Antifungal activity effects of some *lactobacillu s*sp. against some of Labneh spoilage microorganisms

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Abstract: Four antifungal Lactobacilli strains had higher antifungal activity. Their antifungal activities were tested in concentrated yogurt (Labenh), against fungal species of (*Debaryomyces hansenii* NRRL 1448, *Kluyveromyces marxianus* EMCC 101900, *Saccharomyces cerevisiae* NRRL 2291, *Penicillium roqueforti* and *Aspergilus flavus* NRRL 3251) commonly involved in the spoilage of dairy products. The antifungal strains belonged to *Lactobacillus casei, Lactobacillus rhamnosus* TISTR 541, *Lactobacillus acidophilus* AL-5*and Lactobacillus helveticus* EMCC 4193 species and showed different acidifying and growth capacities in labneh samples during storage period at 5^{o}C+1. All tested Lactobacillus strains showed an antifungal activity in labneh samples. *L.rhamnosus* and *L. casei* showed a very strong antifungal effect in labneh by completely inhibiting all tested fungi as compared to control (with yoghurt strter). Both *L. case i*and *L. rhamnosus* completely inhibited the fungal growth of *Aspergillus flavus* NRRL 3251 (aflatoxicogenic strain) assayed. Higher antifungal activity was exhibited by actively growing cells of the four lactobacilli strains compared with the MRS broth supernatants of the four lactobacilli bacterial strains containing metabolites with antifungal activity using the disc assay method. These Lactobacillus cultures showed inhibitory activities against yeasts in Labenh at refrigerator temperatures (5°C+1) without an influence on the quality properties of the food. Initial cell numbers of 5×10^7 cells/g of *Lactobacillus* sp. It was found that 1×10^7 cells/g of lactobacilli were the optimal concentrations to yield a total inhibition of the spoilage yeasts (*Debaryomyces hansenii, Kluyveromyces marxianus,* and *Saccharomyces cerevisiae*).
[Abeer E. A. Amer and Mohamed E. Amer. **Antifungal activity effects of some *lactobacillus*sp. against some of Labneh spoilage microorganisms.** *Nat Sci* 2018;16(11):100-113]. ISSN 1545-0740 (print); ISSN 2375-7167 (online). http://www.sciencepub.net/nature. 15. doi:10.7537/marsnsj161118.15.

Key words: *Lactobacillus* – antifungal activity –yeasts – antifungal Lactobacilli strains-labneh.

1. Introduction

Khurana and Kanawjia, (2007) reported that dairy products such as yogurts and fermented milks are of high economic importance. A large panel of new products are now proposed to the consumers such as like fruit-yogurt beverages, or added-value products with low calorie, reduced-fat varieties or supplemented with physiologically active ingredients including fibers, phytosterols, omega-3-fatty acids, whey based ingredients, antioxidant vitamins and isoflavones**. Mayoral *et al.*, 2005)** reported that despite fermented milks and yogurts are generally considered as microbiologically stable, they may be subjected to contaminations with acid tolerant fungi, which can occur at all stages of food processing from raw materials to finished products. *Candida parapsilosis, Candida diffluens, Debaryomyce shansenii, Kluyveromyces marxianus, Rhodotorula mucilaginosa, Yarrowia lipolytica, Zygosaccharomyces bailii* or *Penicillium brevicompactum* are among the most frequently encountered fungal contaminants in yogurts and fermented milks, particularly in those containing fruits or sugar**.** Fungal spoilage is then a major limiting factor for the stability and the commercial value of these products. It causes significant economic losses. Moreover, evolution of consumers' demand has led industrials to reduce the use of chemical preservatives in fermented dairy products. It is then necessary to find alternative strategies to prevent fungal spoilage and/or to increase their shelf life. In this context, biopreservation, that implies the use of microbial cultures selected for their ability to control the growth of spoilage microorganisms, has taken a considerable development **(Mills, *et al.*, 2011). Schnürer and Magnusson, (2005) found that** LAB are known to possess antimicrobial activities linked to their strong competition for nutrients, to the decrease of pH due to their fermentative metabolism and to the production of inhibitory metabolites. Several species of lactobacilli *Lb. coryniformis,* (*Lb. casei, Lb. paracasei, Lb. plantarum. Lb. rhamnosus,*), *pediococci* (*P. acidilactici P. pentosaceus,*) *and lactococci* (*Lc. lactis*) have been described as antifungal**.** However, few studies **(Schwenninger and Meile, 2004; Suomalainen and Mäyrä-Makinen, 2005; Tawfik, *et al.*, 2004)** have tested in the capacity of these bacteria to prevent fungal spoilage in yogurts or

fermented milks, in spite of the significant commercial interest in using them as natural food preservatives.

Lactic acid bacteria (LAB) have a long history of use in the manufacture of a large variety of fermented foods where they contribute to their preservation and organoleptic properties. During the last decade, there has been increasing interest in the development of LAB bioprotective cultures as alternative to chemical additives in food. This growing interest is mainly driven by consumers' demand for food products without chemical preservatives and/or for preservatives from natural origin. This is why many industrials are moving towards the use of protective microbial cultures, mainly LAB, able to produce antagonistic metabolites such as bacteriocins, peptides and/or low-weight non-proteinaceous compounds (organic acids, fatty acids, H2O, etc.). Many scientific evidences or proof-of-concept in literature 2 underline the great potential of such an approach to combat pathogenic or spoilage microorganisms in various food products such as meat **(Budde *et al*., 2003; Vermeiren *et al*., 2004; Castellano *et al*., 2008),** fish **(Brillet *et al*., 2005 Chahad *et al*.,2012); Tomé *et al*., 2008;** bakery products **(Dal Bello *et al*., 2007; Gerez *et al*., 2009; Ryan *et al*., 2011)** and vegetables **(Trias *et al*., 2008; Randazzo *et al*., 2009).** However, in contrast to probiotics **(Gregoret *et al*., 2013),** only a limited number of commercial protective cultures are marketed today, and this statement is especially true for antifungal bioprotective cultures in dairy products. Yet, dairy products are particularly susceptible to fungal contaminations leading to food spoilage (off-flavour, deterioration of visual appearance) and important economic losses (Nelson, 1993).

The limited number of marketed bioprotective cultures in fermented dairy products can be first explained by the numerous constraints linked to their market implementation. Apart from being safe for human consumption, a selected strain must fulfill several criteria **(Wessels *et al*., 2004)** and it is often difficult to gather them all. Among them, the antimicrobial strain must be active in the desired food without producing any detrimental effects on the growth and functionality of starter bacteria **(Holzapfel et al., 1995).**

The objectives of this study were to:

1- Evaluate the antifungal activity of selected lactic acid bacteria (4 Lactobacillus strains) against several species of yeasts and molds in laboratory agar media.

2- Demonstrate the efficacy of selected antifungal LAB for preventing yeasts and molds growth on concentrated yogurt (Labneh), and study the stability of its antifungal activity during storage period.

2. Materials and methods

Materials:

Fresh buffalo's milk, used in the manufacture of Labneh, was obtained from Animal Production Research Institute, Egypt. Buffalo's milk contained 15.5 % total solids and 5.1 % fat. Commercial grade fine salt was purchased from the local market, produced by El-Nasr Salines Company, Alexandria, Egypt. The salt was used (1%) for cheese manufacture.

Chemicals: Chemicals used in this study were of the analytical grade.

Microorganism

Traditional yogurt culture (*Lactobacillus delbrueckii* sub sp. *bulgaricus* and *Streptococcus salivarius* subsp. *Thermophilus* (1:1), *Lactobacillus casei* 01, *L. acidophilus* AL-5 and *Penicillium roqueforti* were obtained from Chr. Hansen Laboratories, Copenhagen, Denmark. *Aspergillus flavus* NRRL 3251, *Debarryomyces hansenii* NRRL 1448 and *Saccharomyces cerevisiae* NRRL 2291, were kindly provided from the Northern Regional Research Laboratory (NRRL), USA. *Lactobacillus rhamnosus* TISTR 541 was kindly provided in freeze-dried form Thailand Institute of Scientific and Technological Research, Bangkok, Thailand. *L. helveticus* EMCC 4193 and *Kluyveromyces marxianus* EMCC 101900 was obtained from the Egyptian Microbial Culture Collection [EMCC] at Cairo Microbiological Resources Center (Cairo MIRCEN) Faculty of Agriculture, Ain Shams University, were selected for this study.

Growth and preservation media:

DeMan, Rogosa, Sharpe (MRS) broth and agar media were used for the cultivation and enumeration of lactobacilli strains. Lactobacilli strains were propagated for 24h at 37^{o}C in Difco MRS broth while *L. casei* 01 was incubated at 32^{o}C for 48h. Stock cultures of lactobacilli was made by mixing a pure culture that had been grown over night with an equal volume of 20% glycerol solution and storing at -20^{o}C until experimental use (**Van Den Berg *et al*., 1995**). While, *Penicillium roqueforti*, and *A. flavus* NRRL 3251 were grown, activated and enumerated on Oxytetracycline Glucose Yeast extract agar (OGYE agar) at 25^{o}C for 5-7 days. Inocula containing spores or conidia were prepared by growing the mould on Oxytetracycline Glucose Yeast extract soft agar for 5-7 days (or until sporulation) and then collecting spores or conidia after vigorously shaking the slant with sterile sodium chloride water (8.5 gm NaCl per liter). These fungi strains were used for the artificial contamination of labneh.

All tested yeasts (*Debaryomyces hansenii* NRRL 1448, *Kluyveromyces marxianus* EMCC 101900, and *Saccharomyces cerevisiae* NRRL 2291) were grown,

activated and enumerated on a yeast and malt extract agar (YM agar) at 25°C for 48h. This yeast strains was stored in yeast extract (0.3%) and malt extract (2%) medium (YEMA) supplemented with glycerol (30%, v/v) at -80°C, and cultivated aerobically at 25°C for 2 days on YEMA agar. Yeasts suspensions were prepared in sterile 0.1% peptone water by scraping colonies from the surface of YEMA agar after 2 days incubation at 25°C. This yeast strains was used for the artificial contamination of labneh. The microbial count of the lactic acid bacteria in the Labneh samples was determined using Lactobacilli MRS agar (Difco, Detroit, Mich., U.S.A.).

Methods:

Antiyeast activity assay induced by cells embedded into agar:

YM Agar plates were prepared, supplemented with an antiyeast *Lactobacillus*. The cultures were previously grown separately for 24 h at30°C in YM broth media, and then serially diluted in diluent (0.85% NaCl containing 0.1% peptone). Portions of 20 ml of agar (50°C) were prepared to give a final level of 10^5 cells/ml agar. Agar plates were poured and after solidifying fully grown yeast cultures as well as the corresponding dilutions were spot inoculated on the plates yielding 10^3 to100 cells/spot. The agar plates were stored in triplex at 25 °C for up to 48 -72 h and the growth of indicator yeast was controlled every day for25 °C. As a control, agar plates without embedded lactobacilli were prepared as well as agar plates with an addition of 1 g/l potassium-sorbate.

Antifungal activity assay:

Two different methods (the overlay method and the disc assay method), were used to detect antifungal activity.

The overlay method:

The overlay method was performed using MRS agar plates for Lactobacilli strains which LAB were inoculated as two 2-cm-long lines and incubated at 37+1°C for 48h. The plates were then overlaid with 10mlof Oxytetracycline Glucose Yeast extract soft agar containing 10^5 fungal spores (conidia) per ml. The plates were incubated at 25+1°C for 5 days. The plates were examined for clear zones of inhibition around the bacterial streaks, and zone areas was scored as follows: -, no inhibition (visible of mycelium and vegetative spores) +, no fungal growth on 0.1 to 3% of the plate area per bacterial streak; ++, no fungal growth on 3% to 8% of the plate area per bacterial streak; or +++, no fungal growth on > 8% of the plate area per bacterial streak.

The disc assay method:

The disc assay method was performed using MRS agar for Lactobacilli strains. Four drops from an active culture of each bacterial species tested were spotted onto disc assay on agar plates and incubated until will grown colonies could be observed (approximately 48h). The plates were then overlaid with 10 mlof Oxytetracycline Glucose Yeast extract soft agar on which 0.1 ml of mould spore suspension was finally spread out. After incubation for 5 days at 25+1°C, the plates were examined for halo formation around the bacterial colonies. The growth of fungi and specially the extent of sporulation were visually evaluated by comparing the color of the colonies on the control plates. Sporulated colonies differ significantly in color from unsporulated colonies.

The disc assay method for supernatant of lactic acid bacteria

The disc assay method was performed using MRS agar for Lactobacilli strains. The inhibitoriest LAB strains gave the highest antifungal effect against the most common dairy spoilage and toxigenic fungi were chosen to study antifungal properties. These strains were *L. acidophilus*, *L. rhamnosus*, *L. casei*, and *L. helvtius*. The disc assay method was used to test the effect of three treatments on the antifungal activity of LAB supernatant. These three treatments were crude, heat treated, (at 90°C for 10 min then cooled), neutralized (supernatant treated with 0.2N NaOH tell neutralized of acidity). This assay was performed as following: The tested LAB strains were grown on MRS broth tubes and was incubated at 35°C for 48h. One ml of growth was put in flasks contained 100 ml of MRS broth and incubated at 35°C for 48h. Cell-free supernatant was obtained by centrifugation at 5000 rpm for 5 min at 4°C. Cell free supernatant was divided into 3 portions to study the antifungal effect of: (1) crude cell-free supernatant without any treatment, (2) heat treated cell-free supernatant. (3) Neutralized treatment was prepared using sterilized 0.2N NaOH to titrate acidity of cell-free supernatant (titration to pH7).

After the performed 0.1 ml of mould spore suspension was put on petri dish every yeast and fungi two dishes then put (OGYE) agar on it and allow to set after that put the disc assay on agar then put two drops from cell-free culture with treatment of each bacterial species tested were spots onto disc assay on agar plates and incubated at 25°C for 5 days and observed results after incubation the plates were examined for formation around the treatments.

Preparation of Labneh

Labneh was manufactured according to **Robinson and Tammime (1994).** Fresh buffalo's milk was heated at 72°C for 15 sec, cooled to 45°C and divided into sex equal portions. The first portion was served as control was inoculated with 3% of yogurt starter (*S. thermophilus* and *L. bulgaricus*), the 2nd was inoculated with 1.5% of yogurt starter 2% *L. rhamnosus* TISTR 541 (T1), the 3rd was inoculated with 1.5% of yogurt starter and 2% of *L. acidophilus*

AL-5 (T2). The fourth portion was inoculated with 1.5% of yogurt starter and 2% of *L. helvetius* EMCC 4193(T3), the fifth portion was inoculated with 1.5% of yogurt starter and 2% of *Lactobacillus casei* 01(T4). The sixth portion was inoculated with combination of all starter cultures using (1% yogurt starter, 1% *L. acidophilus* AL-5,1% *L. rhamnosus* TISTR 541,1% *L. helvtius* EMCC 4193 and1% *L. casei* 01 (1:1:1:1:1:1) (T5). Samples were kept at 40^{o}C up to reach a PH of 4.5 (6 h). All cultures were inoculated atapproximately 1 × 10^{7}cfu /g into 3 kg milk. Each portion was further divided into sex equal portions, one served as control (having starter culture alone) and the other five portions were artificially contaminated with approximately 10^{5}cfu /ml of each of *Debarryomyce shansenii*, *Kluyveromyces marxianus* and *Saccharomyces cerevisiae* (table 1), the last two portions were contaminated with 1.5% spores solution of each of *Penicillium roqueforti*, and *A. flavus* (table 1). All the milk portions were incubated at 40^{o}C until it was completely coagulated. The mixtures were then put into cheese cloth bags, which were hung in the refrigerator at 5 ± 1^{o}C for 18 h, to allow drainage of the whey. Labneh samples were then stored at 5+2°C for 16 days. Viable lactobacilli in Labneh samples were enumerated every 3 days using MRS agar during storage period. Labneh samples were analysed for chemical analysis, microbiological analysis and organoletic properties during storage period.

Table (1): Contamination of Labneh during its manufacture by 5 yeasts strains and 2 moulds strains.

Yeast and mold strains	Yeast and mould count, cfu/ml (g)
Control	3x10^{4}
K. marxianus	2.5 x10^{4}
S. cerevisiae	1.2 x10^{4}
D. hansenii	1.8 x10^{4}
Penicillium roqueforti	1 x10^{4}
Aspergillus flavus	1 x10^{4}

Chemical analysis for Labneh

Total solids and ash contents and titratable acidity and fat content of Labneh were determined according to **(AOAC, 1990).** Protein was estimated according to the method of **BSI, 1990**. The pH values were measured using JENWAY Digital pH meter Model 3310.

Microbiological analysis for Labneh

The viable count of the lactic acid bacteria was determined using the pour plate method according to **Vinderola *et al.*, (2000)** and the results were expressed as cfu/ml. Selective media were used to quantify the two strains *Lactobacillus* sp. was enumerated by plating the appropriate dilutions on MRS agar medium**.** Total population of viable microorganisms was counted on regular MRS medium (pH 5.5). All plates were incubated anaerobically at 37 °C for 48 h. Yeasts and moulds were determined according to **Blanchette *et al.*, (1996).**

Sensory evaluation

Samples of Labneh were organolepitically scored for flavour (50 points), body and texture (40 points) and appearance (10 points) according to score suggested by **Keating and Randwhite (1990).**

Statistical analysis

All data were analysed according to statistical analysis system User's Guide **SAS (2001) (**SAS Institute, Inc, U. S. A.). Separation among means was carried out by using **Duncan multiple test, (1955).**

3. Results and discussion

Chemical composition of Labneh

Table (2) shows the changes during storage in the total solids (TS) and fat content of Labneh made with several types of lactic acid bacterial culture. The total solids increased slightly in all treatments as the storage period increased. Total solids content was not significantly varied between treatments ($P < 0.05$). During storage, both total solids content increased and could be ascribed to moisture loss. The data is similar those of **Tamime and Robinson (1985)** and **Mehaia and El-Khadragy (1999)**, who reported that the total solids of Labneh ranged between 22-26 %. Protein content was not significantly varied between treatments ($P < 0.05$) comparing with the control.

In general the chemical composition of Labneh made without or contaminated with Labneh yeasts and fungi was within the normal composition range for a similar product made from Buffalos" milk **(El-Samragy and Zall, 1998; Tamime *et al.* 1989 a, 1991 a; Ozer *et al.*, 1999).**

The titratable acidity (TA) is a very important factor, since it affects the shelf life and the acceptability of Labneh. The percentage of titratable acidity and pH values were significantly, in Labneh without or contaminated with added yeasts and fungi and control treatment, suggesting that the effect of the starter culture and total viable count increased the percentage of titratable acidity and decrease the pH values in Labneh during the storage period. Based on the results presented in table (1), it is evident that

acidity values of the treated Labneh increased with an increase in all treatments the during storage period.

Bacterial counts in Labneh

Labneh prepared by adding four different lactic acid bacterial cultures non contaminated Labneh treatments was subjected to microbiological analysis. Analysis of the results obtained for the total bacteria viable counts, lactic acid bacteria and yeast and mould (Table 3). The results indicated that in all cases the respective counts increased gradually up to 7 day of storage and then decreased thereafter.

It is clear that, the total viable count (TC) of various samples significantly varied ($P < 0.0001$). The significantly highest values for total viable count were found along storage period up to 16 days.

Table (2): Effect of using different Lactobacilli strains on chemical composition, titratable acidity and pH values in none contaminated Labneh during storage period.

Storage period (day)	Treatments[1]					
	Control	T_1	T_2	T_3	T_4	T_5
T.S %						
Fresh	22.42[a]	22.37[b]	22.41[b]	22.34[c]	22.36[c]	22.44[a]
4	22.57[b]	22.62[a]	22.65[a]	22.63[a]	22.48[c]	22.58[b]
8	22.75[a]	22.72[a]	22.75[a]	22.55[c]	22.58[c]	22.61[b]
12	23.13[b]	22.96[b]	23.36[a]	22.94[b]	23.16[b]	22.36[c]
16	23.26[a]	22.98[b]	22.84[c]	22.96[b]	22.70[c]	22.47[d]
Protein %						
Fresh	9.29[c]	9.32[b]	9.44[a]	9.35[b]	9.42[a]	9.43[a]
4	9.33[c]	9.46[b]	9.53[a]	9.50[a]	9.53[a]	9.51[a]
8	9.53[b]	9.56[b]	9.59[a]	9.55[b]	9.63[a]	9.56[b]
12	9.84[a]	9.73[b]	9.80[a]	9.80[a]	9.86[a]	9.82[a]
16	9.85[a]	9.75[b]	9.81[a]	9.81[a]	9.87[a]	9.83[a]
Titratable Acidity %						
Fresh	1.40[a]	1.13[c]	1.30[b]	1.25[b]	1.10[c]	1.41[a]
4	1.42[a]	1.15[c]	1.37[a]	1.29[b]	1.11[c]	1.44[a]
8	1.45[a]	1.17[c]	1.40[a]	1.31[b]	1.13[c]	1.48
12	1.58[a]	1.42[b]	1.55[a]	1.40[b]	1.20[c]	1.57[a]
16	1.59[a]	1.43[b]	1.56[a]	1.42[b]	1.24[c]	1.58[a]
pH value						
Fresh	4.52[a]	4.54[a]	4.57[a]	4.68[b]	4.63[b]	4.51[a]
4	4.50[b]	4.53[a]	4.50[b]	4.55[b]	4.63[a]	4.52[b]
8	4.49[b]	4.40[b]	4.52[b]	4.56[a]	4.63[a]	4.51[b]
12	4.46[b]	4.51[a]	4.43[b]	4.52[a]	4.55[a]	4.46[b]
16	4.43[b]	4.48[a]	4.40[b]	4.50[a]	4.52[a]	4.43[b]

* Means with the same letters are not significantly different. Control: Labneh made from yogurt culture (control). T1: Labneh made from yogurt culture+ *L. rahmnosus* (1:1).
T2: Labneh made from yogurt culture+ *L. acidophilus* (1:1). T3: Labneh made from yogurt culture+ *L. helveticus* (1:1). T4: Labneh made from yogurt culture+ *L. casei* (1:1).
T5: Labneh made from combination of yogurt culture+ *L. acidophilus*+ *L. rahmnosus*+ *L.casei*+ *L. helvticus* (1:1:1:1:1).

Table (3): Microbiological properties of none contaminated Labneh during storage period.

Storage period (day)	Treatments[1]					
	Control	T_1	T_2	T_3	T_4	T_5
T.C (log cfu /g)						
Fresh	6.30[a]	6.11[b]	6.18[b]	6.00[c]	5.85[d]	6.00[c]
4	6.53[a]	6.46[b]	5.48	6.41	6.15	6.14
8	6.68[a]	6.56[b]	6.59[b]	6.49[c]	6.30[d]	6.40[c]
12	6.51[a]	6.40[b]	6.46[b]	6.34[c]	6.10[d]	6.53[a]
16	6.52[a]	6.36[b]	6.43[b]	6.32[c]	6.08[d]	6.51[a]
Lact. (log cfu /g)						
Fresh	8.90[a]	8.88[a]	8.91[a]	7.57[b]	7.55[b]	7.47[c]
4	8.94[a]	8.91[a]	8.87[b]	7.94[c]	8.90[a]	7.90[c]
8	8.90[a]	8.91[a]	8.93[a]	7.97[b]	8.95[a]	7.96[b]
12	8.94[a]	8.96[a]	8. 94[a]	7.66[c]	8.70[b]	7.67[c]
16	8.44[b]	8.24[c]	8. 53[a]	7.50[d]	8.61[a]	7.52[d]
M & Y (log cfu/g)						
Fresh	ND	ND	ND	ND	ND	ND
4	ND	ND	ND	ND	ND	ND
8	ND	ND	ND	ND	ND	ND
12	1.10[d]	1.20[c]	1.30[b]	1.43[b]	1.5[a]	1.30[b]
16	1.20[d]	1.30[c]	1.40[b]	1.48[b]	1.6[a]	1.34[c]

* Means with the same letters are not significantly different. T.C: the total viable count. M & Y: Moulds and Yeasts. Lact.: *Lactobacillus* sp. count. Treatments1: See table 2 for details.

The presented data in Table (2) reveal that the max. count for the lactic acid bacteria was found with Labneh treatments at 10 days of storage period, then it decreased as its count was 27, 23, 25, 22, 21 and 20 x 10^6cfu/g in fresh control Labneh, T1, T2, T3, T4, T5 and control, respectively, and increased to 60, 61, 63, 59, 61 and 59 x 10^5cfu/g at 7 days of storage in the same order. The results reveal that, the LAB of various samples not significantly varied with the treatments, in which LAB would not showed significant differences ($P < 0.0001$).

Table (3) also show that yeasts and moulds counts in none contaminated Labneh are considered indicative of the quality and the shelf life of Labneh, in this regard, yeasts and moulds were detected in all treatments but only after 10 days of storage in significantly different counts between treatments. On the other hand the average of yeast and mould of control treatment at 14 days was 2 x 10^1 cfu/g. It was clear that there was no significant between control, and other treatments.

Organoleptic properties:

The organoleptic properties of the different Labneh were investigated and the results are presented in Table (4). There were considerable and significant differences ($P < 0.0001$) in the flavour of these treated samples as compared with the untreated control.

The untreated control Labneh, when fresh and after 7 days of storage preferred compared to the treated Labneh. Nevertheless, Labneh containing *L. rahmnosus* were the most acceptable after the control. In all cases the total scores of the sensory evaluation decreased gradually during storage. As storage progressed the organoleptic scores decreased in all treatment. These results are in agreement with **Taha *et al* (1997).**

It can be concluded that Lactobacilli strains can be used to increase the shelf life of Labneh for up to 16 day at 5 ± 1^{o}C with acceptable flavour and good appearance.

Table (4): Sensory evaluation of Labneh of none contaminated Labneh during storage period.

Storage period (day)	Treatments[1]					
	Control	T_1	T_2	T_3	T_4	T_5
Flavour (50)						
Fresh	48 ^{a}	45	48 ^{a}	47 ^{a}	46	45
4	46 ^{a}	44	47 ^{a}	46 ^{a}	45	44
8	45 ^{a}	43	45 ^{a}	44 ^{a}	42	41
12	43 ^{a}	40	43 ^{a}	42 ^{a}	38	39
16	42 ^{a}	40 ^{b}	42^a	41^{ab}	37^c	35^c
Body & Texture (40)						
Fresh	38 ^{a}	35 ^{d}	37 ^{b}	38 ^{a}	36 ^{c}	35 ^{c}
4	38 ^{a}	33 ^{d}	36 ^{c}	37 ^{b}	35 ^{c}	34 ^{c}
8	35 ^{a}	32 ^{c}	35 ^{a}	35 ^{a}	33 ^{b}	33 ^{b}
12	34 ^{a}	30 ^{c}	32 ^{d}	34 ^{a}	30 ^{c}	29 ^{d}
16	33^a	28 ^{c}	32 ^{b}	31 ^{ab}	28 ^{bc}	27 ^{bc}
Appearance (10)						
Fresh	10 ^{a}	9 ^{b}	10 ^{a}	10 ^{a}	8 ^{c}	8 ^{c}
4	9 ^{a}	9 ^{a}	9 ^{a}	9 ^{a}	8 ^{b}	8 ^{b}
8	8 ^{a}	7 ^{b}	8 ^{a}	7 ^{b}	7 ^{b}	7 ^{b}
12	8 ^{a}	7 ^{b}	8 ^{a}	7 ^{b}	7 ^{b}	6 ^{c}
16	7^a	6^{ab}	7 ^{a}	6 ^{ab}	6 ^{ab}	6^b
Total Score						
Fresh	96 ^{a}	88	94 ^{b}	95 ^{a}	89 ^{c}	88 ^{c}
4	93 ^{a}	87 ^{b}	93 ^{a}	92 ^{a}	88 ^{b}	87 ^{b}
8	88^{ab}	84	91 ^{a}	86	83	82
12	85 ^{a}	75	81	83 ^{b}	7 ^{c}5	68
16	82^a	74^c	81^b	78^{ab}	71^d	72 ^{d}

* Means with the same letters are not significantly different. **Treatments1: See table 2 for details.**

Antiyeast activities of culture incorporated into YM agar

A total of four lactobacilli strains were tested alone for their antiyeast activities alone or in combination of four lactobacilli strains. The strains yeast were embedded in concentrations ranging from 10^5 to 10^6 cfu/ml in YM agar plates on yeasts (*D. hansenii, Kluyveromyces marxianus and*

Saccharomyces cerevisiae) were spotted yielding 10^5-10^3 cells/spot. The plates were incubated at 25°C.

Although the four lactobacilli strains showed only weak inhibitory activities using them alone, their combination revealed high antagonistic values at 5°C. The highest activities were detected with the four lactobacilli strains were combination together is represented in Table 5. Weak activities were observed with *L. acidophilus* AL-5alone. In all approaches, cell numbers of 10^7cfu/ml were necessary for the four *Lactobacillus* strains were necessary for high inhibitory activities.

Testing the antiyeast activities of mixtures of the four lactobacilli strains (*L. casei*, *L. rhamnosus*, *L. acidophilus* and *L. helveticus*) together in combination, give an increase in inhibition in yeast growth was recognizedin (Table 5). In comparison to the trials in which only one single *Lactobacillus* strain was used (Table 5). A complete inhibition of the yeast growth of *D. hansenii*, *S. cerviciea*, and *Kluyveromyce smarxianus*, was achieved by using combination of the four lactobacilli strains (*L. casei*, *L. rhamnosus*, *L. acidophilus* and *L. helveticus*) together, while *L. rhamnosus* reduced the growth of *D. hansenii* and *Kluyveromyces marxianus*, but *L. casei* only significantly reduced the growth of *D. hansenii*.

Table (5): Growth inhibition of yeasts using the disc assay method expressed in log10 cfu/ml by 4 different *Lactobacillus* sp. held at 5°C.

Yeast strain	Lactobacilli strain				
	L. rhamnosus	***L. acidophilus***	***L. helveticus***	***L. casei***	**Combination of 4 *Lactobacillus* sp.**
D. hansenii	(+/–)	+	(+/–)	–	–
K. marxianus	(+/–)	+	+/–	+/–	–
S. *cerviciea*	(+/–)	+/–	+/–	+/–	–

L. rhamnosus (10^7cfu/ml); *L. acidophilus* (10^7cfu/ml); *L. helveticus* (10^7cfu/ml); *L. casei*01 (10^7cfu/ml)
++ very strong growth; + strong growth; +/– weak growth; (+/–) very weak growth; – no growth;
spot-inoculation (10^3 cells/spot)

Behavior of different dairy yeasts spoilage in labneh:

To evaluate the effect of different lactic acid bacterial cultures on ***D***. *hanseni*ig rowth in labneh along the storage (16 days at 5°C+1) the data is presented in table (6). The data presented that lactic acid bacterial were slight different among cultures in fresh samples. The highest LAB counts was observed in labneh containing *L. rhamnosus* or *L. acidophilus* (7.7 and 7.04 log cfu/g respectively). Generally, Lactic acid bacteria counts slightly decreased among the first week of the storage followed by gradual decrease till the end of the storage. The highest decrease Lactic acid bacteria counts were found in control samples followed by labneh with *L. helveticus* (4.4 and 4.7 log cfu/g respectively) at the end of storage.

On the other hand, there were significant differences in yeast counts along storage. The yeast counts slightly decreased in control labneh as the storage period progressed. The highest yeast counts were recorded in control labneh followed by labneh with *L. helvetius.* It was observed that the antifungal activity of *Lactobacillus* sp. cultures was maintained with other yoghurt starter cultures of *L. bulgaricus* and *S. thermophilus*.

To evaluate the effect of different lactic acid bacterial cultures on *K. marxianus* growth in labneh along the storage (16 days at 5°C+1) the data is presented in table (7). The data presented that lactic acid bacterial were slight different among cultures in fresh samples. The highest LAB counts were observed in labneh fresh containing *L. rhamnosus* and control (7.4 and 7.2 log cfu/g respectively). Generally, Lactic acid bacteria counts slightly decreased among the first week of the storage followed by gradual decrease till the end of the storage. The highest decrease Lactic acid bacteria counts were found in control samples followed by labneh with *L. casei* (5.0 and 4.7 log cfu/g respectively) at the end of storage. On the other hand, there were significant differences in yeast counts along storage. The yeast counts slightly decreased in control labneh as the storage period progressed. The highest yeast counts were recorded in labneh with *L. casei* followed by control. On the other hand using combination of the four *Lactobacillus* sp. strains showed the highest inhibition of fungal growth.

To evaluate the effect of different lactic acid bacterial cultures on *S. cerviciae* growth in labneh along the storage (16 days at 5°C+1) the data is presented in table (8). The data presented that lactic acid bacterial were slight different among cultures in fresh samples. The highest LAB counts were observed in labneh fresh containing *L. rhamnosus* and *L. casei* (7.4 and 7.3 log cfu/g respectively). Generally, Lactic

acid bacteria counts slightly decreased among the first week of the storage followed by gradual decrease till the end of the storage. The highest decrease Lactic acid bacteria counts were found in control samples followed by labneh with *L. casei* (4.4 and 4.2 log cfu/g respectively) at the end of storage. On the other hand, there were significant differences in yeast counts along storage. The yeast counts slightly decreased in control labneh as the storage period progressed. The highest yeast counts were recorded in control followed by labneh with *L. acidophilus*.

Table (6): Effect of using 4 different *Lactobacillus* sp. cultures in growth inhibition of *D. hansenii* expressed in log 10 cfu/g during storage period.

Storage period (day)		Treatments[1]					
		Control	T_1	T_2	T_3	T_4	T_5
Fresh	LAB counts (log10 cfu/g)	7.4+0.5^{a}	7.5+ 0.2^{a}	6.8+ 0.24^{b}	6.8+ 0.5^{b}	6.6+ 0.2^{c}	6.8+ 0.5^{b}
	yeast counts (Log cfu /g)	4.4+0.2^{a}	4.3+0.1^{a}	4.3+ 0.2^{a}	4.2+0.1^{a}	4.3+0.1^{a}	4.2+0.2^{a}
4	LAB counts (log10 cfu/g)	7.2+ 0.1^{a}	7.3+ 0.2^{a}	7.6+ 0.3^{a}	6.5+ 0.2^{b}	6.0+ 0.5^{c}	7.6+ 0.3^{a}
	yeast counts (Log cfu /g)	4.7+0.2^{a}	4.4+0.1^{c}	4.5+0.2^{b}	4.5+0.1^{b}	4.4+0.1^{c}	4.3+0.2^{c}
8	LAB counts (log10 cfu/g)	6.3+0.1^{b}	6.0+ 0.3^{b}	7.2+ 0.2^{a}	5.3+ 0.2^{c}	5.3+ 0.1^{c}	7.2+ 0.1^{a}
	yeast counts (Log cfu /g)	4.8+0.1^{a}	4.5+0.1^{c}	4.6+0.2^{a}	4.6+0.1^{b}	4.5+0.1^{c}	4.4+2^{c}
12	LAB counts (log10 cfu/g)	5.3+0.1^{b}	4.8+ 0.4^{c}	7.2+ 0.2^{a}	5.3+0.1^{b}	5.0+ 0.2^{c}	7.2+ 0.1^{a}
	yeast counts (Log 10cfu /g)	4.85+0. 1^{a}	4.65+ 0.1^{c}	4.77+0.1^{a}	4.68+0.1^{b}	4.5+0.1^{d}	4.6+1^{c}
16	LAB counts (log10 cfu/g)	5.1+ 0.4^{b}	4.4+ 0.3^{c}	5.7+ 0.1^{a}	4.2+0.1^{c}	4.4+ 0.3^{b}	5.6+ 0.1^{a}
	yeast counts (Log cfu /g)	4.9+0. 1^{a}	4.7+ 0.1^{b}	4.8+0.1^{a}	4.7+0.1^{b}	4.6+0.1^{d}	4.7+1^{c}

Values with different small letters in a row are significantly different (P< 0.05). **Treatments1: See table 2 for details.**

Table (7): Effect of using 4 different *Lactobacillus* sp. cultures in growth inhibition of *K. marxianus* expressed in log 10cfu/g during storage period.

Storage period (day)		Treatments[1]					
		Control	T_1	T_2	T_3	T_4	T_5
Fresh	LAB counts (log10 cfu/g)	7.2+0.1^{b}	7.4+ 0.2^{a}	7.1+ 0.2^{b}	7.1+ 0.2^{b}	7.0+ 0.1^{c}	7.4+ 0.1^{a}
	yeast counts (Log cfu /g)	5.0+0.1^{a}	5.0+0.1^{a}	5.0+0.1^{a}	5.0+0.1^{a}	5.0+0.1^{a}	5.0+0.1^{a}
4	LAB counts (log10 cfu/g)	6.9+ 0.1^{c}	7.2+ 0.2^{a}	7.1+ 0.3^{a}	7.1+ 0.1^{b}	7.0+ 0.2^{b}	7.2+ 0.2^{a}
	yeast counts (Log cfu /g)	5.1+0.2^{a}	4.8+0.1^{c}	4.7+0.2^{b}	4.6+0.1^{b}	4.4+0.1^{b}	4.4+0.1^{c}
8	LAB counts (log10 cfu/g)	5.9+0.1^{c}	6.5+ 0.3^{b}	7.0+ 0.1^{a}	5.2+ 0.4^{d}	5.3+ 0.2^{d}	6.4+ 0.3^{b}
	yeast counts (Log cfu o/g)	5.1+0.2^{a}	4.8+0.1^{c}	4.7+0.2^{b}	4.5+0.1^{b}	4.5+0.1^{b}	ND
12	LAB counts (log10 cfu/g)	5.4+0.1^{c}	5.3+ 0.4^{b}	6.2+ 0.1^{a}	5.4+0.1^{c}	5.1+ 0.1^{d}	6.0+ 0.1^{a}
	yeast counts (Log cfu /g)	3.0+0.2^{b}	3.1+0.1^{b}	3.5+0.2^{b}	3.6+0.1^{b}	3.8+0.1^{b}	ND
16	LAB counts (log10 cfu/g)	5.0+ 0.1^{a}	5.4+ 0.2^{a}	5.0+ 0.2^{c}	5.1+0.2^{b}	4.7+ 0.2^{d}	5.2+ 0.2^{c}
	yeast counts (Log cfu /g)	2.6+0.1^{c}	2.2+0.1^{d}	3.1+0.1^{a}	2.5+0.1^{c}	3.0+0.1^{b}	ND

Values with different small letters in a row are significantly different (P< 0.05). **Treatments1: See table 2 for details.**

Table (8): Effect of using 4 different *Lactobacillus* sp. cultures in growth inhibition of *S. cerviciae* expressed in log 10cfu/g during storage period.

Storage period (day)		Treatments[1]					
		Control	T_1	T_2	T_3	T_4	T_5
Fresh	LAB counts (log10 cfu/g)	7.1+0.2^{b}	7.4+ 0.2^{a}	7.1+ 0.1^{c}	7.2+ 0.1^{b}	7.3+ 0.2^{b}	7.1+ 0.1^{c}
	yeast counts (Log cfu /g)	5.0+0.1^{a}	5.0+0.1^{a}	5.0+0.1^{a}	5.0+0.1^{a}	5.0+0.1^{a}	5.0+0.1^{a}
4	LAB counts (log10 cfu/g)	6.8+ 0.1^{d}	7.3+ 0.1^{a}	7.2+ 0.1 ^{a}	6.9+ 0.1^{c}	6.9+ 0.1^{c}	7.1+ 0.1^{b}
	yeast counts (Log cfu /g)	5.0+0.1^{a}	4.7+0.1^{b}	4.6+0.1^{c}	4.5+0.1^{d}	4.3+0.1^{d}	4.5+0.1^{c}
8	LAB counts (log10 cfu/g)	6.2+0.1^{c}	6.3+ 0.2^{b}	7.1+ 0.1^{a}	5.9+ 0.2^{c}	6.4+ 0.1^{b}	6.5+ 0.1^{b}
	yeast counts (Log cfu /g)	4.7+0.1^{a}	4.5+0.1^{b}	4.5+0.1^{b}	4.3+0.2^{c}	4.2+0.1^{d}	4.1+0.1^{d}
12	LAB counts (log10 cfu/g)	5.4+0.2^{d}	5.9+ 0.1^{b}	6.2+ 0.1^{a}	5.7+0.2 ^{b}	5.5+ 0.1^{d}	5.8+ 0.1^{c}
	yeast counts (Log cfu /g)	4.6+0.1^{a}	4.4+0.1^{b}	4.3+0.1^{c}	4.1+0.1^{d}	4.1+0.1^{d}	4.1+0.1^{d}
16	LAB counts (log10 cfu/g)	4.4+ 0.1^{c}	4.9+ 0.2^{a}	5.o+ 0.1 ^{a}	4.2+0.1^{d}	4.5+ 0.1^{c}	4.6+ 0.1^{b}
	yeast counts (Log cfu /g)	4.5+0.1^{a}	4.1+0.1^{c}	4.3+0.1^{b}	4.1+0.1^{c}	4.0+0.1^{c}	4.1+0.0^{c}

Values with different small letters in a row are significantly different (P< 0.05). **Treatments1: See table 2 for details.**

Antifungal activity of some different Lactic acid bacterial strains using disc assay and overlay methods:

Generally, overlay and disc assay methods gave the same antifungal activity data with all tested strains. Furthermore, there were no fungal spore formations observed throughout the upper agar of *L. rhamnosus* TISTR541 and *L. casei* 01 or using disc assay method. *Aspergillus flavus* showed the most resistance to antifungal activity of tested *Lactobacillus* strains. The four *Lactobacillus* strains *L. rhamnosus* TISTR 541, *L. helveticus* and *L. casei*01 *L. plantarum* NRRL B-4496 totally inhibiting the growth of *Aspergillus flavus*.

L. rhamnosus and *L. casei* were able to inhibit the growth and production by *A. flavus* in vitro. Table (9) shows the inhibition of growth of one *A. flavus* by *L. rhamnosus, L. acidophilus, L. helveticus, L. casei* and using combination of the four *Lactobacillus* sp. strains together via the agar disc assay method using (OGYE) agar. *L. rhamnosus, L. helveticus, L. casei* and using combination of the four *Lactobacillus* sp. strains showed the highest inhibition of fungal growth. *L.* rhamnosus was able to reduce the growth of *A. flavus* assayed whereas *L. helveticus* inhibited the growth of 90% of fungal strains. (60%) of *A. flavus* growth were totally inhibited byeither *L. helveticus or L. casei*. Also results showed that *L. rhamnosus* and *L. helveticus* was able to inhibit the sporulation production on *A. flavus* in disc assay method. Also *L. rhamnosus, L. helveticus, L. casei* and using combination of the four *Lactobacillus* sp. strains showed the highest inhibition of *Penicillium roqueforti* growth. Also results showed that *L. rhamnosus* was able to inhibit the sporulation production on *P. roqueforti* in disc assay method.

Table (9): Antifungal activity of some different Lactic acid bacterial strains against the most dairy spoilage and toxigenic fungi using an overlay and disc assay methods.

Fungi strain	Lactobacilli strain					
	Technique	*L. rhamnosus*	*L. acidophilus*	*L. casei*	*L. helveticus*	Combination of 4 *Lactobacillus* sp.
Aspergillus flavus	Overlay	+++	++	+	+	+++
	Disc assay	+++ NSF	++	+	+ NSF	+++
Penicillium roqueforti	Overlay	+++	+	++	+++	+++
	Disc assay	+++ NSF	+	++	+++	+++

+, no fungal growth on 0.1 to 3% of the plate area per bacterial streak or the surface of colony;++, no fungal growth on< 8% of the plate area per bacterial streak or inhibition on all plate and NSF, no spore formation observed throughout the upper agar. (+++, inhibition on allstrong mould growth inhibition, no mycelium present)

Exploring the antifungal activity of some different treatments of Lactic acid bacterial strains:

To explore the main antifungal effect of both *L. rhamnosus, L. casei,* and *L. helvtius* crude cell free supernatant were tested for antifungal effect against the most dairy spoilage and toxigenic fungi (of *A. flavus and Penicillium roqueforti*) the data were tabulated in table (10). It could be noticed that *L. rhamnosus,* followed by *L. helveticus* cell free supernatant, had the highest antifungal activity. While *L. acidophilus* cell free supernatant had the least antifungal activity. Using combination of the four crude Lactobacilli cell free supernatant showed the

highest inhibition of fungal growth of *A. flavus and Penicillium roqueforti*.

Table (10): Antifungal effect of crude Lactobacilli cell free supernatant some on the most dairy spoilage and toxigenic fungi.

Fungi strain	Lactobacilli Strains				
	L. rhamnosus	*L. acidophilus*	*L. casei*	*L. helveticus*	Combination of 4 *Lactobacillus* sp.
Aspergillus flavus	++ NSF	++	++	+ NSF	+++ NSF
Penicillium roqueforti	+++	+	+++	+++	+++

+, no inhibition on the surface of the colony (on obvious mycelium growth from 25 %of plate surface) no spore formation ++, very clear and large halo (strong mould inhibition, some mycelium present) +++, inhibition on all strong mould growth inhibition**,** no mycelium present) and NSF, no spore formation observed throughout the upper agar.

Table (11) presented the antifungal effect of neutralized Lactobacilli cell free supernatant on *A. flavus and Penicillium roqueforti*.

Antifungal activity of neutralized cell free supernatant of *L. rhamnosus* was disappeared against *A. flavus.* Also antifungal activity of neutralized cell free supernatant of *L. rhamnosus* was reduced against *Penicillium roqueforti*. Generally, cell free supernatant of *L. casei*was had the greatest antifungal activity along all other neutralized cell free supernatant. **Magnusson, *et al.* (2003)** and **Lavermicocca, *et al.* (2003)** reported that *L. casei* EMCC 1093and *L.* acidophilus EMCC 1892 produce several metabolites that may act together to inhibit mould growth in liquid culture. Many reports have suggested that antifungal activity is a combination of organic acids such as lactic acid and phenyllactic acids or bacteriocins and low molecular weight antimicrobial agents and peptides **(Strom, *et al.*, 2002).**

Table (11): Antifungal effect of neutralized Lactobacilli cell free supernatant some on the most dairy spoilage and toxigenic fungi

Fungi strain	Lactobacilli Strains				
	L. rhamnosus	*L. acidophilus*	*L. casei*	*L. helveticus*	Combination of 4 *Lactobacillus* sp.
Aspergillus flavus	++	++	+++	+	+++
Penicillium roqueforti	-	+	++	+++	+++

-, no inhibition (visible growth of mycelium and vegetative spores)+, no inhibition on the surface of the colony (on obvious mycelium growth from 25 %of plate surface) no spore formation++, very clear and large halo (strong mould inhibition, some mycelium present) +++, inhibition on all strong mould growth inhibition**,** no mycelium present)

The data in table (12) presented the antifungal effect of heated Lactobacilli cell free supernatant on *A. flavus and Penicillium roqueforti*. Heating of Lactobacilli cell free supernatant had no remarkable effect on their antifungal activity. The data could be indicated that, the main cause for antifungal activity of the tested Lactobacilli did not effected by heat treatment (at 90^{o}C for 10 min).

Niku-Paavola, *et al.*, (1999) reported that lactic acid bacteria culture to 16 h decreased the inhibitory effect of the compounds. Lactic acid bacteria affect mould growth and mycotoxin production by different mechanisms including production of organic acids or other heat stable compounds having low molecular weight, depletion of nutrients, or microbial competition **(Lund, *et al.*, 1995a).**

Table (12): Antifungal effect of heated Lactobacilli cell free supernatant some on the most dairy spoilage and toxigenic fungi.

Fungi strain	Lactobacilli Strains				
	L. rhamnosus	*L. acidophilus*	*L. casei*	*L. helveticus*	Combination of 4 *Lactobacillus* sp.
Aspergillus flavus	++	++	+++	+, NSF	+++
Penicillium roqueforti	+++	+	+++	+++	+++

+, no inhibition on the surface of the colony (on obvious mycelium growth from 25 %of plate surface) no spore formation ++, very clear and large halo (strong mould inhibition, some mycelium present) +++, inhibition on all strong mould growth inhibition**,** no mycelium present) and NSF, no spore formation observed throughout the upper agar.

Behavior of different dairy spoilage and toxigenic fungi in labneh:

To evaluate the effect of different lactic acid bacterial cultures on *A. flavus*growthin labneh along the storage (16 days at 5°C+1) the data is presented in table (13). Thedata presented that lactic acid bacterial were slight different among cultures in fresh samples. The highest LAB counts was observed in labneh containing *L. rhamnosus* or *L. acidophilus* (7.86 and 7.85 log cfu/g respectively). Generally, Lactic acid bacteria counts slightly decreased among the first week of the storage followed by gradual decrease till the end of the storage. The highest decrease Lactic acid bacteria counts were found in control samples followed by labneh with *L. casei* (4.4 and 4.7 log cfu/g respectively).

On the other hand, there were significant differences in mould counts along storage. The mould counts slightly decreased in control labneh as the storage period progressed. The highest mould counts were recorded in control labneh followed by labneh with *L. helvetius.*

Table (13): Effect of using 4 different Lactobacillus sp. cultures in growth inhibition of *A. flavus* expressed in log 10cfu/g in labneh during storage period.

Storage period (day)		Treatments[1]					
		Control (yoghurt)	T_1	T_2	T_3	T_4	T_5
Fresh	LAB counts (log10 cfu/g)	7.73+0.3^{a}	7.86+ 0.2^{a}	7.85 + 0.1^{a}	7.7+ 0.5^{a}	7.6+ 0.2^{a}	7.6+ 0.1^{a}
	Mould counts (log10 cfu /g)	4.0+0.2 ^{a}	4.1+0.1 ^{a}	4.1+0.1^{a}	4.1+0.1 ^{a}	4.1+0.1 ^{a}	4.0+0.2 ^{a}
4	LAB counts (log10 cfu/g)	7.43+ 0.3^{b}	7.6+ 0.3^{a}	7.55 + 0.2 ^{a}	7.6+ 0.1^{a}	7.3+ 0.7^{b}	7.33+ 0.1^{a}
	Mould counts (log10 cfu /g)	4.6+0.1^{a}	4.65+0.1^{a}	4.0+0.1^{a}	4.35+0.1^{a}	4.1+0.2^{a}	4.0+0.2^{a}
8	LAB counts (log10 cfu/g)	7.0+0.2^{c}	7.5+ 0.2^{a}	7.22+ 0.2^{b}	7.1+ 0.4^{a}	6.97+ 0.1^{d}	7.17+ 0.1^{c}
	Mould counts (log10 cfu /g)	4. 88+0.2^{a}	4.16+0.1^{b}	4.55+0.2^{b}	4.86+0.1^{a}	4.0+0.1^{c}	4.25+0.1^{b}
12	LAB counts (log10 cfu/g)	6.5+0.1^{a}	6.63+ 0.1^{a}	6.6+ 0.1^{a}	6.4+0.1 ^{b}	6.3+ 0.2^{b}	6.52+ 0.2^{a}
	Mould counts (log10 cfu /g)	5.10+0.1^{a}	4.45+0.1^{b}	4.35+0.1^{b}	5.16+0.1^{a}	4.50+0.1^{c}	4.45+0.1^{b}
16	LAB counts (log10 cfu/g)	4.4+ 0.1^{d}	5.2+ 0.2 ^{c}	6.4+ 0.1 ^{a}	5.9+0.1^{b}	4.7+ 0.1^{c}	5.3+ 0.1 ^{a}
	Mould counts (log10 cfu /g)	5.49+0.1^{a}	4.45+0.1^{d}	4.2+0.1^{a}	5.31+0.2^{a}	4.72+0.2^{b}	5.2+0.2^{b}

Values with different small letters in a row are significantly different (P< 0.05). **Treatments1: See table 2 for details.**

Inhibitory effect of lactic acid bacterial cultures in growth inhibition of *Penicillium roqueforti*:

Effect of different lactic acid bacterial cultures on *Penicillium roqueforti* growth in labneh along the storage (16 days at 5°C+1) is presented in table (14). Lactic acid bacterial counts ranged from 7.96 to 7.31 log cfu/g was observed in fresh labneh with *L. rhamnosus* and *L. acidophilus*, respectively. Also there were slight significant differences (P< 0.05) among all treatments and control sample along the storage. The gradual decreases were recorded in all labneh samples along storage. At the end of storage, LAB counts ranged from 6.7 to 5.5 log cfu/g for labneh with *L. rhamnosus* and control, respectively.

The highest decrease in lactic acid bacterial counts was found in control followed by labneh with *L. acidophilus* followed by labneh with *L. helvetius.* Generally, the maximum LAB counts were recorded in labneh with *L. rhamnosus* along the storage period.

On the other hand, mould counts slightly decreased as the storage period progressed in control labneh samples. *Penicillium roqueforti* counts slightly increased in control labneh as the storage period progressed and this increase was less than 1 log cycle. On the contrary, viability of *P. roqueforti* slightly decreased with the increase of the storage period in labneh with *L. rhamnosus* followed by labneh with *L. helvetius.*

Table (14): Effect of using 4 different *Lactobacillus* sp. cultures in growth inhibition of *P. roqueforti* expressed in log 10 cfu/g in labneh during storage period.

Storage period (day)		Treatments[1]					
		Control	T_1	T_2	T_3	T_4	T_5
Fresh	LAB counts (log10 cfu/g)	7.73+0.3^{a}	7.96+ 0.2^{a}	7.55 + 0.1^{a}	7.34+ 0.5^{a}	7.31+ 0.2^{a}	7.4+ 0.1^{a}
	Mould counts (log10 cfu /g)	4.36+0.2 ^{a}	4.1+0.1^{a}	4.26+0.1^{a}	4.75+0.1^{a}	4.2+0.2^{a}	4.3+0.1^{a}
4	LAB counts (log10 cfu/g)	7.5+ 0.1 ^{b}	7.7+ 0.1^{a}	7.1+ 0.2^{c}	7.2+ 0.1^{c}	7.1+ 0.1^{d}	7.2+ 0.1^{d}
	Mould counts (log10 cfu /g)	4.56+0.2 ^{a}	3.6+0.1^{a}	4.0+0.1^{c}	4.45+0.2^{b}	4.1+0.1^{c}	4.0+0.1^{d}
8	LAB counts (log10 cfu/g)	6.8+ 0.1 ^{b}	7.4+ 0.1^{a}	6.8+ 0.1^{b}	6.8+ 0.1^{b}	6.5+ 0.1^{c}	6.8+ 0.1^{b}
	Mould counts (log10 cfu /g)	4.66+0.2 ^{a}	3.9+0.1^{b}	3.9+0.1^{b}	3.9+0.1^{b}	3.8+0.1^{c}	3.8+0.1^{c}
12	LAB counts (log10 cfu/g)	6.7+ 0.1^{b}	7.5+ 0.1^{a}	5.8+ 0.1^{b}	5.8+ 0.1^{b}	5.5+ 0.1^{d}	5.9+ 0.1^{c}
	Mould counts (log10 cfu /g)	4.85+0.1 ^{a}	3.8+0.1^{b}	3.8+0.1^{b}	3.7+0.1^{c}	3.7+0.1^{c}	3.6+0.1^{d}
16	LAB counts (log10 cfu/g)	5.5+ 0.1^{c}	6.7+ 0.1^{a}	5.1+ 0.1^{d}	5.8+ 0.1^{b}	4.9+ 0.1^{d}	5.4+ 0.1^{c}
	Mould counts (log10 cfu /g)	6.7+ 0.1^{b}	7.5+ 0.1^{a}	5.8+ 0.1^{b}	5.8+ 0.1^{b}	5.5+ 0.1^{d}	5.9+ 0.1^{c}

Values with different small letters in a row are significantly different (P< 0.05). **Treatments1: See table 2 for details.**

Conclusions

The aim of this study was the development of new protective cultures based on 4 lactobacilli strains with a focus on the suppression of yeasts and moulds in labneh samples. In a preceding study, they recognised comparably high antifungal properties in members of the *Lactobacillus casei* group as well as in some strains of *Lb. plantarum*, **Miescher Schwenninger**, ***et al.* (2003)**.

In the food industry, potassium-sorbate is used as a preservative with qualified approval for certain food mainly to prevent outgrowth of undesired fungi. Its preservative effect was compared to the different protective cultures developed in this study.

In this study, the inhibitory cultures were inoculated to the milk together with the starter culture that did not influence the fermentation process of the concentrated yoghurt (labneh). Furthermore, different sensory evaluations with yoghurt supplemented with protective cultures *Lactobacillus casei*, *Lactobacillus rhamnosus* TISTR 541, *Lactobacillus acidophilus* AL-5 *and Lactobacillus helveticus* EMCC 4193 species in combination with yoghurt culture did not reveal any perceptible differences to texture of labneh samples. As these examples show, biopreservation is a useful tool as a "natural" and gentle way to preserve food. Lactobacilli strains have a promising potentialin this field. But nevertheless, cultures applied to food or feed should clearly be identified and characterized to confirm their status as food grade. Furthermore, information on inhibitory mechanisms including synergistic actions between the strains should be available that will help to use unobjectionable strains for an increased food safety. Bacteriocins of lactobacilli are well described as reviewed by **Holo *et al.* (2002).**

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9/5/2018