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IMPACT OF PROBIOTICS ON YEASTS AND MOULDS TO IMPROVE QUALITY OF SOME DAIRY PRODUCTS

By

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ABSTRACT

A total of 180 samples of yoghurt (Small- and large-scale- sized container), soft cheese (Tallaga and Feta) and rayeb milk (small- and large-scale -sized container) 30 of each were arbitrarily collected from different retails as dairy shopes and markets in Beni-Suef city, Egypt. Results revealed that, the mean values of total yeasts and moulds count were $2.1x10^5$ $\pm0.74x10^5$, $6.6x10^3\pm3.2x10^3$, $2.6x10^5\pm0.96x10^5$, $2.3x10^4\pm0.6x10^4$, $2.2x10^5\pm0.75x10^5$ and $4.1x10^3 \pm 1.5 \times 10^3$, CFU/ml or g respectively.

Also, the present work evaluated the influence of probiotics as live organisms on quality of some dairy products as Tallaga cheese and rayeb milk through estimation of chemical and microbial changes during refrigeration storage especially against *C. albicans* pathogen.

Keywords:

Yoghurt, Soft cheese, Rayeb milk, *C .albicans*, probiotic, *L .acidophilus* and *B. bifidum*.

INTRODUCTION

Milk and dairy products play an essential role in human nutrition therefore, it is preferred that 25% of the daily nutrition intake contains dairy products **(Hoven, 1987).**

Fermented milk precognitive as a dairy product obtained by the fermentation of milk through the action of Lactic Acid Bacteria "LAB" which resulted in lowering of pH with or without coagulation. These bacteria must be viable, active and abundant in the product. The popular fermented milk products are Yoghurt, Labneh, Acidophieus milk, Rayeb and Butter milk, etc. **(Ahmed *et al*., 2014).**

Yoghurt is a classical fermented milk product which consumed all over the world mainly in northern European countries, Balkans,Middle-East and Indian sub-continents **(Tamine, 2002).** Yoghurt is manufactured by the addition of healthy bacteria and live cultures to milk as

Streptococcus thermophilus and *Lactobacillus delbrueckii subsp. Bulgaricus* **(Steinkraus, 1997 and Tamine and Robinson, 2007).**The main reason pointed out for yoghurt and other fermented dairy products consumption which contain cultural as improves lactose digestion and eliminates the symptoms of lactose intolerance, high in protein, calcium, phosphorus, magnesium, potassium, riboflavin and vitamin A **(Álvarez-León *et al*., 2006 and Cueva and Aryana, 2008).**

Soft cheese is processed from cow's or buffalo's milk or a mixture of them according to the Egyptian cheese-making technology. Production may be artisanal or industrial, depending on whether the cheeses are manufactured with raw theorized (heated below pasteurization level) or pasteurized milk **(Robinson and Tamime 2002).**

Laban Rayeb is one of the fermented milk products consumed by different ages in Egypt and other countries, for its highly nutritive value and therapeutic properties **(Sayed, 2012).**

The bacteriological quality of milk and dairy products is virtue by various factors as the initial flora of raw milk, the processing conditions, post-heat treatment contamination during packaging and handling. Undesirable bacteria that can cause spoilage of dairy products include coliforms, yeasts and moulds **(Nduka, 2007).**

Yeasts and moulds prefer more acidic products so they are the major cause of spoilage of yoghurt and fermented milks **(Fleet, 1990 and Rohm *et al*., 1992).** Moulds can grow well on the surfaces of dairy products when oxygen is present so, its growth is limited in packaged products, but some moulds can grow under low oxygen tension **(Hocking and Faedo, 1992).** Presence of yeasts and moulds in milk and dairy products are undesirable even when found in few numbers as they resulting in taint changes that render the products of poor quality **(Abdel Hameed, 2011).** Yeasts and moulds growing in fermented product produce lowering in the acidity as they utilize some of the acid which may result in the growth of putrefactive bacteria **(Oyeleke, 2009).**

The microflora of the gastrointestinal tract includes various species of lactic acid bacteria "LAB", which have an important function to prevent disease **(Sullivan and Nord, 2005 and Galdeano and Perdigon, 2006).** Also, they play an effective role in manufacturing of fermented food that provides its characteristic flavor, texture and its nutritional value. However, for many centuries, LAB has been an effective form of natural food preservation. This bio-preservative activity is refluxed to production of bacteriocin **(Simova *et al*., 2009).** Dairy products are considered as probiotics carrier foods especially fermented milk products

(Bergamini *et al*., 2005). *Lactobacillus* and *Bifidobacterium* genera are the most known probiotic microorganisms **(Prasad *et al*., 2000).**

Lactobacilli and *Bifidobacteria* are recognized as a good example for health-promoting constituents of the microflora. Lactobacilli have a health importance in gastrointestinal disturbance as they reduce constipation, infantile diarrhea, traveler's diarrhea, irritable bowel syndrome (IBS), and lactose-intolerant individuals and resist infections such as *Salmonellae.* Bifidobacteria also has benefit effect as stimulating the immune system, and inhibiting pathogen growth, helping to restore the normal flora after antibiotic therapy, production of B vitamins and reducing blood ammonia and blood cholesterol levels **(Gibson, 2002).**

pH is an important factor which can dramatically affect bacterial growth, *Lactobacillus spp*. as a probiotic can tolerate a wide range of pH (1-9) and grow well at acidic pH 1-5 **(Chowdhury *et al*., 2012).**

Food containing such LAB should contain at least 10^7 live microorganisms per gm. or ml. at the time of consumption, in order to benefit the consumer **(Ishibashi and Shimamura, 1993 and Hathout and Aly, 2010).**

Lactobacillus acidophilus is considered as one of the body's primary defense mechanisms against Candida, as it has protection effect against pathogenic yeast infections **(Mercenier *et al*., 2003).** Also**,** many researches indicated that L. acidophilus is the most popular species of probiotic bacteria produces substances that slow or prevents the growth of Candida **(Yang, 2000 and Mohamed *et al*., 2010)**.

Lactic acid bacteria have antagonistic effect due to producing some substances such as organic acids (lactic, acetic, propionic acids), carbon dioxide, hydrogen peroxide, diacetyl, low molecular weightantimicrobial substances and bacteriocins **(Quweh and Vesterlund, 2004).**

Therefore, our study was planned on the impact of probiotics on yeasts and moulds to improve quality of some dairy products

MATERIAL AND METHODS

1- Collection and handling of the samples:

A total of 180 samples of yoghurt (small- and large-scale sized containers), soft cheese (Tallaga and Feta sized containers) and rayeb milk (small- and large-scale sized containers), 30 of each were arbitrarily collected from different retails as dairy shopes and markets in Beni-Suef city, Egypt. The collected samples were delivered as soon as possible to the laboratory in an insulated ice-box and examined in the same day.

2- Total Yeasts and Moulds count was done according to **Roberts and Greenwood (2003).**

3-Estimating the impact of probiotic bacteria on the growth and survival of C. albicans in vitro:

3-a- Bacterial strains:

The bacterial strains used in this study were *C. albicans***,** *Lactobacillus acidophilus and Bifidobacterium.Bifidum* which obtained from the Animal Health Research Institute (Dokii, Egypt).

3-b-Media used for growth of the bacteria:

Probiotic strains were propagated in de Man Rogosa and Sharpe (MRS) broth supplemented with 0.05% L-cysteine hydrochloride (Sigma, Buchs, Switzerland) at 37^0C for 24 h under an atmosphere of 5% Co_2 for L. acidophilus and anaerobically for *B. bifibum*, *C. albicans* strain was propagated in 10 ml. of Brain-Heart Infusion broth (CM1135B, Oxoid) at 25^0C for 24 hrs.

3-c- Suspension inoculations for Bacterial strains:

Pathogenic strains counts were adjusted at concentration of 10^8cfu /ml, while probiotic strains were adjusted at concentration of 10^9 cfu/ml.

3-d- Tallaga cheeses manufacturing:

Cheese was prepared and processed according to the method outlined by **Abou-Donia (1986).**

3-e- Rayeb-milk manufacturing:

Rayeb-milk was prepared according to the method outlined by **Sayed (2012) and Ramesh and Arun (2013).**

3-f-Chemical analysis of manufactured products:

The pH of all cheese and rayeb samples was measured using (AD 111 digital pH meter 609) at 25±1 ºC **APHA (1974).**

The salt percentage (sodium chloride content) in cheese samples was measured by the standard method described by **APHA (1992).**

3-g-Enumeration of probiotic strains in manufactured cheese and rayeb was performed according to **Souza and Saad (2009).**

3-h- Enumeration of pathogenic strains in manufactured cheese and rayeb was done according to **Roberts and Greenwood (2003).**

RESULT

Table (1): Statistical analytical results of the examined samples based on their total yeasts and moulds count /ml. or gm.

Samples	Examined Samples	Positive samples		Mean	±SEM
		No.	%		
Small scale yoghurt	30	20	66.7	$2.1x10^5$	$0.74x10^5$
Large scale yoghurt	30	12	40	$6.6x10^3$	$3.2x10^3$
Talaga cheese	30	20	66.7	$2.6x10^5$	$0.96 x10^5$
Feta cheese	30	15	50	$2.3x10^4$	$0.6x10^4$
Small scale rayeb milk	30	27	90	$2.2x10^5$	$0.75x10^5$
Large scale rayeb milk	30	10	33.3	$4.1x10^3$	$1.5x10^3$

Table (2): Frequency distribution of the examined samples based on their total yeasts and moulds count /ml. or gm.

Large scale rayeb milk		Small scale rayeb milk		Feta cheese		Talaga cheese		Large scale yoghurt		Small scale yoghurt		Intervals
%	No	%	No	%	No	%	No	%	No	%	No	
66.7	20	10	3	50	15	33.3	10	60	18	33.3	10	< 10
6.7	2	30	9	6.7	2	13.3	4	13.3	4	3.3	1	10 - $<10^2$
3.3	1	20	6	3.3	1	10	3	10	3	10	3	10^2 - $<10^4$
23.3	7	40	12	40	12	26.7	8	16.7	5	43.4	13	10^4 - $<10^6$
0	-	0	-	0	-	16.7	5	0	-	10	3	10^6 - $<10^8$
100	30	100	30	100	30	100	30	100	30	100	30	Total

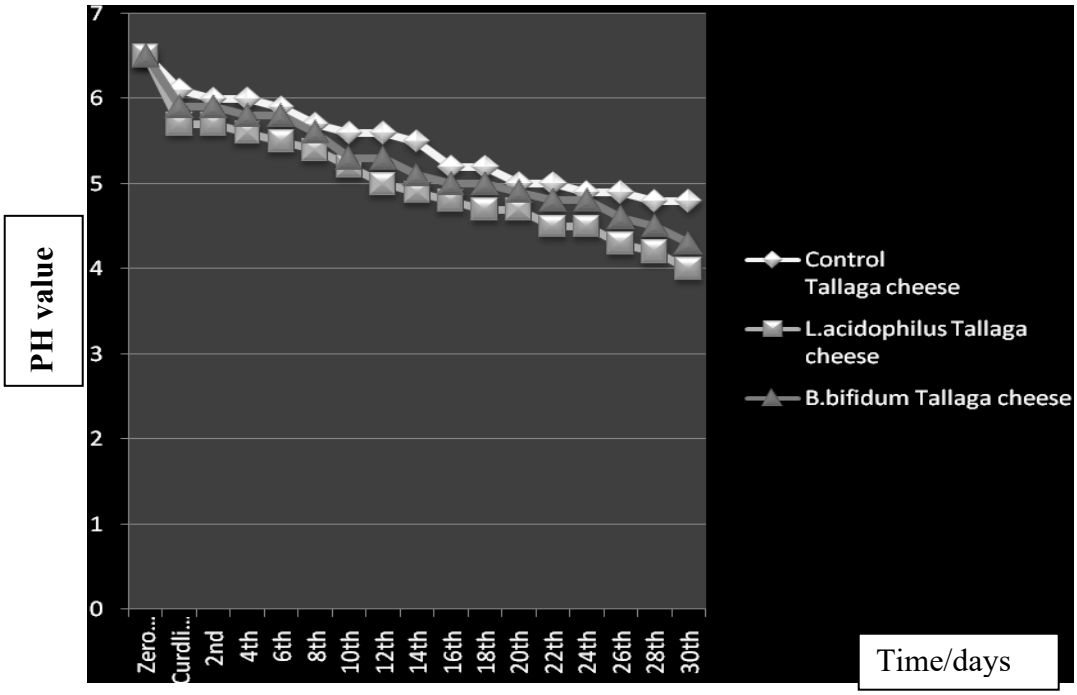

Fig. (1): PH of control and probiotic Tallaga cheese during production and refrigerator storage at 5°c

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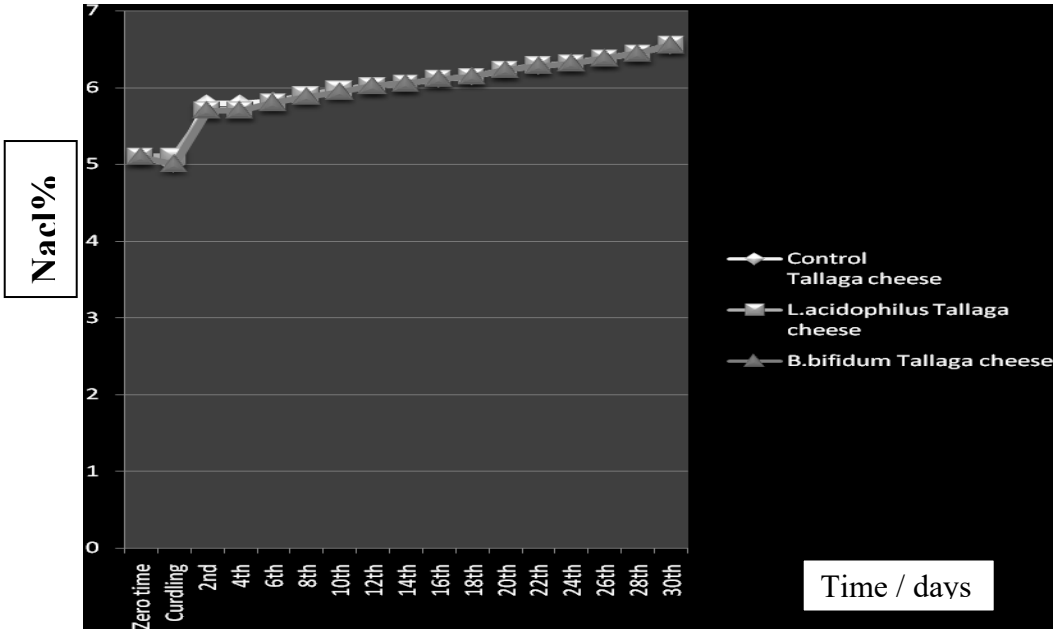

Fig. (2): Salt content (Nacl %) of control and probiotic Tallaga cheese during production and refrigerator storage.

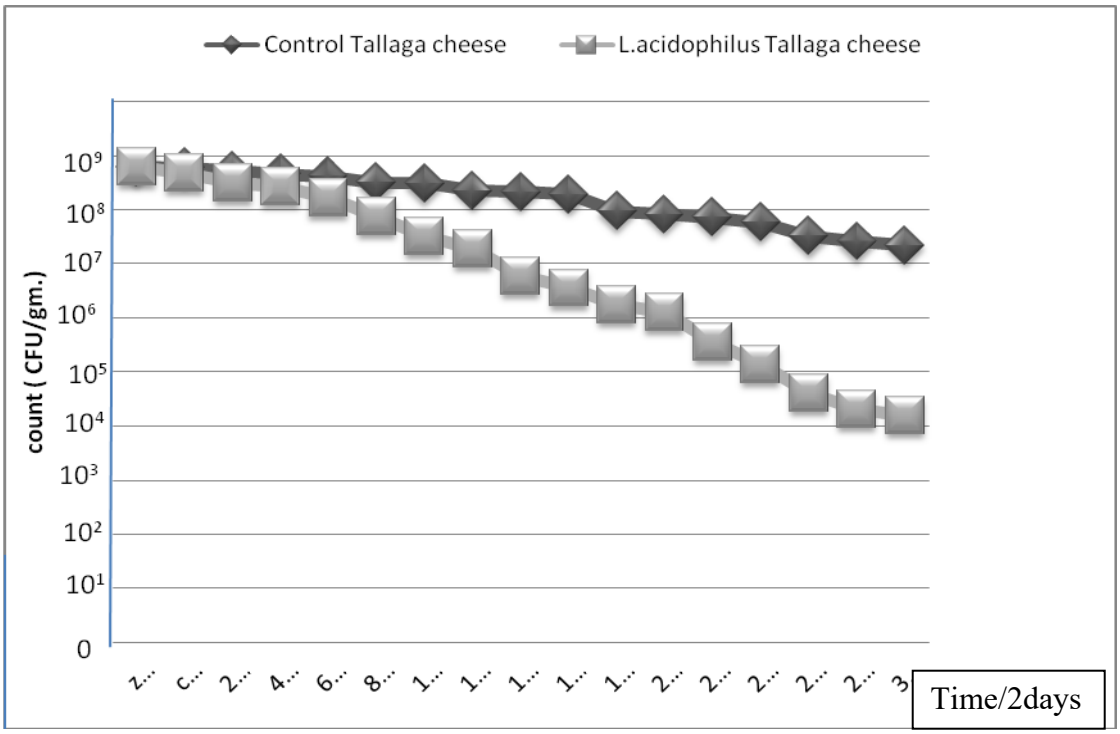

Fig. (3): Impact of *L.acidophilus*on *C.albicans* organism in Tallaga cheese during production and refrigerator storage.

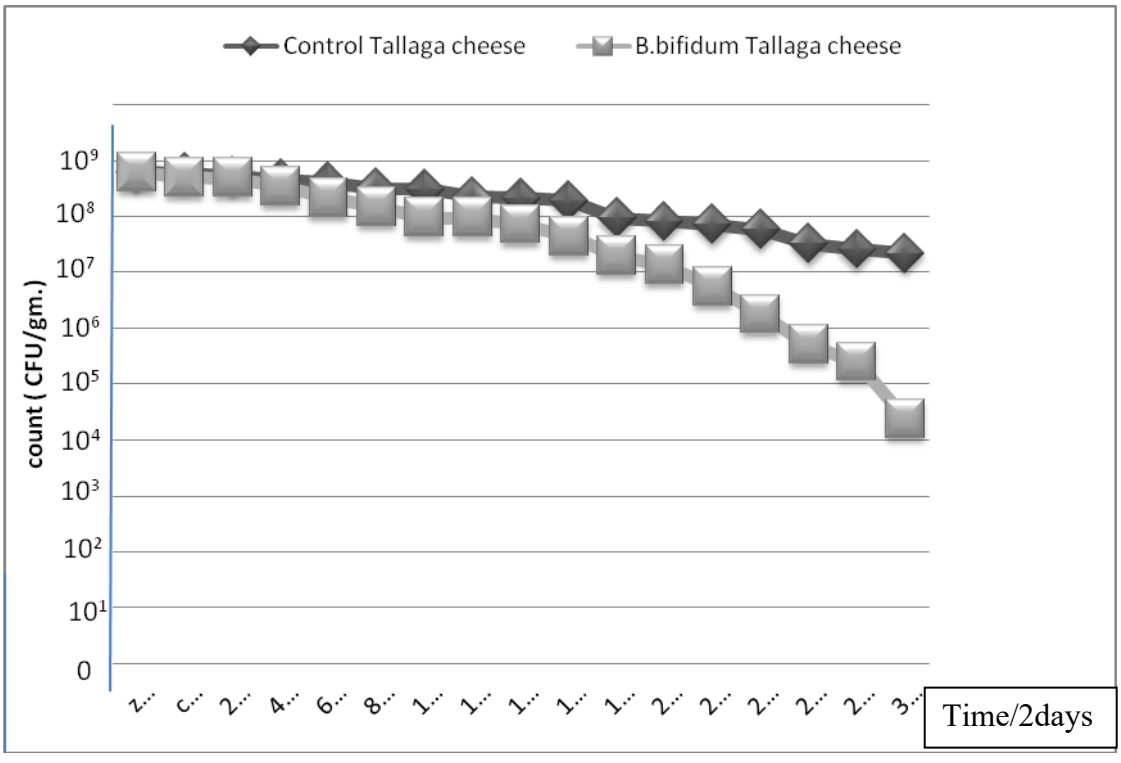

Fig. (4): Impact of *B.bifidum*on *C.albicans* organism in Tallaga cheese during production and refrigerator storage.

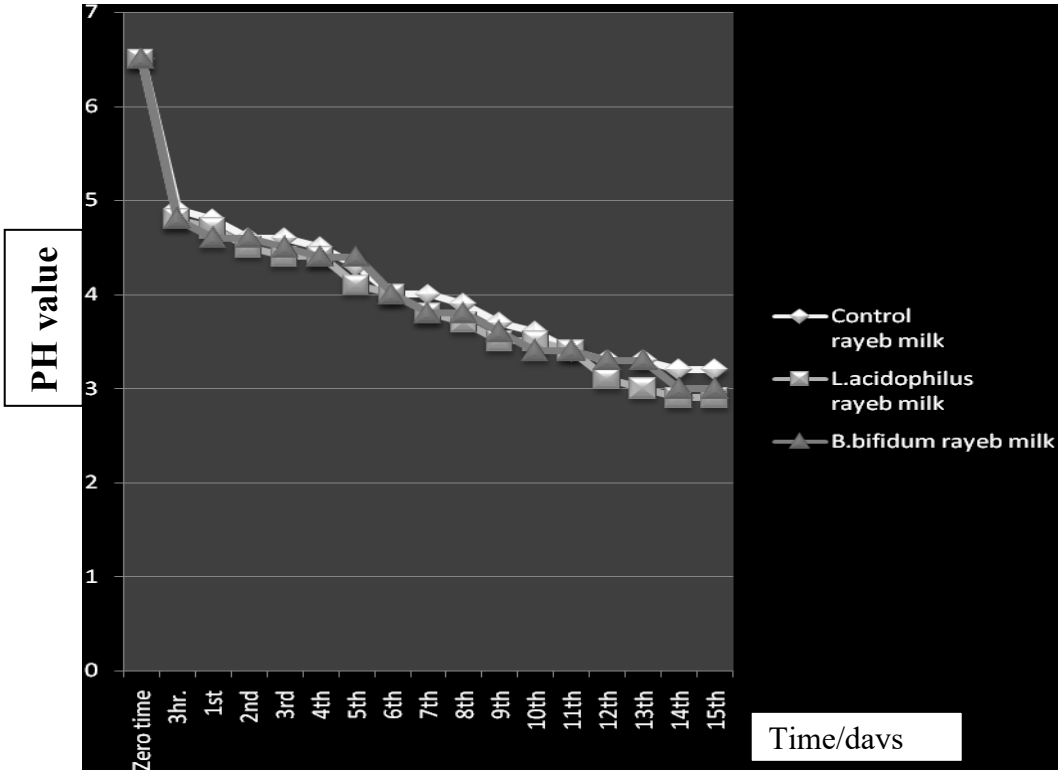

Fig. (5): PH of control and probiotic rayeb milk during production.

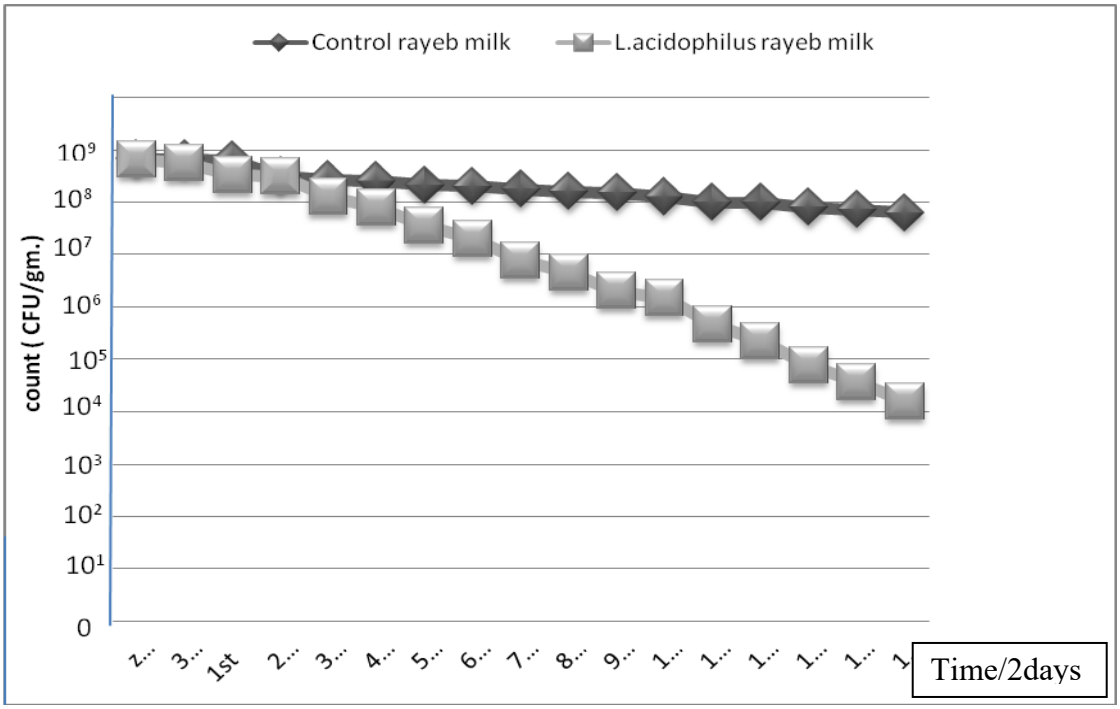

Fig. (6): Impact of *L.acidophilus*on C.albicans organisms in rayeb milk during production and refrigerator storage.

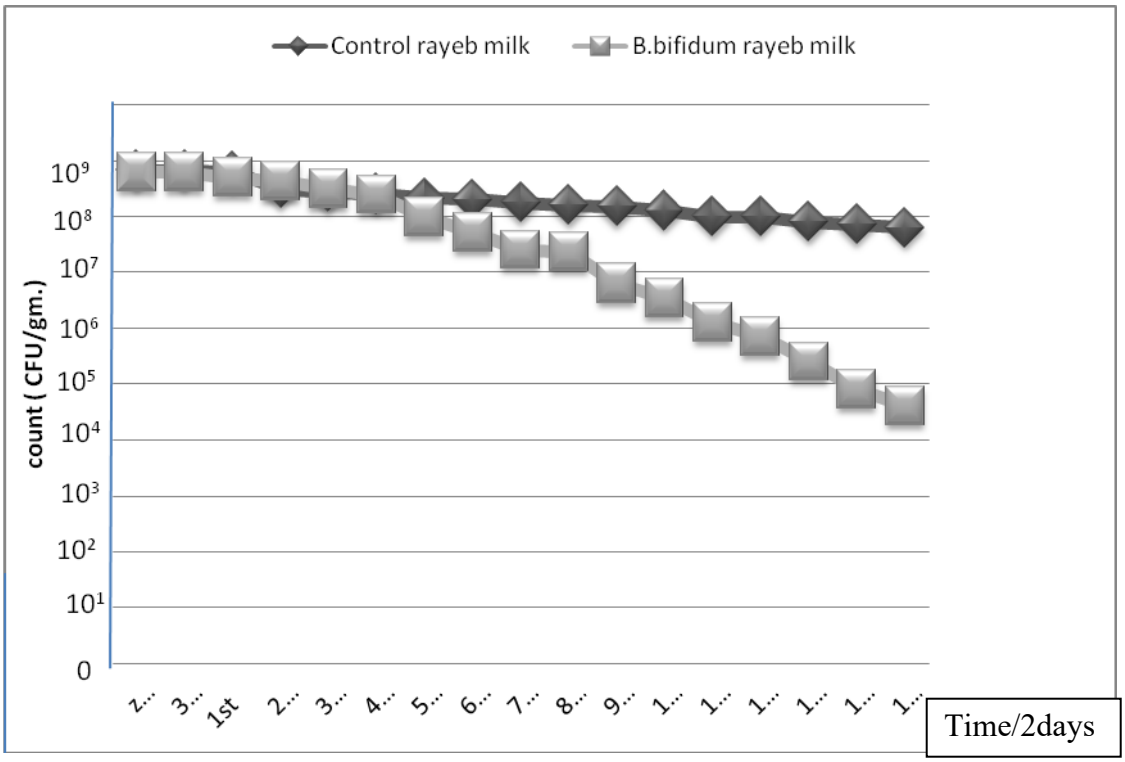

Fig. (7): Impact of *B.bifidum*on *C.albicans* organisms in in rayeb milk during production and refrigerator storage.

DISCUSSION

It is preferable to enumerate yeasts and moulds in unpasteurized dairy products that produced from raw milk or from Pasteurized products as their presence indicate recontamination during manufacturing or processing and bad storage as exposed to high humidity. Additionally their existences has both economic and health problems as some group can produce mycotoxins which consider as a public health hazard, as well as other members can ferment lactose which give a characteristic fruity or yeasty flavor and evident gas **(Chapman and Sharpe, 1990; Viljoen and Greyling, 1995 and Valerie *et al.*, 2001).**

The statistical analytical results in (Table 1) showed that, the mean values of yeasts and moulds that detected in the examined yoghurt (small and large), soft cheese (Tallaga and Feta) and rayeb milk (small and large) samples were 2.1 x 10^5 ± 0.74 x 10^5, 6.6 x10^3 ±3.2 x10^3, 2.6 x10^5 ±0.96 x10^5, 2.3x10^4±0.6 x10^4, 2.2x10^5±0.75x10^5 and 4.1x10^3±1.5x10^3cfu/gm. or ml., respectively.The highest frequency distribution 53.4, 60, 56.6, 50, 60 and 66.7 % lies within the ranges10^4 - <10^8, <10, <10 - <10^4, <10, 10^2 - <$10^6$and <10, respectively (Table 2).

The obtained results of total yeasts and moulds count in the small-scale yoghurt samples were

nearly similar with those reported by **Dardashti *et al.* (2001),** but higher values were observed by **Oksuztepe *et al.* (2007) and Abou El-Makarem (2013).**

Nearly similar count of total yeasts and moulds in the large-scale yoghurt samples was reported by **Dardashti *et al.*(2001)** and higher results was obtained by **Abou El-Makarem (2013).**

The finding of total yeasts and moulds counts in the Tallaga cheese samples were agreed with **Ceylan *et al.* (2003).** However, there were instances whereas much higher counts were recorded by **Amer *et al.* (2005)** and lower results were reported by **Ghada *et al.* (2004) and Al -Tahiri (2005)**.

Nearly similar counts of total yeasts and moulds in the Feta cheese samples were recorded by **El-Sayed *et al.* (2011)**, while higher result were detected by **Amer *et al.* (2005)** but, lower finding were obtained **by Ghada *et al.* (2004) and Al -Tahiri (2005).**

The total yeasts and moulds count in the small scale rayeb milk samples was higher in the works of **Shawer (2013).**

The count of total yeasts and moulds in the large scale rayeb milk samples was parallel to the findings of **Ahmed *et al.* (2014)** inspite lower value was recorded by **Shawer (2013).**

Yeasts and moulds consider that, the main causes of yoghurt and fermented milks as pH is low which provides a preferable environment for their growth. Also they can grow and contaminate cheese factory environment as cheese-making equipment, in air and in curd and whey. Good manufacturing practices conditions for yoghurts production should produce product that contain not more than 10 cells with shelf life of 3 - 4 weeks at 5°C. **(Lund *et al.*, 2003 and Ledenbach and Marshall, 2009)**

Fungi consider ubiquitous pathogens that can cause spoilage and bio-deterioration of many foods especially dairy food as they produce metabolic byproducts which cause off-odors and flavors as well as visible changes in color or texture may also cause gassing defects in cheeses **(Filtenborg *et al.*, 1996)**. Some moulds can also adversely affect human and animal health, as they may produce mycotoxins that have been related to a range of pathologies, from gastroenteritis to cancer **(Hussein and Brasel, 2001).**

Probiotics are live organisms that produce benefit to health of the consumer or host when ingested through their ability to implant themselves on the intestinal microflora**,** resist gastric acidity and bile digestion **(Sfakianakis and Tzia, 2014).** Also, Probiotics are added with concentrations of 10^7-10^8cfu/gm or ml. as adjunct cultures to food and products, if no longer

participating or participating in the fermentation, they are able to reach a concentration of 10^8-10^9cfu/gm or ml. after the fermentation happened **(Vinderola *et al.,* 2011)**.

The mechanism of lactic acid bacteria "LAB" in the controlling of microbial and pathogens growth is their production of lactic acid in addition to other antimicrobial compounds which exerted causing inhibition on the growth of pathogen **(Tadesse *et al.,* 2005).**

Numerous antibiotic-like elements similar as bacteriocin which are produced by LAB includes, acidophilin, bulgarican as well as other broad-spectrum inhibitors. These substances are considered very effective against *C. albicans* growth, in addition to the intensity of inhibition action that produced by LAB is vary through the organism's strains **(Gilliand and Speck, 1977; Anderson *et al*., 1998 and Segun, 2015)**.

The results obtained in Fig. (1) showed that after 30 days refrigerator storage control Tallaga cheese had PH value "4.8"**,** *L. acidophilus* Tallaga had "4.0" and *B. bifidum* Tallaga cheese "4.3". From the data presented in Fig. (2) it is evident that, the Salt content (Nacl%) nearly similar in *L. acidophilus* Tallaga cheese ,*B. bifidum* Tallaga cheese and control one during production and after 30 days refrigerator storage.

The result given in Fig. (3) recorded that *L. acidophilus* strain had antagonistic effect against ***C.albicans*** which depressed in its count from 7.9 x$10^8$to 1.9x10^4cfu/gm. than control one from7.9 x10^8 to 3.4x10^7cfu/gm. that agree with **Falagas *et al.* (2006)**, **Cartwright (2010)**, **Mohamed *et al.* (2010) and Ebrahimi *et al.* (2012)**. Also, in Fig. (4) *B. bifidum* strains had inhibitory effect on *C. albicans* has decreased its count from 8.0 x10^8 to 3.8x10^4cfu/gm. than control one from 7.9 x10^8 to 3.4x10^7cfu/gm.

The antimicrobial properties of yoghurt samples were mostly higher than these of the cell free and these activities may due to lactic acid production, decreasing in pH and other antimicrobial compounds that may be present in the yoghurt **(Hassan *et al*., 2013).**

The data illustrated in Fig. (5) showed that after 15 days refrigerator storage control rayeb milk had PH values "3.2", *L.acidophilus*"2.9" and *B. bifidum*rayeb milk "3.0" and these result agree with **Abd El-Gawad *et al.* (2014).**

It is evident from the results which presented in Fig. (6) that *L. acidophilus* strains had antagonistic effect against *C.albicans* pathogen as lowering its count from 8.2 x10^8 to 2.0x10^4cfu/ml. than control one from 8.2 x$10^8$to 8.0x10^7cfu/ml. which were nearly similar the findings reported by **Cartwright (2010)**, **Mohamed *et al.* (2010) and Ebrahimi *et al.* (2012)**. Also, in Fig. (7) *B. bifidum* strains had antagonistic effect on *C. albicans* as lowering its count

from 8.0 x10^8 to 6.0x10^4cfu/ml. than control one from 8.2 x$10^8$to 8.0x10^7cfu/ml.

Finally, it is clear the importance of probiotics to health of the consumers when ingested in the dairy products and all the dairy products must be processed with addition of probiotics.

CONCLUSION

It was concluded from this work that some of dairy products as yoghurt, soft cheese and rayeb milk sold in Beni-Suef city, Egypt were of bad quality as heavy yeasts and moulds contaminated that rendering them threating to public health and may causes many diseases specifically food poisoning to consumer and/or undesirable changes in the dairy products that rendering them unsatisfactory for human consumption and cause economic losses.

It is recommended to establish hygienic measures that improve the microbiological quality of these products as educational programs for farmers, dairy products handlers and consumers, sanitary production and proper handling of either milk or milk utensils, rapid and adequate cooling of milk and proper heat treatment of milk during processing.

Also, the information which given in this work demonstrated that for improvement the microbiological quality of dairy product we can use probiotics strains as *L. acidophilus*and *B. bifidum* as it is have antagonistic effect against many species like *C.albicans* strains so, these confirm the health benefits derived from the human consumption of fermented dairy products.

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