 | 2.86 | 2.53 | 2.40 | 2.40 | 15.53 | 1 |
| 81RZ | 6 | 2.26 | 2.53 | 280 | 2.53 | 16.13 | 1 |
| 84RZ | 5.2 | 2.80 | 2.06 | 1.60 | 2.60 | 13.26 | 1 |
| 88RZ | 5.26 | 2.73 | 2.33 | 2.20 | 2.20 | 14.73 | 1 |
| 91RZ | 5.73 | 3.13 | 2.53 | 1.93 | 1.86 | 15.20 | 1 |
| 92RZ | 5.26 | 2.86 | 2.13 | 1.80 | 1.73 | 13.86 | 1 |
| 93RZ | 6.73 | 5.66 | 4.96 | 6.46 | 6.73 | 30.56 | 1 |
| 94RZ | 5.26 | 2.80 | 2.80 | 2.66 | 2.404 | 15.93 | 1 |
| 128RZ | 4.40 | 2.26 | 2.40 | 2.26 | 2.13 | 13.46 | 1 |
| 129RZ | 5.26 | 2.80 | 2.80 | 2.06 | 2.40 | 15.93 | 1 |
| 134RZ | 5.06 | 3.00 | 2.73 | 3.13 | 3.00 | 16.93 | 1 |
| 143RZ | 4.66 | 4 | 3 | 2.73 | 2.66 | 17.06 | 1 |
| 144RZ | 5.66 | 3.33 | 2.60 | 2.93 | 3.00 | 17.53 | 1 |
| 146RZ | 5.06 | 3.33 | 2.60 | 2.66 | 2.80 | 16.46 | 1 |
| 147RZ | 4.20 | 2.93 | 2.40 | 2.13 | 2.13 | 13.80 | 1 |
| 149RZ | 5.26 | 2.80 | 2.80 | 2.66 | 2.40 | 15.93 | 1 |
| 151RZ | 4.80 | 3.46 | 2.93 | 2.33 | 2.33 | 15.86 | 1 |
| 152RZ | 5.26 | 3.46 | 2.86 | 2.40 | 2.46 | 16.46 | 1 |
| 153RZ | 5.06 | 3.53 | 2.93 | 2.86 | 2.53 | 16.93 | 1 |
| 165RZ | 4.60 | 2.40 | 1.93 | 1.73 | 2.06 | 12.73 | 1 |
| 166RZ | 4.73 | 2.60 | 4.73 | 2.06 | 2.33 | 14.00 | 1 |
| 169RZ | 5 | 3.13 | 2.60 | 2.40 | 2.26 | 15.40 | 1 |
| 174RZ | 5.26 | 3.26 | 2.73 | 2.93 | 2.93 | 17.13 | 1 |
| 178RZ | 4 | 2.80 | 2.40 | 2.40 | 2.20 | 13.80 | 1 |
| 179RZ | 4.73 | 2.80 | 2.13 | 2.06 | 2.33 | 14.06 | 1 |
| 180RZ | 4.73 | 3.06 | 2.40 | 2.26 | 2.33 | 14.80 | 1 |
| 181RZ | 5.73 | 2.93 | 1.93 | 2.93 | 3.00 | 16.53 | 1 |
| 183RZ | 4.86 | 2.93 | 2.33 | 2.20 | 2.46 | 14.80 | 1 |
| 185RZ | 5.53 | 2.93 | 2.33 | 3.46 | 3.46 | 17.73 | 1 |
| 186RZ | 5 | 3.26 | 2.53 | 2.53 | 2.60 | 15.86 | 1 |
| 187RZ | 4.66 | 2.93 | 2.33 | 2.40 | 2.20 | 14.53 | 1 |
| 188RZ | 4.66 | 2.93 | 2.33 | 2.40 | 2.20 | 14.53 | 1 |
| 189RZ | 4.66 | 2.93 | 2.26 | 2.46 | 2.40 | 14.73 | 1 |
| 193RZ | 4.86 | 3.40 | 2.80 | 1.73 | 1.60 | 14.40 | 1 |
| 194RZ | 5.33 | 2.93 | 2.13 | 2.53 | 2.66 | 15.60 | 1 |
| 195RZ | 4.60 | 3.20 | 2.40 | 1.73 | 1.80 | 13.73 | 1 |
| 196RZ | 4.40 | 2.86 | 2.26 | 2.26 | 2.26 | 14.13 | 1 |
| 197RZ | 4.46 | 3.06 | 2.26 | 2.00 | 2.06 | 13.86 | 1 |
| 198RZ | 5.2 | 3.06 | 2.26 | 2.80 | 2.80 | 16.13 | 1 |
| 199RZ | 5.26 | 3.40 | 2.33 | 1.93 | 1.93 | 14.86 | 1 |
| 200RZ | 4.80 | 3.06 | 2.46 | 2.60 | 2.66 | 15.60 | 1 |
| | | | | | | | |
| 204RZ | 5.33 | 3.20 | 2.66 | 2.66 | 2.80 | 16.66 | 1 |

Overall 1: non acceptable Overall 1: non acceptable Overall 2: acceptable

Overall; 2> overall>1; refer to different overall acceptability between panelists

Con. Table 6.

| Con. Tab | <i>ic</i> 0. | | | Mean | | | |
|--------------|--------------|------------|------------|--------------|-----------------|------------|----------|
| Isolates | Color (10) | Taste (10) | Smell (10) | Texture (10) | Appearance (10) | Total (50) | Over all |
| 205RZ | 5.60 | 3.33 | 2.26 | 1.86 | 1.93 | 15.00 | 1 |
| 206RZ | 5.06 | 3.20 | 2.53 | 2.06 | 1.93 | 14.80 | 1 |
| 214RZ | 5.13 | 3.13 | 2.46 | 2.06 | 2.00 | 14.80 | 1 |
| 216RZ | 5.73 | 3.66 | 2.66 | 2.00 | 2.06 | 16.20 | 1 |
| 217RZ | 4.73 | 3.06 | 2.26 | 2.00 | 1.93 | 14.00 | 1 |
| 218RZ | 5.40 | 3.26 | 2.60 | 1.73 | 1.60 | 14.60 | 1 |
| 223RZ | 5.26 | 3.20 | 2.46 | 2.00 | 2.00 | 14.93 | 1 |
| 226RZ | 5.53 | 3.46 | 2.33 | 2.26 | 2.13 | 15.73 | 1 |
| 230RZ | 5.06 | 3.26 | 2.60 | 2.06 | 2.13 | 15.13 | 1 |
| 234RZ | 5.26 | 3.40 | 2.33 | 1.86 | 1.80 | 14.66 | 1 |
| 239RZ | 5.26 | 3.26 | 2.73 | 2.73 | 2.73 | 16.73 | 1 |
| 241RZ | 4.93 | 2.73 | 1.80 | 1.80 | 1.73 | 13.00 | 1 |
| 242RZ | 4.73 | 3.06 | 2.26 | 1.80 | 1.60 | 13.46 | 1 |
| 243RZ | 5.53 | 3.40 | 2.20 | 1.73 | 1.66 | 14.53 | 1 |
| 251RZ | 5.26 | 3.40 | 2.33 | 1.80 | 1.73 | 14.53 | 1 |
| 263RZ | 4.46 | 3.06 | 2.26 | 2.00 | 2.06 | 13.86 | 1 |
| 265RZ | 5.26 | 3.46 | 2.73 | 2.13 | 1.93 | 15.60 | 1 |
| 266RZ | 5.46 | 3.73 | 2.73 | 1.73 | 1.60 | 15.26 | 1 |
| 269RZ | 5.13 | 3.26 | 2.66 | 2.06 | 1.93 | 15.06 | 1 |
| 270RZ | 5.26 | 3.40 | 2.33 | 1.73 | 1.73 | 14.46 | 1 |
| 271RZ | 4.73 | 3.00 | 2.40 | 1.66 | 1.66 | 13.46 | 1 |
| 272RZ | 5.06 | 3.26 | 2.66 | 2.06 | 1.86 | 14.93 | 1 |
| 273RZ | 5.26 | 3.40 | 2.33 | 1.93 | 1.93 | 14.86 | 1 |
| 274RZ | 7.93 | 8.26 | 7.80 | 7.66 | 7.90 | 39.56 | 2 |
| 275RZ | 6.60 | 5.60 | 4.93 | 5.43 | 5.33 | 27.90 | 1.33 |
| 276RZ | 7 | 7.46 | 7.86 | 7.23 | 7.56 | 37.13 | 1.53 |
| 26KS | 5.2 | 3.80 | 2.80 | 1.73 | 1.60 | 15.13 | 1 |
| 28KS | 4.46 | 3.06 | 2.33 | 1.66 | 1.53 | 13.06 | 1 |
| 30KS | 4.66 | 2.93 | 2.46 | 2.20 | 2.00 | 14.26 | 1 |
| 33KS | 5.33 | 3.46 | 2.73 | 1.86 | 1.86 | 15.26 | 1 |
| 34KS | 4.93 | 3.40 | 2.80 | 1.93 | 1.80 | 14.86 | 1 |
| 35KS | 5.2 | 3.60 | 2.53 | 2.00 | 1.93 | 15.26 | 1 |
| 38KS | 5.53 | 3.13 | 2.33 | 1.80 | 1.60 | 14.40 | 1 |
| 39KS | 5.06 | 3.33 | 2.73 | 2.13 | 1.80 | 15.06 | 1 |
| 40KS | 4.46 | 2.66 | 2.06 | 1.66 | 1.53 | 12.40 | 1 |
| 43KS | 5.06 | 3.00 | 2.26 | 1.93 | 2.00 | 14.26 | 1 |
| 56KS | 5.13 | 3.26 | 2.20 | 1.46 | 1.53 | 13.60 | 1 |
| 57KS | 5.2 | 3.06 | 2.46 | 1.93 | 1.80 | 14.46 | 1 |
| 61KS | 6.26 | 4.73 | 4.46 | 5.26 | 5.40 | 26.13 | 1.06 |
| 62KS | 7.13 | 5.13 | 5.93 | 6.40 | 5.93 | 30.53 | 1.20 |
| 63KS | 5.06 | 3.20 | 2.26 | 1.26 | 1.46 | 13.26 | 1 |
| 65KS | 5 | 3.00 | 2.53 | 1.66 | 1.66 | 13.86 | 1 |
| 66KS | 5.53 | 3.46 | 2.33 | 1.80 | 1.66 | 14.80 | 1 |
| 67KS | 5.46 | 3.26 | 2.20 | 1.73 | 1.66 | 14.33 | 1 |
| 72KS | 5 | 3.66 | 2.46 | 1.86 | 1.86 | 14.86 | 1 |
| Mean | 5.67 | 3.28 | 2.84 | 3.03 | 2.94 | 17.77 | 1.01 |
| $LSD_{0.01}$ | 0.81 | 0.81 | 0.68 | 0.69 | 0.63 | 2.15 | - |
| | | | | | | | |

Overall 1: non acceptable

Overall: 2> overall 1>1; refer to different overall acceptability between panelists



Table 7. Sensorial evaluation means of fermented milk by Lactococci isolates

| | Mean | | | | | | | | | |
|---------------------|------------|------------|------------|------------|---------------|------------|----------|--|--|--|
| Isolates | Color (10) | Taste (10) | Smell (10) | Texture(10 | Appearance(10 | Total (50) | Over all | | | |
| 37RZ | 6.73 | 4.86 | 5.2 | 5.73 | 5.8 | 28.33 | 1 | | | |
| 66RZ | 7.4 | 5.13 | 5.46 | 5.13 | 5 | 28.13 | 1 | | | |
| 72RZ | 5.5 | 1.13 | 1.80 | 5.80 | 5.33 | 19.6 | 1 | | | |
| 124RZ | 8.7 | 8.6 | 8.53 | 8.13 | 8.4 | 42.4 | 2 | | | |
| 23KS | 7.46 | 5.33 | 4.86 | 4.33 | 3.93 | 25.93 | 1 | | | |
| Mean | 7.17 | 5.01 | 5.17 | 5.82 | 5.69 | 28.88 | 1.2 | | | |
| LSD _{0.01} | 0.50 | 0.65 | 0.60 | 0.72 | 0.65 | 2.01 | - | | | |

Overall 1: non acceptable

Overall 2: acceptable

Overall; 2> overall>1; refer to different overall acceptability between panelists

Table 8. Sensorial evaluation means of fermented milk by Enterococci isolates

| Isolates | | | | Mean | | | |
|----------|------------|-----------|------------|-------------|----------------|------------|----------|
| isolates | Color (10) | Taste(10) | Smell (10) | Texture(10) | Appearance(10) | Total (50) | Over all |
| 18RZ | 5.86 | 1.73 | 1.00 | 1.73 | 1.93 | 12.26 | 1 |
| 19RZ | 6.86 | 1.66 | 0.93 | 3.13 | 2.26 | 14.86 | 1 |
| 26RZ | 6.73 | 1.53 | 0.73 | 4.26 | 4.06 | 17.33 | 1 |
| 29RZ | 6.40 | 1.26 | 0.93 | 6.33 | 6.33 | 21.26 | 1 |
| 30RZ | 7.00 | 1.46 | 0.93 | 6.06 | 6.20 | 21.66 | 1 |
| 33RZ | 7.26 | 1.26 | 0.86 | 5.93 | 5.40 | 20.73 | 1 |
| 35RZ | 6.86 | 1.33 | 0.80 | 6.46 | 6.00 | 21.46 | 1 |
| 36RZ | 7.06 | 1.26 | 0.86 | 6.66 | 6.60 | 22.46 | 1 |
| 38RZ | 6.60 | 1.66 | 1.66 | 5.26 | 5.13 | 20.33 | 1 |
| 43RZ | 7.00 | 2.60 | 2.46 | 6.40 | 6.46 | 24.93 | 1 |
| 44RZ | 6.66 | 1.53 | 1.86 | 4.80 | 4.53 | 19.40 | 1 |
| 45RZ | 6.66 | 1.53 | 1.13 | 6.13 | 5.86 | 21.33 | 1 |
| 49RZ | 6.53 | 1.20 | 1.00 | 5.60 | 5.06 | 19.40 | 1 |
| 50RZ | 6.80 | 2.20 | 1.13 | 6.33 | 6.33 | 22.80 | 1 |
| 51RZ | 7.86 | 2.80 | 1.66 | 7.06 | 7.26 | 26.66 | 1 |
| 58RZ | 7.20 | 2.40 | 1.06 | 6.86 | 6.33 | 23.86 | 1 |
| 60RZ | 7.06 | 1.40 | 1.13 | 6.86 | 6.46 | 22.93 | 1 |
| 64RZ | 6.86 | 1.93 | 1.26 | 3.00 | 3.80 | 16.86 | 1 |
| 73RZ | 5.53 | 1.46 | 1.00 | 5.53 | 5.00 | 18.53 | 1 |
| 74RZ | 6.80 | 2.26 | 3.80 | 4.46 | 4.66 | 22.00 | 1 |
| 82RZ | 6.86 | 1.73 | 0.93 | 4.20 | 4.06 | 17.80 | 1 |
| 83RZ | 6.86 | 1.13 | 0.73 | 3.93 | 3.93 | 16.60 | 1 |
| 89RZ | 6.93 | 2.46 | 1.86 | 4.93 | 5.13 | 21.33 | 1 |
| 90RZ | 7.26 | 2.73 | 1.80 | 5.53 | 5.20 | 22.53 | 1 |
| 98RZ | 6.06 | 1.20 | 0.80 | 4.33 | 4.13 | 16.53 | 1 |
| 99RZ | 7.20 | 2.80 | 3.33 | 6.13 | 6.20 | 25.66 | 1 |
| 100RZ | 6.80 | 3.40 | 3.93 | 6.46 | 5.80 | 26.40 | 1 |
| 119RZ | 4.86 | 1.73 | 1.00 | 0.93 | 1.06 | 9.60 | 1 |
| 136RZ | 5.26 | 3.53 | 3.66 | 4.40 | 4.06 | 20.93 | 1 |
| 137RZ | 5.53 | 2.20 | 2.33 | 5.06 | 4.93 | 20.06 | 1 |
| 141RZ | 6.86 | 3.53 | 2.80 | 6.20 | 6.26 | 25.66 | 1 |
| 142RZ | 7.00 | 3.73 | 3.13 | 5.86 | 5.13 | 24.86 | 1 |
| 148RZ | 6.73 | 3.86 | 3.20 | 6.33 | 5.80 | 25.93 | 1 |

| 150RZ 6.73 3.20 2.73 1.93 1.73 16.33 1 | | | | | | | |
|--|------|------|------|------|------|-------|---|
| | 6.73 | 5.20 | 2.73 | 1.93 | 1.73 | 16.33 | 1 |

Overall 1: non acceptable Overall 2: acceptable

Con. Table 8.

| _ | | | | Mean | | | |
|----------|-------|-------|-------|---------|------------|-------|----------|
| Isolates | Color | Taste | Smell | Texture | Appearance | Total | Over all |
| | (10) | (10) | (10) | (10) | (10) | (50) | Over an |
| 155RZ | 6.73 | 3.66 | 2.53 | 6.06 | 6.40 | 25.40 | 1 |
| 167RZ | 6.73 | 3.66 | 1.66 | 6.66 | 5.93 | 23.66 | 1 |
| 168RZ | 7.00 | 0.86 | 0.53 | 1.00 | 0.80 | 10.20 | 1 |
| 219RZ | 6.73 | 2.80 | 2.60 | 3.66 | 3.46 | 19.26 | 1 |
| 220RZ | 6.80 | 3.06 | 2.13 | 6.26 | 5.53 | 23.80 | 1 |
| 227RZ | 6.73 | 3.26 | 1.73 | 5.13 | 5.00 | 21.86 | 1 |
| 228RZ | 6.73 | 3.06 | 2.60 | 5.26 | 4.86 | 22.53 | 1 |
| 233RZ | 6.73 | 3.26 | 2.00 | 6.00 | 5.26 | 23.26 | 1 |
| 254RZ | 6.13 | 2.60 | 2.86 | 2.66 | 3.20 | 17.46 | 1 |
| 255RZ | 7.00 | 1.80 | 1.66 | 3.46 | 3.13 | 17.06 | 1 |
| 256RZ | 6.06 | 1.60 | 1.06 | 3.33 | 3.46 | 15.53 | 1 |
| 260RZ | 6.00 | 2.06 | 2.13 | 3.06 | 3.06 | 16.33 | 1 |
| 261RZ | 5.40 | 1.93 | 1.26 | 3.20 | 3.06 | 14.86 | 1 |
| 262RZ | 5.40 | 2.00 | 1.26 | 1.93 | 1.86 | 12.46 | 1 |
| 267RZ | 6.53 | 2.13 | 1.86 | 4.80 | 4.26 | 19.60 | 1 |
| 268RZ | 7.06 | 1.66 | 1.13 | 2.33 | 2.40 | 14.60 | 1 |
| 277RZ | 7.86 | 1.80 | 1.13 | 6.73 | 6.60 | 24.13 | 1 |
| 278RZ | 7.53 | 1.60 | 1.13 | 6.06 | 5.80 | 22.13 | 1 |
| 279RZ | 7.13 | 1.60 | 1.06 | 3.06 | 2.73 | 15.60 | 1 |
| 283RZ | 7.40 | 2.00 | 0.73 | 4.20 | 4.00 | 18.33 | 1 |
| 24KS | 7.33 | 1.66 | 1.06 | 1.73 | 1.60 | 13.40 | 1 |
| 25KS | 7.46 | 1.13 | 0.86 | 1.93 | 1.00 | 12.40 | 1 |
| 36KS | 7.26 | 2.13 | 1.26 | 1.86 | 0.93 | 13.46 | 1 |
| 37KS | 7.40 | 1.73 | 1.13 | 3.13 | 3.06 | 16.64 | 1 |
| 41KS | 6.00 | 1.93 | 1.60 | 6.93 | 5.53 | 22.00 | 1 |
| 42KS | 6.53 | 2.80 | 1.66 | 6.93 | 5.53 | 23.46 | 1 |
| 44KS | 7.20 | 1.80 | 1.00 | 2.73 | 2.53 | 15.26 | 1 |
| 46KS | 6.26 | 1.73 | 1.00 | 2.46 | 2.80 | 14.26 | 1 |
| 47KS | 6.86 | 1.80 | 1.26 | 5.73 | 5.26 | 20.93 | 1 |
| 48KS | 7.13 | 1.86 | 1.13 | 5.86 | 5.33 | 21.33 | 1 |
| 49KS | 5.93 | 1.66 | 1.06 | 3.40 | 3.80 | 15.86 | 1 |
| 50KS | 6.73 | 1.66 | 1.06 | 4.13 | 4.00 | 17.60 | 1 |
| 51KS | 6.53 | 1.80 | 0.80 | 6.13 | 5.40 | 20.66 | 1 |
| 52KS | 6.93 | 1.86 | 0.73 | 3.46 | 4.06 | 17.06 | 1 |
| 54KS | 7.40 | 1.73 | 1.00 | 3.20 | 2.73 | 16.06 | 1 |
| 55KS | 6.80 | 1.73 | 1.00 | 4.20 | 4.06 | 17.80 | 1 |
| 58KS | 7.53 | 1.73 | 1.00 | 4.00 | 3.80 | 18.06 | 1 |
| 59KS | 7.40 | 2.06 | 1.20 | 6.13 | 6.13 | 22.93 | 1 |
| 60KS | 7.33 | 1.93 | 1.40 | 4.06 | 3.26 | 18.00 | 1 |
| 64KS | 7.40 | 1.60 | 0.66 | 2.46 | 2.86 | 15.00 | 1 |
| 68KS | 7.13 | 1.53 | 0.66 | 4.40 | 4.20 | 17.93 | 1 |
| 69KS | 5.26 | 1.33 | 0.93 | 4.66 | 4.40 | 16.60 | 1 |
| 71KS | 5.86 | 1.53 | 0.86 | 5.80 | 5.20 | 19.26 | 1 |

| Mean | 6.75 | 2.15 | 1.60 | 4.71 | 4.46 | 19.70 | 1.01 |
|--------------|------|------|------|------|------|-------|------|
| $LSD_{0.01}$ | 0.57 | 0.58 | 0.55 | 0.76 | 0.68 | 1.87 | - |

Overall 1: non acceptable

Overall 2: acceptable

Group C isolates; the smell and the taste were not very pleasant, the curd was very bitter, this was found with isolates (16RZ, 55RZ and 266RZ). Haddadin, 2005 found similar result with some tested strains of lactobacilli. Other G.C isolates in this study, produced sweet taste and strong odor in fermented milk. There are significant differences between panelists to accept this taste Tables (3 and 6). The isolate (274 RZ) was acceptable by all panelists. Its curd was soft, smooth, and homogenous; and the sweet taste was accepted.

Lactococci isolates confer to the curd certain pleasant odor and compact texture except isolate (72RZ). The (124RZ) isolate was received the highest overall acceptability level when compared to all other lactococci. The obtained results in Table (7) clearly showed that there are significant differences ($P \le 0.01$) in sensorial evaluation means for *lactococci* isolates.

Enterococci isolates, the curd was very unpleasant taste and cowshed odder. Using the isolate (246RZ); the curd presents marked defect with a strong rancidity, on the other hand, (238RZ) was the best isolates in flavor acceptability without defects. Data in Table(8) indicate the differences between the sensorial evaluation means of enterococci isolates.

CONCLUSION

The domination of obligatory heterofermentative and Enterococcus spp. among the associated LAB was established during fermentation process of Egyptian Kishk, while the facultative heterofermentative isolates were predominant. These were significant differences in organoleptic properties among isolates in fermented milk. Hemolytic activity of most isolates were γhemolytic. To obtain cereal fermented milk with flavor characteristics similar to those of artisanal product, the isolated lactic acid bacteria should first be identified completely and tested in mixed cultures based on their technological properties. In this study, 15 isolates, which had a good organoleptic characteristics and no hemolytic activity, were selected for complied identification. Based on these results, future works will be done to selecte a new starter culture to produce fermented milk like Laban Zeer for its using in Kishk manufacturing.

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