Role of lactic acid bacteria as a biopreservative agent of Talbina

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Abstract: Talbina is a mixture of barley flour and milk. The aim of this study is to evaluate the role of probiotic bacteria (*L. gasseri*, *L. reuteri*) compared to yoghurt starter bacteria (*S. thermophilus* and *L. delbreukii* sub sp. *bulgaricus*) as a biopreservative agent of Talbina samples. Shelf life of refrigerated Talbina processed by lower count (ratio 1:3 LAB : Talbina) of *L. gasseri* or *L. reuteri* increased and reached over 21 days at 6±2°C, compared to yoghurt starter bacteria which ranged between 6 and 14 days depending on the type of milk used. Storage temperatures are considered the main factors for biopreservation action of lactic acid bacteria (LAB). Increasing storage temperature to 12±2°C increased total fungal count and greatly changed fungal profile. It could be concluded that the potential of LAB to inhibit the growth of common food spoiling fungi opens up new perspectives for the bio-preservation of food products.
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1. Introduction

Bio-preservation has gained increasing attention as natural means for controlling the shelf life and safety of food products. The application of bioprotective cultures to ensure the hygienic quality is a promising tool although, it should be considered only as an additional measure to good manufacturing, processing, and storage and distribution practices (Holzapfel et al., 1995). LAB have shown a major potential for use in bio-preservation because of safety for human consumption (GRAS status) and the prevalent microflora during storage in many foods (Vignolo et al., 2008). LAB can produce a wide range of antimicrobial metabolites, i.e. organic acids, diacetyl, acetoin, hydrogen peroxide and bacteriocins. These antimicrobial activities can contribute in the microbiological safety by controlling the growth of other microorganisms, and inhibition of pathogenic bacteria such as *Listeria monocytogenes*, *Staphylococcus* sp. and *Clostridium* sp. (Stiles 1996; Caplice and Fitzgerald, 1999)

The nutritious and therapeutic benefits of probiotic microorganisms have been most extensively investigated in dairy products such as milk, yogurt (Khalil and Mansour, 1998), and cheese (Ong et al., 2006; Meyer et al., 2007). Vasiljevic and Shah (2008) added that probiotic bacteria have been exploited extensively by the dairy industry as a tool for the development of novel functional products. Probiotics have been also incorporated in edible spreads (Charteris et al., 2002); meat (Arihara et al., 1998); Ras cheese (Abdalla et al., 2008); and beef burger (Mohsen et al., 2009).

In recent years there has been a growing research interest for the utilization of barley in a wide range of food applications (Bilgi and Çelik, 2004) as it can be processed into a number of palatable, nutritious food products. Barley flour is suitable for a wide range of food applications, and it is used in a wide range of traditional Arabic, Kurdish, Persian, and Turkish foodstuffs including *kashkak*, *kashk* and *murri*. In Saudi Arabia, barley soup is traditionally eaten during Ramadan (Long, 2005). Historically, barley has been an important food source in northern and Eastern Europe and Asia (Newman and Newman, 2006).

Talbina is a popular traditional food product in the Arab world prepared by mixing barley flour and milk and cooking for 10 to 15 minutes. In Islam, Talbina was prescribed for seven diseases (Hadith), these include grief, high cholesterol levels, heart disease, treatment of cancer, effects of aging, diabetes and hypertension. Recently Miller et al. (2000) confirmed these facts, whereas Majchrzak et al. (2004) added that barley contains a number of antioxidants that can reduce the incidence of chronic diseases, including cancer. Because dairy products provide the ideal food system for delivery of probiotic bacteria (Shehata et al., 2004), therefore, the objectives of this study are to evaluate the effect of probiotic bacteria compared to yoghurt starter bacteria on the shelf life of Talbina produced and on fungal profile during refrigerated storage period. The viability of LAB was also evaluated.

2. Material and Methods
2.1. Materials

Raw fresh cow milk having 12.26% TS, 3.51% fat, 3.42% protein, 0.17% titratable acidity and pH 6.50 was obtained from a private farm in Calyoubia Governorate, whereas UHT (Ultra Heated treatment) milk as well as barley flour were obtained from supermarkets. Raw milk was pasteurized at 72°C for 15 s before using in the processing of Talbina.

2.2. Cultures

Lactobacillus delbreukii sub sp *bulgaricus* and *S. thermophilus* were obtained from Chr. Hansen's lab. Denmark, while the probiotic bacteria *L. gasseri* LA39 and *L. reuteri* LA6 were kindly donated by Dr. T. Saito, Faculty of Biological Resource Science, Tohoku University, Japan.

2.3. Organism preparation

De Man Rogosa Sharpe (MRS) broth (Difco Labs., Detroit, MI, USA) was used for propagation of the LAB. For each culture, MRS broth was inoculated at 1% using a freshly prepared culture of the desired strain of LAB and incubated at 37°C. Fermented milk was prepared by inoculating the colonies in reconstituted 10% no-fat dry milk (NFDM) until coagulation.

2.4. Preparation of Talbina

Talbina samples were prepared by mixing milk (UHT milk, fresh cow milk) and barley flour (10%) and cooking for 10 minutes. After cooling LAB>10^7 CFU/g was added to the mixture. The LAB added to the Talbina was divided into two different concentrations: Lower count (1:3 LAB: Talbina) and higher count (1:1 LAB: Talbina).

Different treatments (T) used in this study were as follows:-
T 1: *L. gasseri*, lower count, UHT milk
T2: *L. gasseri*, higher count, UHT milk
T3: *L. gasseri*, lower count, fresh milk
T4: *L. gasseri*, higher count, fresh milk
T5: *L. reuteri*, lower count, UHT milk
T6: *L. reuteri*, higher count, UHT milk
T7: *L. reuteri*, lower count, fresh milk
T8: *L. reuteri*, higher count, fresh milk
T9: Yoghurt starter bacteria, lower count, UHT milk
T10: Yoghurt starter bacteria, higher count, UHT milk
T11: Yoghurt starter bacteria, lower count, fresh milk
T12: Yoghurt starter bacteria, higher count, fresh milk
T13: Control Talbina, UHT milk
T14: Control Talbina, fresh milk

All Talbina samples were distributed in plastic cups (100g each) and stored at 6±2 and 12±2°C for 21 days. Samples were prepared in three replicates.

2.5. Microbiological analysis

Samples were prepared for microbiological examination according to ICMSF (1996). Samples were examined for total fungal count (CFU/g); according to American Public Health Association (APHA, 1992). Isolated fungi were identified according to Nelson et al., (1983). Viable cells of Lactobacilli in Probiotic Talbina were determined on MRS agar (Dave and Shah, 1996).

2.6. Titratable acidity and pH

Samples were analyzed during storage for titratable acidity (%) according to the method of AOAC (1990). Samples were also analyzed during storage for pH value using a wireless Mess-Stab 656 pH meter (Knick, Berlin, Germany).

2.7. Statistical analysis

Statistical analysis was performed using SPSS statistical program for windows (Version 16) (SPSS Inc., Chicago, IL, and USA). Standard deviation of mean was used to describe data, and a Student's T-test was used to evaluate the significant differences between control and Talbina samples. P value was considered significant if less than 0.05 at 95%.

3. Results
3.1. Microbiological analysis

Talbina is a new traditional food processed from barley flour, milk and LAB. A specific factor for Talbina shelf life is to prevent fungal growth, therefore, it is necessary to discuss the role of different factors affecting fungal occurrence during refrigeration storage.

3.1.1. Total fungal count

Fungal counts in processed Talbina samples were demonstrated in Figure (1). Lower counts of fungi were isolated from Talbina samples stored at both 6±2 and 12±2°C compared to control. Fungi were not observed in Talbina samples (Figure 1) processed by UHT milk with lower LAB count (ratio 1:3) of *L. gasseri* or *L. reuteri* during the storage period (21 days) at 6±2°C revealing significant difference to control samples. On the other hand, the period reached to 14 days for samples processed by yoghurt starter bacteria. Using higher LAB count (ratio 1:1), (Figure 1a, c) decreased the shelf life period to 14 and 7 days for samples processed by *L.*

gasseri and *L. reuteri* respectively. Increasing storage temperatures to 12±2°C lead to higher fungal count (Figure 1) in processed Talbina.

3.1.2. Occurrence and distribution of fungi

Data in Table (1) showed that increasing the storage temperature to 12±2°C caused a great change in fungal profile, where *Aspergillus* species were the most dominant fungi, whereas *Penicillium* species were the most dominant on those stored at 6±2°C. In addition, *Fusarium* species appeared only on Talbina processed by fresh milk. This variation of fungal species may be due to the difference of optimum temperature for each genus of fungal growth.

The occurrence of *A. parasiticus*, *A. flavus, A. niger* and *A. carbonarius* (Table 2) differed according to the type of milk used. During storage at 6±2°C, *A. flavus* was the most existing fungi followed by *A. niger* and *A. carbonarius* respectively in Talbina samples processed by raw milk. Meanwhile in Talbina samples processed by UHT milk, *A. niger* was the dominant species followed by *A. carbonarius* and *A. parasiticus* respectively. On the other hand, *A. parasiticus* was the most prevailing fungi in both Talbina samples processed by either fresh milk or UHT milk at 12±2°C.

3.1.3. Lactic acid bacteria viable count

Results in Table (3) showed that Talbina processed by either low or high viable count of *L. gasseri* or *L. reuteri* were over 10^7 CFU/g (7.00 $\log_{10}$ CFU/g). Data also indicated that Lactobacilli increased during the development of the storage period at 6±2°C. After 7 days of storage viable count recorded 7.681 and 7.301 $\log_{10}$ CFU/g for *L. gasseri* and *L. reuteri* respectively for the lower starting count (1:3). The viable counts continuously increased during storage period as shown in Table (3).

3.2. Chemical analysis
3.2.1. Titratable acidity (%)

Samples with higher count of bacteria, UHT milk and stored at 6±2°C (Figure 2) showed higher titratable acidity (1.14%) for *L. gasseri* samples at zero time compared with those processed by lower count of bacteria (0.69%). These findings were recorded for all treatments; this may be due to metabolites produced by LAB, which were proportional to the bacterial count in fermented milk.

Figure (2a, b) also revealed that Talbina processed by yoghurt starter bacteria and UHT milk showed the highest titratable acidity (0.96%) followed by *L. gasseri* (0.69%) and *L. reuteri* (0.64%) respectively for samples stored at 6±2°C at zero time. Samples stored at 12±2°C showed that increasing titratable acidity was also recorded for samples processed by

fresh milk (Figure 2d) compared to those processed by UHT milk (Figure 2c).

3.2.2. pH value

Table (4) showed that the pH value of control Talbina was similar to that of milk, and that there was no great change in the pH value during storage period. The unchanged pH value of control Talbina during storage is evidence that Talbina is processed under sanitation conditions. On the other hand, the pH values ranged from 5.44 to 5.25 and from 5.11 to 5.00 for *L. gasseri* Talbina processed by UHT milk and fresh milk respectively during storage period at 6±2°C revealing significant difference to control samples. Furthermore, *L. gasseri* Talbina samples had higher pH values than those of *L. reuteri* followed by yoghurt starter. These findings are related to the change in titratable acidity (%).

3.3. Shelf life

Figure (3) revealed that *L. gasseri* and *L. reuteri* Talbina samples stored at 6±2°C showed long shelf life followed by yoghurt Talbina. All samples stored at 12±2°C showed a lower shelf life, which reached up to 7 days (data no shown). Furthermore, the UHT milk (Figure 3) had a long shelf life in all samples compared to fresh milk. Shelf life means that samples are without any unfavourable changes in flavour, taste, appearance and no fungal growth observed, low titratable acidity, high pH values .Results also indicated that probiotic bacteria had a positive effect on the shelf life of samples compared to either control or samples processed by yoghurt starter bacteria. On the other hand, lower bacterial count for both *L. gasseri* and *L. reuteri* showed the best results such as long shelf life (21 days) compared to those of higher bacterial count (Figure 3).

4. Discussion

LAB are considered to be important components of the microbiota, playing a large variety of health-promoting functions. Strains belonging to the *Lactobacillus* genera have traditionally been used as probiotics and added as functional components to various food products. Talbina samples processed by fresh milk were highly contaminated by fungi compared to those processed by UHT milk. These results are in agreement with Brenier-Pinchart et al. (2006) who surveyed different products for fungal contamination and found that UHT milk is not contaminated by filamentous fungi, since UHT milk is heated to 135°C for a couple of seconds to kill harmful bacteria that may be present. In addition UHT milk is sometimes called 'long-life milk,' and it is slightly different from fresh milk and has an extra

treatment that enables it to be stored at room temperature (as long as it is unopened) for extended periods. Meanwhile the heat treatment for fresh milk (pasteurization or boiling) may not be enough to ensure the safety of the fresh milk against microorganisms. The effect of heat treatment of milk was reported by Ismail et al. (2004) who indicated that heat treatment might reduce the curd tension by partial precipitation of calcium salts and changes in protein. On the other hand, local fresh milk may be contaminated with antibiotic residues or preservative agents that can affect the growth of LAB in dairy product (Saneez et al., 1995). In an effort to overcome this problem for home or Simi pilot scale industries, the possibility is considered to replace totally or partially fresh milk in dairy product by UHT milk, which is considered as a preferable consumer's health milk.

The storage conditions, especially temperature, represent an important factor affecting the microbiological quality of foods and feeds, and the improper storage temperature may prolong survival of the microorganisms or even enhance their multiplication (Zmysłowska and Lewandowska 2000). In agreement, Collombo et al. (1992) reported that low temperature was more effective for prolonged cheese storage than high temperature. Therefore, it could be demonstrated that storage temperature is considered the first factor affecting the shelf life of Talbina, thus refrigerating temperature is important to control spoilage or fungal growth of probiotic Talbina.

Our results stated that *L. gasseri* followed by *L. reuteri* were able to delay and/or decrease fungal contamination in Talbina compared to control These findings may be due to the production of the bacteriocins gassericin A and reutericin 6 from *L. gasseri* LA39 and *L. reuteri* LA6 respectively (Kawai et al., 2004). The bacteriocin gassericin A showed the highest antimicrobial activity against gram-positive food borne pathogenic bacteria, *Listeria monocytogenes*, and *Staphylococcus aureus* (Kawai et al., 2006). Arakawa (2008) added that this bacteriocin can be produced in natural media such as milk and milk based media. Arakawa et al. (2009) explained that bacteriocins (gassericin A, reutericin 6) might act on the cytoplasmic membrane of the target cell and cause death of the cell by efflux of potassium ion.

Previously AbdAlla et al. (2008) found that two probiotic bacterial strains (*L. reuteri* and *L. casei*) inhibited the fungal growth of *A. parasiticus* on Ras cheese during 90 days of storage. Recently Mohsen et al., (2009) reported that the use of LAB completely inhibited the pathogenic microorganisms

and total fungal count in beef burger during 45 days of storage at 4°C.

Various media have been studied as carriers for probiotic bacteria, such as cheese (Bergamini et al., 2005), and yoghurts (Vinderola et al., 2000), but there is no data for Talbina as probiotic vehicles. Our results stated that when Lactobacilli were inoculated in Talbina, it sustained a cell count at recommended concentrations of probiotic in food (10^7 or 10^8 CFU/g) (Reid, 2001). These results are in good harmony with those recorded by Ishibashi and Shimamura (1993) who reported that food containing such bacteria should contain at least 10^7 live microorganisms per g or per ml at the time of consumption, in order to benefit the consumer. On the other hand, the count of LAB were still in the limit up to day 14 of storage period as recommended by Svensson (1999) who reported that probiotic cultures should be able to withstand food processing and storage conditions encountered during the manufacture of functional foods under industrial conditions.

Results showed that titratable acidity (%) was higher for yoghurt starter bacteria followed by *L. reuteri* and *L. gasseri* respectively in descending order, whereas control samples showed the lowest titratable acidity and no remarkable change during storage. It was also noticed that titratable acidity increased sharply during storage period. Results are in synchronization with those reported by Joshi and Sharma (2009) who revealed that titratable acidity increased with the advancement of fermentation period up to 16 days, meanwhile El Owni and Hamed (2009) added that the titratable acidity of cheese samples stored at room temperature was higher in comparison with those stored at refrigerator temperature. Our results were confirmed by Rivera-Espinoza and Gallardo-Navarro (2010) who indicated that *L. delbreukii* produced significantly more titratable acidity expressed as lactic acid than *L. casei*, and that *L. delbreukii* was capable of surviving at low pH and high acidity. These findings were discussed previously by Carr et al., (2002) who stated that LAB encompass a heterogeneous group of microorganisms, which have a common metabolic property that produces lactic acid from the fermentation of carbohydrates. The decrease in pH of Talbina during storage was related to titratable acidity (%). It is also necessary to know that pH measures free hydrogen ion concentration, thus it is a more direct measurement, as it circumvents "apparent acidity" and is usually less subject to error or misinterpretation than titratable acidity measurement. This may have been due to the production of lactic and organic acids by LAB, which had an effect on lowering pH value as reported by Kuipers et al.

(2000) and Shah (2007). Thus, the pH value of the product was in the range of 6.4 to 4.5, which is the best pH for encouraging lactic acid bacterial growth as reported by Rivera-Espinoza and Gallardo-Navarro, (2010). Therefore, pH value plays an important role for the microbiological growth affecting the shelf life of the products.

Our investigation revealed that LAB play an important role as a preservative agent depending on the type of bacteria and its count during application leading to the increase in shelf life of the Talbina. These findings were confirmed by Shah (2007) who reported that LAB produce some components, which have a bacteriocidal and bacteriostatic effect, and therefore, lead to the delaying and/or disappearance of fungal growth in samples during storage period.

Results also revealed that storage temperature is the most important factor affecting the preserving action of LAB in the product, which is higher at 6±2°C, followed by the type of LAB used depending on the preserving action of bacteriocin. The third factor is the count of bacteria that produce metabolites parallel with the count of bacteria such lactic acid, and organic acid related to titratable acidity during storage.
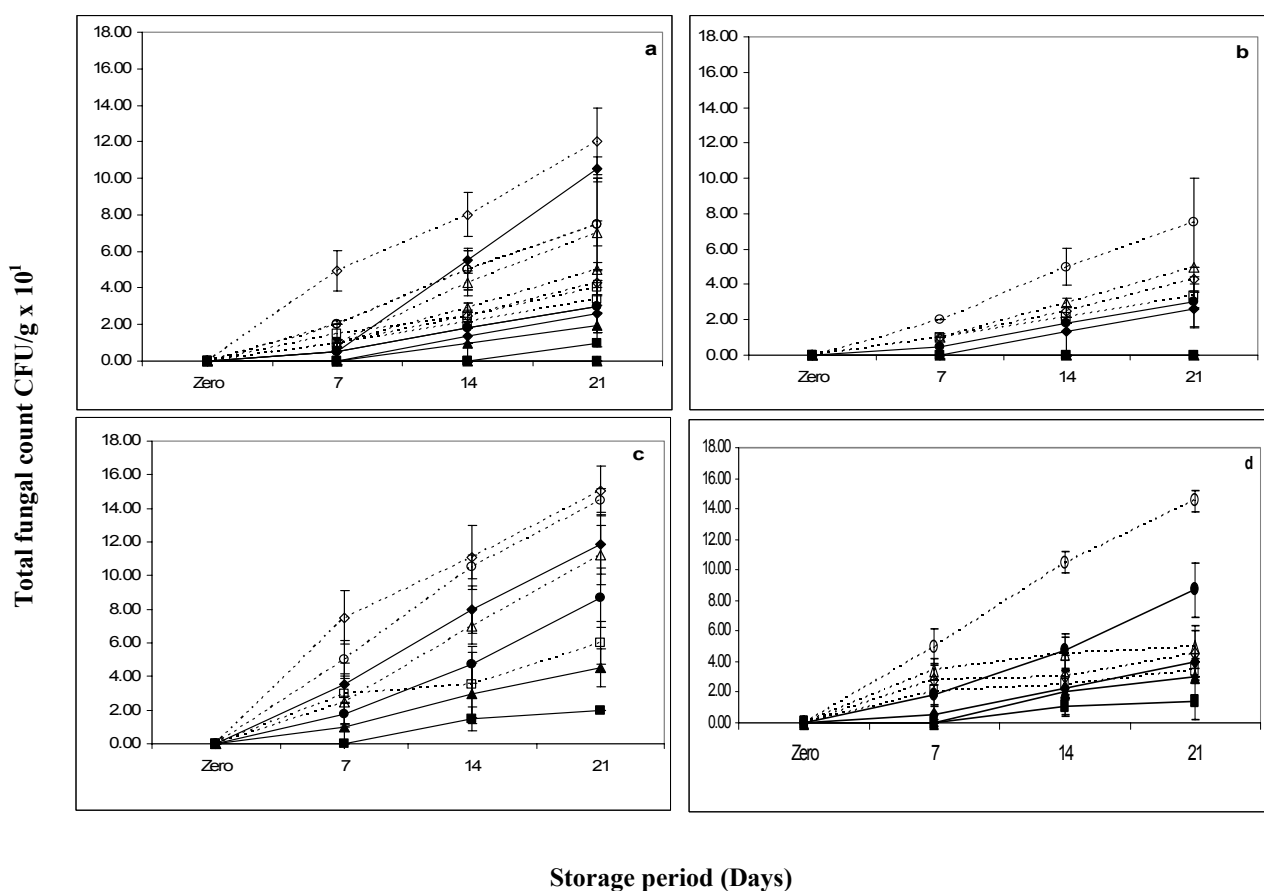

Storage period (Days)

Fig.1. Changes in total fungal count CFU/g in Talbina

a)UHT milk and higher count of LAB, b)UHT milk and lower counts of LAB, c)Fresh milk and higher counts of LAB, d) Fresh milk and lower counts of LAB during storage at 6±2°C ___ and 12±2°C ---. Data are Mean ± SD. Results revealed no significant difference. ● Control at 6±2°C, ○ Control at 12±2°C, ■ *L. gasseri* at 6±2°C, □ *L. gasseri* at 12±2°C, ▲ *L. reuteri* at 6±2°C, △ *L. reuteri* at 12±2°C, ◆ Yoghurt starter at 6±2°C, ◇Yoghurt starter at 12±2°C

Table 1
Occurrence and distribution of fungi in samples of Talbina during storage

Fungi	6±2°C				12±2°C			
	UHT milk		Raw milk		UHT milk		Raw milk	
	CFU/g	%	CFU/g	%	CFU/g	%	CFU/g	%
Aspergillus	10	45.45	22	59.46	27	84.37	39	82.98
Penicillium	11	50.00	10	27.03	2	6.25	3	6.38
Fusarium	0	0.00	4	10.81	0	0.00	1	2.13
Rhizopus	1	4.55	1	2.7	3	9.37	4	8.51
Total	22	100	37	100	32	100	47	100

Table 2
Occurrence and distribution of *Aspergillus* species in samples of Talbina during storage

Aspergillus species	UHT milk				Raw milk			
	6±2°C		12±2°C		6±2°C		12±2°C	
	CFU/g	%	CFU/g	%	CFU/g	%	CFU/g	%
A. parasiticus	2	20	11	40.74	3	13.64	16	41.03
A. flavus	0	0	4	14.81	8	36.36	8	20.51
A. niger	5	50	7	25.93	6	27.27	10	25.64
A. carbonarius	3	30	5	18.52	5	22.73	5	12.82
Total	10	100	27	100	22	100	39	100

Table 3
Viability of *Lactobacillus* (log$_{10}$ CFU/g) in Talbina stored at 6±2°C

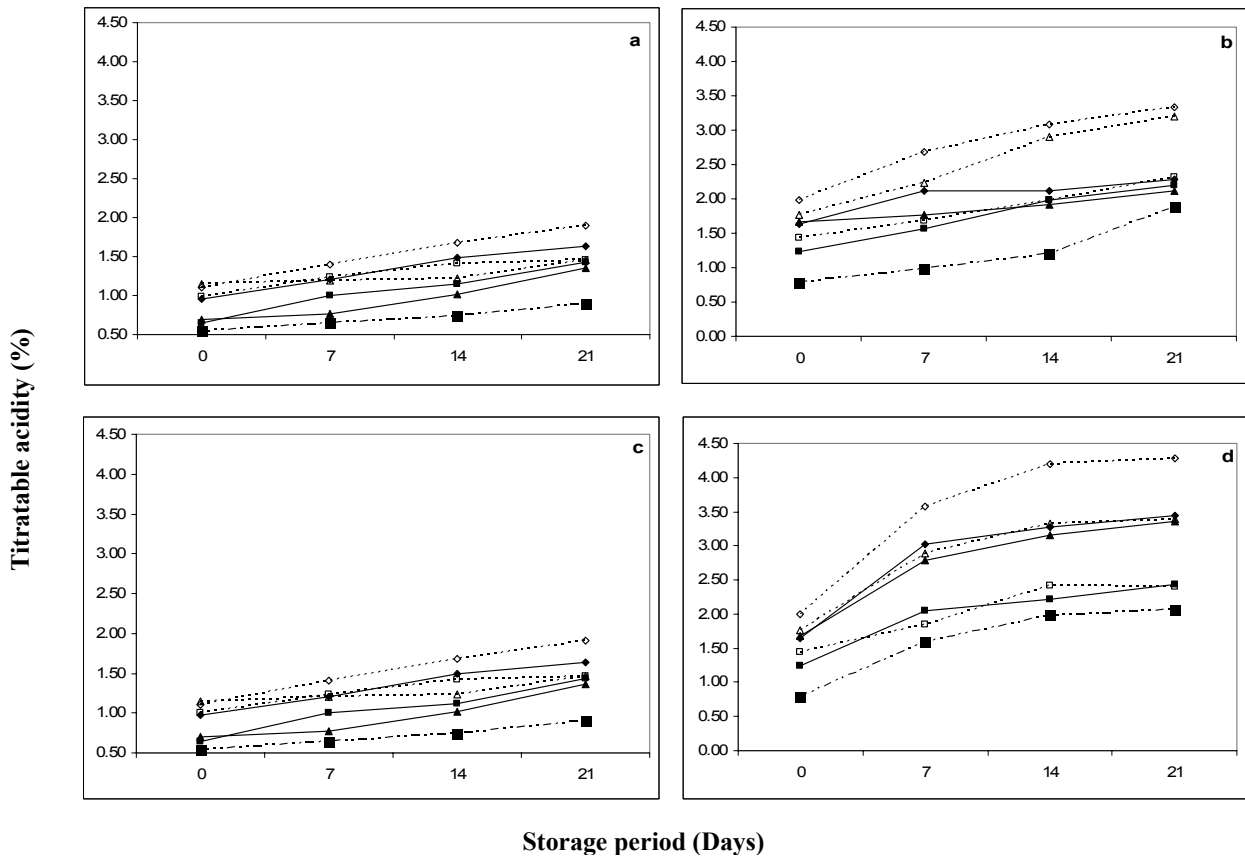| LAB	Ratio of LAB: Talbina	*Lactobacillus* count (log$_{10}$ CFU/g)		
		Zero time	Day 7	Day 14
L. gasseri	1:1	7.740	8.113	8.901
	1:3	7.082	7.681	8.719
L. reuteri	1:1	7.698	8.079	8.880
	1:3	7.000	7.031	8.602

Fig.2. Changes in titratable acidity (%) in Talbina

a) UHT milk and stored at 6±2°C, b) Fresh milk and stored at 6±2°C, c) UHT milk and stored at 12±2°C, d) Fresh milk and stored at 12±2°C during storage period.   ------ Ratio of LAB: Talbina 1:1 v/v, _____ Ratio of LAB: Talbina 1:3 v/v. Results revealed real significant difference *P<0.05*. ■ Control, ▲△*L. gasseri,* ■□*L. reuteri,* ◆◇Yoghurt starter
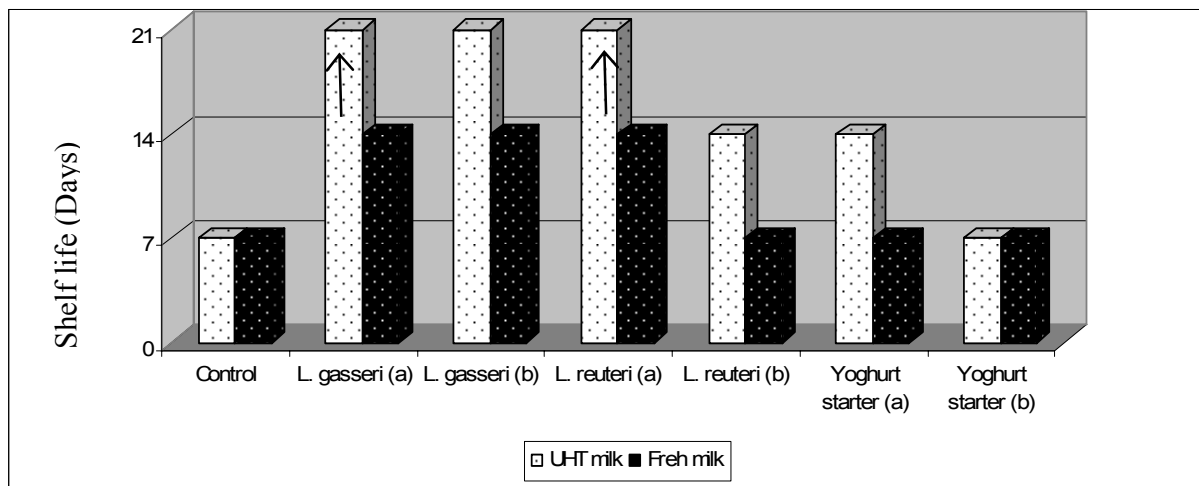

Fig.3. Shelf life of Talbina processed by UHT and fresh milk during storage at 6±2°C

Arrows means more than 21 days. a) Ratio of LAB: Talbina (1:3 v: v), b) Ratio of LAB: Talbina (1:1 v: v)

Table 4

Changes in pH value of Talbina after 21 days of storage

Treatments	Storage period (Days)	6±2°C		12±2°C	
		UHT milk	Fresh milk	UHT milk	Fresh milk
Control	Zero	6.45	6.02	6.44	5.54
	21	6.16	5.67	5.57	4.89
L. gasseri a	Zero	5.44*	5.11*	5.11*	4.79
	21	5.25*	5.00*	4.98*	4.63
L. gasseri b	Zero	5.27*	4.89*	4.95*	4.54*
	21	5.00*	4.62*	4.63*	4.40*
L. reuteri a	Zero	5.18*	5.43*	5.00*	4.93
	21	4.95*	4.95*	4.85*	4.59
L. reuteri b	Zero	4.97*	5.10*	4.79*	5.09*
	21	4.83*	4.96*	4.62*	4.10*
Yoghurt starter a	Zero	5.10*	5.11*	4.67*	4.37*
	21	4.50*	4.88*	4.19*	4.19*
Yoghurt starter b	Zero	4.70*	4.69*	4.55*	4.20*
	21	4.50*	4.20*	4.10*	4.10*

a) Ratio of LAB: Talbina (1:3 v: v)
b) Ratio of LAB: Talbina (1:1 v: v)
*Indicates real significant differences *P<0.05*

4. Conclusion

LAB provides a high preservative effect especially at low temperature <6°C causing longer shelf life to the product over 21 days. It could also be concluded that the potential of LAB to inhibit the growth of common food spoiling fungi opens up new perspectives for the bio-preservation of food products. Furthermore, Talbina as a dairy product provides the ideal food system for the delivery of these beneficial bacteria to human gut. Further research needed includes studying more LAB as biopreservative agents as well as identifying and isolation of bacteriocins produced by LAB.

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