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Low Cholesterol Fermented Milk Beverage by Probiotic Bacteria

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Abstract

Fermented milk are widely consumed for their benefits and refreshing effects. It was made with cow's milk produced by three probiotic bacteria *Lactobacillus plantarum*, *Bifidobacterium bifidum* and *Lactococcus lactis subsp. lactis biovardiacetylactis*MD 099(1:1:1). Low cholesterol fermented milk beverage was prepared from 3%fat (control), and clear low cholesterol butter oil by adding 5% β cyclodextrin was mixed with skim milk and homogenized to obtained 1, 2 and 3% low cholesterol milk. The sensory properties and cell viability of the fermented products were evaluated. The gross chemical composition, pH, flavor components (acetaldehyde and diacetyl) and viscosity were determined. The results it is possible to successfully prepared Low cholesterol fermented milk beverage of 3% fat (2.4mg cholesterol /100ml) which is lower than the control 3% fat (12.4 mg cholesterol /100ml). Counts increased gradually during first day of incubation tell the end of 5th days then decreased gradually. The control and treatments samples contained the recommended levels of survival cells (10^6–10^7 CFU/g) probiotic bacteria at the end of 10 days. Statistical analysis of odor intensity, acidity, creaminess and viscosity were sensory evaluated. This fermented milk T3 was of excellent organoleptic characteristics which were almost very close to the control one.

Keywords;Fermented milk; beverage; probiotic; sensory evaluation; cholesterol.

Introduction

Although dairy products in general have the image of healthy foods, this image is often not perceived for products with a high fat content such as butter, cream, and certain type of cheeses and beverage [1, 2]. The World Health Organization and the American Heart Association have recommended that consumers reduce their consumption of saturated fatty acids and cholesterol to lower the risk of coronary heart disease. Today there is growing interest in the manufacture of cholesterol-reduced dairy products. Several studies on a laboratory scale have indicated that cholesterol removal from homogenized milk and cream was most effectively achieved by β-cyclodextrin (β-CD) powder [3].

Fermented milk products are widely consumed for their benefits and refreshing effects. It could be saying that their popularity attributed to the effective use of consumer-driven flavors and milder cultures [4-6]. These products already have a positive health image [7, 8], which can be further enhanced by the addition of probiotic bacteria with therapeutic properties [9, 10]. Probiotics – living microorganisms that when consumed in sufficient amounts provide health benefits beyond basic nutrition are emerging as important dietary ingredients in functional foods. The majority of probiotics are lactic acid bacteria, especially lactobacilli, and bifidobacteria. Factors related to technological and sensory aspects of the probiotic food products are of utmost importance since only by satisfying the demands of consumers can the food industry succeed in promoting the consumption of functional products in the future [11,12].

It has been almost medically settled that hypercholesterolemia is precursory to breast cancer in women, cancerous growths in tissues, atherosclerosis, thrombosis, biliary cirrhosis, liver necrosis, gallstones, cardiovascular disorders and other adverse biological effects. Boyed and Mcguire [13],

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Oakenfull*et al* [14] and Peter [15]; are amongst those many who emphasized the momentousness of cholesterol level diminution in diets.

Experimental

Fresh cow's milk was (12.61%) total solids (T.S), (3.3%) fat, (3.2%) total protein (T.P), (4.85%) lactose, (0.65%) Ash. It was obtained from the farm of Faculty of Agriculture, Cairo University, Egypt.

-Strains belonging to *Lactobacillus plantarum* and *Bifidobacterium bifidum* were obtained from the agent of Chr. Hansens Laboratory Denmark A/S. and *Lactococcus lactis subsp. lactis biovardiacetylactis*MD 099 were obtained from EZAL Group Rhone-Polanc Z. Ade Buxires BP-10, 88220 DangeSainaii-Romain-France.

- The β- Cyclodextrin was obtained from FlukaChemieGmbh CH -9471 Buchs, 081/75525 11 Sigma – Aldrich PFd – 89552 Steinheim 07329/970, Switzerland.

Activation of the bacterial strains

Cow's milk heated to 80°C for 15 min, and then rapidly cooled to 40°C. Three probiotic bacteria: *Lactococcus lactis subsp. lactis biovardiacetylactis*, *Lactobacillus plantarum* and *B. bifidum* (1:1:1) were added at the level of 3% (w/v) served as mixed active starter culture into the milk.

BEVERAGE Manufacture:

The milk was divided to four portions: The first part was standarezed to 3% fat and used as a control as one control. The second, third and fourth treatments were added low cholesterol butter oil with different concentrations (1, 2, and3%) by adding from 5% solution of β- cyclodextrin as described by [3, 16], were mixed with skim milk and homogenized to 1% (T1), 2%(T2) and 3%(T3) low cholesterol fat.

The 4 treatments was heated at 90°C / 5 min , then cooled at 35°C. *Lactobacillus plantarum*, *Lactococcus lactis subsp. lactis biovardiacetylactisand B. bifidum(1:1:1) were* added at the rate of 3% (w/v) served as mixed starter culture into the milk. The inoculated milk were stirred gently and incubated at 35° C until the acidity reached 0.7%, The resultant coagulation of all treatments was homogenized, all treatments filled into bottles and were stored in refrigerated at 8±1°C . The resultant low cholesterol fermented milk beverage was analyzed as fresh and after of 3, 5, 7 and 10 days of storage.

Method of analysis:-

Physicochemical determinations: The total solids, titratable acidity, total protein and non protein nitrogen contents of low cholesterol fermented milk beverage were determined according to A.O.A.C. [17]. The pH value of the fermented milk was measured using digital pH meter (HANNA, Instrument, Italy) with combined glass electrode. Total cholesterol was determinates as described by Pantulu*et al* [18]. Acetaldehyde was determined as mentioned by Less and Jago [19], while diacetyl content was estimated as described by Less and Jago [20]. Samples were analyzed at 1, 5, 7 and10 days. Viscosity was measured in fresh samples at 7^{o}C using a Brookfield digital viscometer (Model DV-II+VISCOMETER, Spindle-00). The speed was set from 3to100 rpm. Three readings, 30 s apart, were recorded for each sample.

Activation of the bacterial strains:

Bifidobacterium bifidum, *L. plantarum and Lactococcus lactis subsp. lactis biovardiacetylactis*were activated individually by three successive transfers in modified MRS and M17 followed by three successive transfers in sterile 10% reconstituted skim milk powder and incubated at 37°C for 48 h under anaerobic conditions cultures were prepared 24 h before used .

Bacteriological Analysis

Samples preparation:

Twenty five ml of each sample was mixed and homogenized in sterile mixer, and diluted with buffered peptone water to make the sufficient dilutions for the microbiological analysis. Ten-fold dilutions of homogenates samples were prepared and inoculated onto plates of selective media. Viable cell count of *L. lactis*ss*diacetylactis*were enumerated on M17 agar (oxoid) after aerobic incubation at 30^{o}c for 48h [21]. *L. plantarum* was determined on MRS selective agar [22]. plates were incubated at 37^{o}C for 4 days.*Bifidobacterium bifidum* was determined according to El-Shenawy et al. [23], using modified MRS agar (oxoid) supplemented with 0.05% L-cystein- HCl (Merck, Germany). Plates were incubated at 37^{o}C for 48h. under anaerobic conditions (BBL Gas pak, Becton Dickinson, Cockeysvile MA

USA). Coliform group was determined using solid medium method onto plates of violet red bile agar medium; plates were incubated for 24 hrs at 35ºC [24]. Enumeration of *Staphylococcus aureus* in samples was carried out by spreading 0.l ml of each of sufficient (expected) dilution onto the surface agar medium Mannitol salt agar (Oxoid Ltd., England), media supplemented with egg yolk and potassium tellurite solution. Plates were incubated at 37ºC for 48 hrs [25]. Enumeration of yeasts and moulds were carried out using the potato dextrose agar medium. Plates were incubated at 25°C for 3-7 days [26,27].

Sensory evaluation

Panel selection: Eleven trained panelists were selected among the staff of Dairy Science Department, National Research Center, Cairo, Egypt.

Statistical analyses

Statistical analyses were performed using the GLM procedure with SAS [28]. Software. Duncan's multiple comparison procedure was used to compare the means. A probability to P≤0.05 was used to establish the statistical significance.

Results and discussion

Physicochemical determinations

Viscosity:

Figure (1) shows that the viscosity of resultant Low cholesterol fermented milk beverage increased significantly as fat percent was increased in the prepared Low Cholesterol Low cholesterol fermented milk (LCFMB) either fresh or at all the storage period up to the 7thday of storage, and then slightly decreased up to the end of the storage period . Also, viscosity of LCFMB of 3% fat did not significantly differed in compare with control one. Similar results were also noticed by Tamime and Robison[29] and Alroubaiya [30].

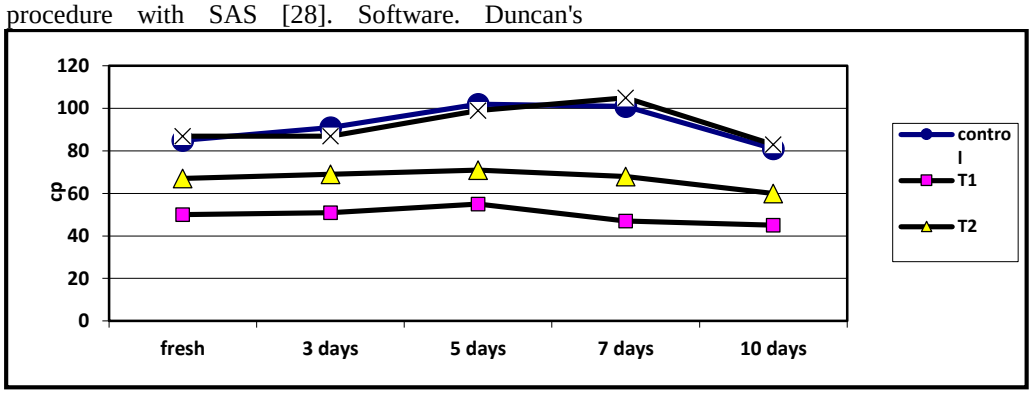


Figure (1): Changes in Viscosity (cP) of the low cholesterol fermented milk beverage during the storage periods at 8 ± 1º C.

Total solids content:

Results in Table (1) the total solids content of LCFMB decreased significantly as the total fat of the fermented milk was decreased from T1 to T2 and up to T3 at fresh and during storage period. Also, total solids content tended to increase slightly as the storage period prolonged. The obtained slight increase in total solids may be attributed to the development of acidity and also, to the natural evaporation. These obtained results are in agreement with those reported by Abou-Dawood and Abdou [31], Ibrahim *et al*, [32]. and Salem *et al*, [33].

Table (1): Changes of total solids percent of the low cholesterol fermented milk beverage during the Storage periods at 8 ± 1º C.

Treatment	Storage period (days)				
	Fresh	3	5	7	10
Control 3% fat	12.60^{a}	12.62^{a}	12.65^{a}	12.71^{a}	12.79^{a}
T1	11.40^{c}	11.40^{c}	11.43^{c}	11.44^{c}	11.49^{c}
T2	11.65^{b}	11.69^{b}	11.75^{b}	11.79^{b}	11.84^{b}
T3	12.15^{a}	12.18^{a}	12.21^{a}	12.25^{a}	12.28^{a}

The same capital letters between columns or rows are not significantly (p≤ 0.05).
T1= 1% Low cholesterol fat T2= 2% Low cholesterol fat T3= 3% Low cholesterol fat

pH and titratable acidity(T.A) :

Table (2) shows that no significant differences were noticed between all treatments of LCFMB and control in the pH values. Meanwhile, results reveal a slight decreased in pH during storage period of all treatments. The obtained results are in agreement with those reported by Ghalab*et al* [34], and Alroubaiya [30]. Table (3) indicates that the acidity of all treatments slightly increased as the storage period prolonged. Tamime and Deeth [35] and Abd El Salam *et al* [36] reported similar results.

Total protein content (T.P):

No significant differences in total protein, between the different treatments when fresh and during storage period.

Non- protein nitrogen (N.P.N) / Total nitrogen (TN) percent.

Results in Table (4) indicate no significant differences in NPN/TP percent, between all treatments at fresh and during storage period. It is also clear from table (6) that the NPN /TP percent increased with advance of storage period. Similar results were noticed by Rosic and Kurmann [37] and Tamime and Deeth [35].

Table (2): Changes of pH value of the low cholesterol fermented milk beverage during the storage periods at 8 ± 1º C.

Treatment	Storage period (days)				
	Fresh	3	5	7	10
Control	4.80^{a}	4.77^{a}	4.67^{a}	4.51^{a}	4.49^{a}
T1	4.77^{ab}	4.69^{b}	4.61^{ab}	4.46^{a}	4.42^{a}
T2	4.74^{b}	4.66^{b}	4.55^{b}	4.53^{a}	4.46^{a}
T3	4.77^{ab}	4.69^{b}	4.56^{b}	4.49^{a}	4.45^{a}

The same capital letters between columns or rows are not significantly ($p \leq 0.05$).
T1= 1% Low cholesterol fat T2= 2% Low cholesterol fat T3= 3% Low cholesterol fat

Table (3): Changes of titratable acidity of the low cholesterol fermented milk beverage during the Storage periods at 8 ± 1º C.

Treatment	Storage period (days)				
	Fresh	3	5	7	10
Control	0.77^{b}	0.78^{b}	0.83^{a}	0.88^{a}	0.92^{a}
T1	0.79^{a}	0.84^{a}	0.87^{a}	0.89^{a}	0.93^{a}
T2	0.83^{ab}	0.83^{a}	0.87^{a}	0.92^{a}	0.95^{a}
T3	0.79^{ab}	0.82^{a}	0.85^{a}	0.91^{a}	0.95^{a}

The same capital letters between columns or rows are not significantly ($p \leq 0.05$).
T1= 1% Low cholesterol fat T2= 2% Low cholesterol fat T3= 3% Low cholesterol fat

Table (4): Change in Non protein nitrogen (N.P.N) / Total nitrogen (TN) percent of low cholesterol fermented milk beverage during the storage period at 8 ± 1ºC.

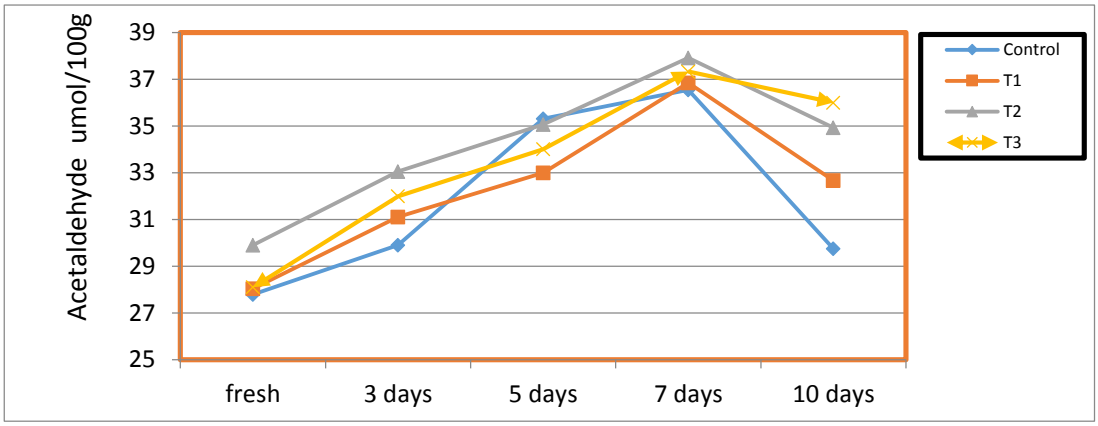
Treatment	Storage period (days)				
	Fresh	3	5	7	10
Control	7.86	9.01	10.12	15.00	17.13
T1	7.26	8.41	9.53	13.34	16.33
T2	7.55	8.94	10.50	14.38	17.56
T3	7.71	8.64	9.42	15.81	17.66

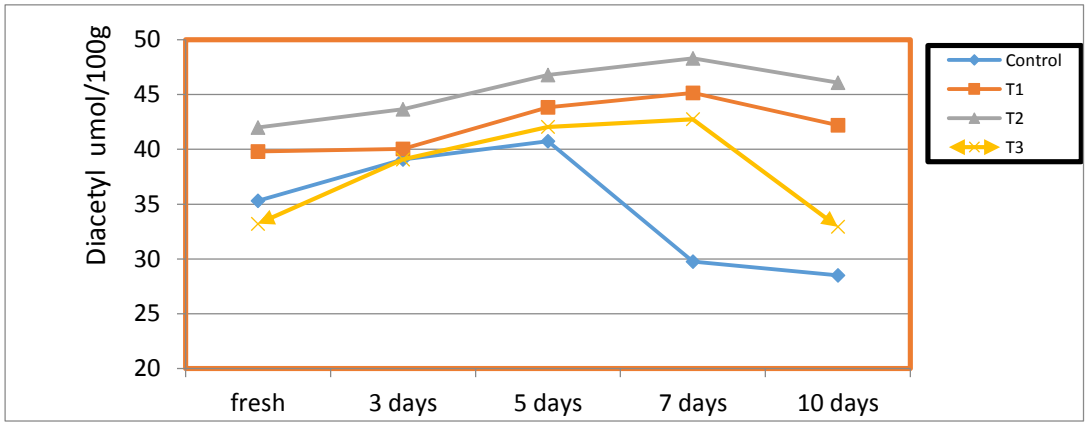
T1= 1% Low cholesterol fat T2= 2% Low cholesterol fat T3= 3% Low cholesterol fat

Acetaldehyde content:

Results indicate that the acetaldehyde content significantly developed as the storage period was advanced. This is true at any of the prepared LCFMB of different fat contents as it is clear in Figure (2). Also, LCFMB T3 was very close to the control one. Similar results were reported by Mehanna*et al*, [38],

Rhom*et al*, [39], Salama [40], Zedan*et al*., [41] and Alroubaiya [30].





Figures (2, 3): Changes of Acetaldehyde and diacetyl (µmol/100gm) content in low cholesterol fermented milk beverage during storage at 8 ±1ºC.

Diacetyl content:

Diacetyl content of the resultant LCFMB in Figure (3) gradually increased as the storage period prolonged up to the 5thday , then strongly dropped at the 7 thand 10 thday. Such decreased in diacetyl content could be due to transferring the diacetyl to acetaldehyde. As previously reported by Tamime and Robinson [42]. Highest diacetyl content was for the control fermented milk is close to the prepared LCFMB T3. Similar results were recorded by Yossef [43] and EL- Senaity [44].

Cholesterol content:

Data in Table (5) shows that the cholesterol content of all LCFMB treatments was substantially increased as the fat content increased. But, they were significantly lower than control one. This decrease is more likely due to the role of β-cyclodextrin in reducing cholesterol content. Cholesterol content decreased at the end of storage period for all types of LCFMB and the control one, such decrease was more likely due to the role of lactic acid bacteria and its fermentation in reducing cholesterol content [34].

Table (5): Changes in cholesterol (mg/100 ml) of the low cholesterol fermented milk beverage during the storage periods at 8 ± 1º C.

Treatment	Storage periods (days)		Reduction%	
	Fresh	10	Fresh	10
Control	12. 4^{a}	6. 59^{a}	0.0^{c}	46.85^{b}
T1	1 .78^{b}	1. 06^{b}	85.65^{a}	91.45^{a}
T2	2. 1^{b}	1. 22^{b}	83.06^{ab}	90.16^{a}
T3	2 .46^{b}	1. 33^{b}	80.16^{b}	89.27^{a}

The same capital letters between columns or rows are not significantly ($p \leq 0.05$).

T1= 1% Low cholesterol fat T2= 2% Low cholesterol fat T3= 3% Low cholesterol fat

Microbiological analysis

The Effect of low cholesterol fermented milk beverage treatments on the viability of probiotic bacteria is presented in Figure (4). Their maximum increased was at the 5thday of storage, then trend decrease gradually till the end day of storage. Whereas the control sample reached the maximum level of viability in the 3th days then decreased gradually to the end day. The reduction in number of probiotic strains may be due to the sensitively of these bacteria to the acid produced during the storage period. Viability of *B. bifidum*, *L. plantarum* and *L. lactisssdiacetylactis*couldbe due to the effect of zinc salts which activated their growth. In this respect these results are in agreement with Magdoub*et al*, [45].who reported that zinc accelerate the growth rate of another bacteria strains were tested,(*L. lactis* spp. *Lactis*, *S. thermophilus*, *L. delbruckii*ssp*bulgaricus*) . Generally, numbers of all probiotic bacteria remained more than 106cfu/ml in all treatments, until the end of storage period. A minimum of 10^6 -10^7 viable microorganisms per gram or milliter should be present in food product in order to meet the requirements of a probiotic food, as by the Japanese fermented milk and lactic acid bacteria beverages association [46]. All samples were free of Coliforms, Moulds and Yeasts when fresh and hroughout storage period (10 days) at refrigerator temperature 8 ±1 ° C as result of hygienic condition during the preparation with, or by the fact that diacetyl is generally considered to be an antimicrobial agent because of the presence of two reactive carbonyl moieties in the molecule as by Lopez de Felipe *et al*., [47].

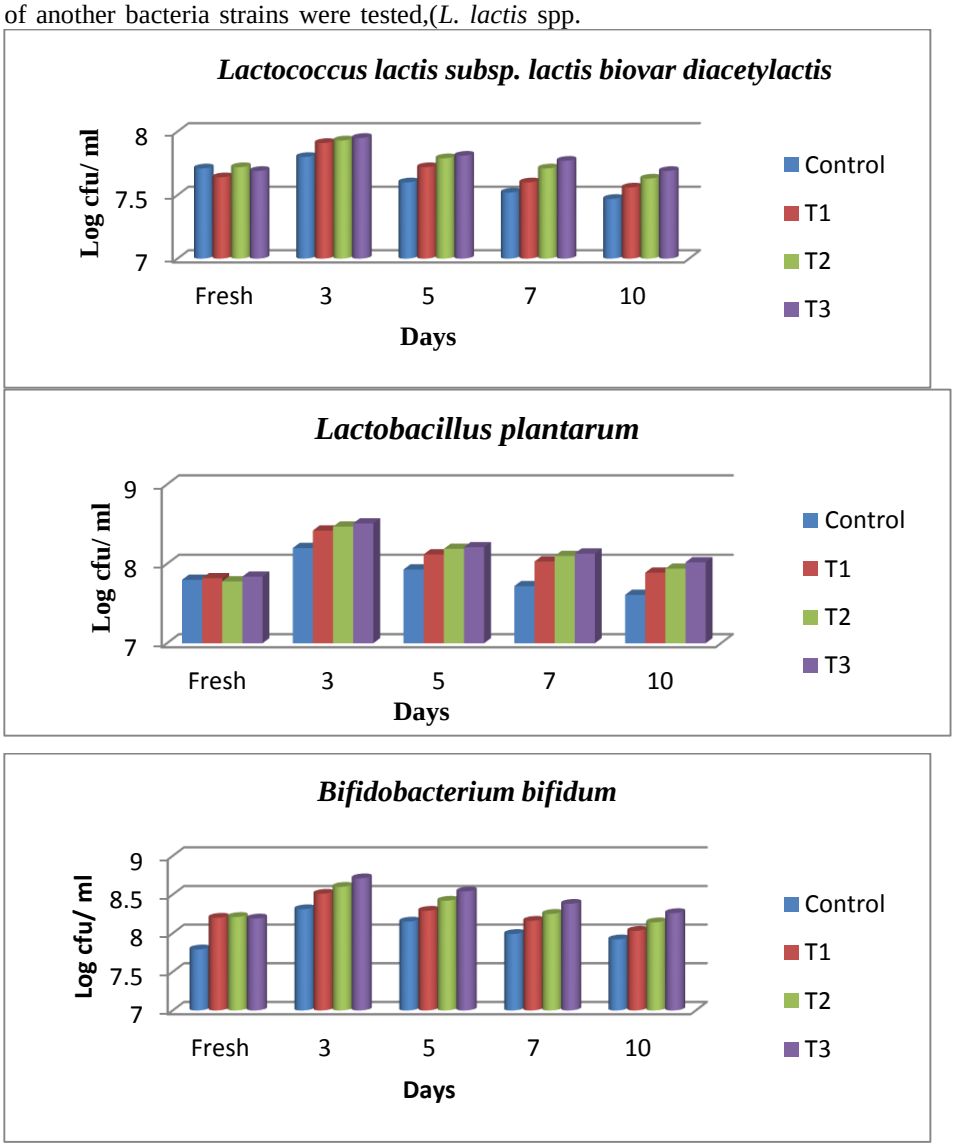


Figure (4): Effect of low cholesterol fermented milk beverage on *L. lactis ssp. diacetylactis*; *B. bifidum* and *L. plantarum*during the storage periods at 8 $\pm$ 1° C

Organoleptic properties:

Table (6) showed that the flavor score of T3 LCFMB was of good flavor and very close to the control both fresh and during storage period. No appreciable differences were obtained for the acidity of the LCFMB was more likely close to the control. Similar results were recorded by Alroubaiya [30].

Prepared LCFMB of high viscosity was noticed when its T2 and T3 which were almost similar to the control treatment of 3% fat. Also, viscosity of the LCFMB and the control were slightly decreased as the storage period increased. The appearance score of the resultant LCFMB was similar for the T2 or T3, which are more or less similar to the control. In general the highest total score of the organoleptic properties resultant LCFMB was for the control followed by 3, 2 and 1% fat LCFMB when fresh and at 3 days of storage. However, the LCFMB of T3 was of higher score than the control one up to the end of storage period.

Table (6): Organoleptic properties of low cholesterol fermented milk beverage during the storage periods at 8 ± 1° C.

Storage period (days)	Treatments	Organoleptic properties				
		Flavor (45)points	Viscosity (30)points	Acidity (10)points	Appearance (15) points	Total (100) points
Fresh	Control	40^{a}	26^{a}	8^{a}	14^{a}	88^{a}
	T1	34^{a}	24^{a}	7^{a}	12^{a}	77^{a}
	T2	39^{a}	25^{a}	6^{a}	13^{a}	83^{a}
	T3	39^{a}	25^{a}	7^{a}	13^{a}	84^{a}
3	Control	42^{a}	24^{a}	8^{a}	13^{a}	87^{a}
	T1	33^{b}	22^{b}	7^{a}	13^{a}	75^{a}
	T2	41^{a}	25^{a}	6^{a}	13^{a}	85^{a}
	T3	40^{a}	25^{a}	8^{a}	13^{a}	86^{a}
5	Control	40^{a}	23^{a}	8^{a}	12^{a}	83^{a}
	T1	33^{b}	21^{a}	6^{b}	13^{a}	73^{a}
	T2	38^{a}	23^{ab}	6^{b}	12^{a}	79^{a}
	T3	40^{a}	24^{a}	8^{a}	13^{a}	85^{a}
7	Control	40^{a}	21^{a}	8^{a}	12^{a}	81^{a}
	T1	32^{b}	21^{a}	5^{b}	13^{a}	71^{a}
	T2	37^{a}	23^{a}	6^{ab}	12^{a}	78^{a}
	T3	39^{a}	22^{a}	7^{ab}	13^{a}	81^{a}
10	Control	39^{a}	22^{a}	7^{b}	12^{a}	80^{a}
	T1	30^{b}	20^{a}	5^{ab}	12^{a}	67^{a}
	T2	36^{a}	22^{a}	6^{ab}	12^{a}	76^{a}
	T3	39^{a}	23^{a}	7^{a}	12^{a}	81^{a}

The same capital letters between columns or rows are not significantly ($p \leq 0.05$).
T1= 1% Low cholesterol fat T2= 2% Low cholesterol fat T3= 3% Low cholesterol fat

CONCLUSION

β -Cyclodextrins can be used for cholesterol removal from milk and dairy products.Nutritional, textural, and flavour characteristics of milk are not considerably affected.

. So, while long-lasting consumption of low-cholesterol milk and dairy products can play a significant role in the decrease of CVD occurrence, production of these products should rise in the next years, which would be in line with current trends of functional foods consumer' interest.

References

[1] Ahmed Mohamed Saied Hussein, Mohamed TawfeekFouad, Mahmoud Abd El-Aziz, Nagah El NoemanyAshour and Esam Ahmed Mohamed Mostafa (2017). Evaluation of Physico-chemical Properties of Some Date Varieties and Yoghurt Made with its Syrups. J. Biol. Sci., 17:213-221.

[2] Abosereh N.A., Salem Abd El Ghani, Rasha S. Gomaa and Mohamed T. Fouad (2016). Molecular Identification of Potential Probiotic Lactic Acid Bacteria Strains Isolated from

Egyptian Traditional Fermented Dairy Products. Biotechnology 15 (1-2): 35-43.

[3] Alonso, L., Cuesta, P. Fontecha, J. Juarez, M.and Gilliland, S. E. (2008). Use of β-cyclodextrin to decrease the level of cholesterol in milk fat. J. Dairy Sci. 92:863–869.

[4] Jensen R. G. and M. Kroger. (2000). The importance of milk and milk products in the diet. Pages 51–52 in Handbook of Dairy Foods and Nutrition. 2nd ed.(ed Miller G. D., Jarvis J. K., McBean L. D.,). CRC Press, Boca Raton, FL.

[5] Akin Zeynep and OzcanTulay. (2017). Functional properties of fermented milk produced with plant proteins.LWT .Volume 86, December , Pages 25-30.

[6] Ghazya, O.A., M.T. Fouad, H.H. Saleha, A.E. Kholif, T.A. Morsy. (2021). Ultrasound-assisted preparation of anise extract nanoemulsion and its. Food Chemistry. 341,1-7.

[7] Jelen P., Gallmann P. and T. Coolbear (2003). Current and future applications of fermentation technology in the dairy industry. Page 10 in Proc. IDF Seminar on Aroma and Texture of Fermented Milk, Kolding, Denmark. International Dairy Federation, Brussels, Belgium

[8] Valli C. and W.B. Traill (2005), Culture and food: A model of yoghurt consumption in the EU. Food Qual. Pref., 16:291–304.

[9] Fouad M.T, AmrMoustafa, Laila Hussein, RamyRomeilah, Mostafa Gouda. (2015). *In-Vitro* Antioxidant and Antimicrobial Activities of Selected Fruit and Vegetable Juices and Fermented Dairy Products Commonly Consumed in Egypt. Research Journal of Pharmaceutical, Biological and Chemical Sciences 6 (2), 541-550.

[10] Lourens-Hattingz A. and B. Viljoen (2001). Yoghurt as probiotic carrierfood. Int. Dairy J. 11:1–17.

[11] Mattila-Sandholm, T., P. Myllarinen, R. Crittenden, G. Mogensen, R. Fonden and M. Saarela (2002). Technological challenges for future probiotic foods. Int. Dairy J., 12: 173–182.

[12] KhorshidianNasim ;MojtabaYousefi and Amir M.Mortazavian.(2020). Probiotic and Prebiotics in Foods: Challenges, Innovations and Advances. Advances in Food and Nutrition Research.Volume 94, Pages 91-114

[13] Boyd, N. F and V. Mcguira (1990). Evidence of association between plasma high-density lipoprotein cholesterol and risk factors for breast cancer. J. of the Nat. Cancer Instit. 82(6)460.

[14] Oakenfull , D.G.; R.J. Pearce and M.L. Rooney (1991). Cholesterol removal. PCT Intern. Patent Appli.; WO 91/ 16824. Cited in DSA 54 (10) 6803.

[15] Peter, S. (1993). Effective removal of cholesterol from butter fat. A paper presented at the 1993 Conference on Fat and Cholesterol Reduced Foods, Florida.

[16] Abd El-Malek , F. A. (1999). Studies on improving some technological and nutritional properties of milkfat. Ph.D. Thesis, Dairy Science and Technology . Fac. Agric., Ain Shams Univ.

[17] A.O.A.C. (2012). Official Methods of Analysis. Association Official Analytical Chemists,19th Ed. Washington, D C, USA.

[18] Pantulu,P.C.; M.B. Rao.; S.D. Ro and C.P. Anatakrishman (1975). Application of liebermannburchard reaction to the unsaponifiable protein of milk lipids for the determination of cholesterol in milk and milk products. MilchWissenschaft 3rd 12:735.

[19] Less, G .J and G . R. Jago (1969). Methods for the estimation of acetaldehyde in cultured Dairy products. Australian J .of Dairy Technology. 12:181-185.

[20] Less, G .J and G . R. Jago (1970)The estimation of diacetyle in the presence of other carbonyl compounds. J. Dairy Res . 37:129.

[21] El-Kholy, W., Azzat B. Abd El-Khalek, Sahar H.S. Mohamed, Mohamed T. Fouad and Jihan M. Kassem (2016). Tallaga Cheese as a New Functional Dairy Product. American J. Food Technology, 11(5):182-192.

[22] El-ShenawyMoustafa, Mohamed T. Fouad, Laila Hassan, FatenSeleet and Mahmoud Abd El-Aziz (2019). A probiotic beverage made from Tiger-nut extract and milk permeate. Pakistan Journal of Biological Sciences. 22(4): 180-187.

[23] El-Shenawy, Moustafa, Mahmoud Abd El-Aziz, WagihElkholy and Mohamed T. Fouad (2016). Probiotic Ice Cream Made with Tiger-nut (Cyperusesculentus) Extract. American J. Food Technology, 11(5):204-212.

[24] El-Shenawy, Moustafa A., Rashed A. Zaghloul, Ibrahim H. Abbass, Amira I. Esmail and Mohamed T. Fouad (2016). Incidence of some epidemiologically relevant food-borne pathogens in street-vended sandwiches. Journal of Pharmaceutical, Biological and Chemical Sciences 7 (2), 468-474.

[25] Hussein H. A. & Mohammed T. Fouad&Khaled A. AbdEl-Razik&Amal M. Abo El-Maaty& C. D'Ambrosio4 & A. Scaloni& A. M. Gomaa. (2020). Study on prevalence and bacterial etiology of mastitis, and effects of subclinical mastitis and stage of lactation on SCC in dairy goats in Egypt. 52:3091–3097.

[26] Abbas, H. M., Nadia M. Shahein, Nabil S. Abd-Rabou, Mohammed T. Fouad and Wafaa M. Zaky (2017). Probiotic-fermented Milk

Supplemented with Rice Bran Oil. International Journal of Dairy Science, 12, 204-2010.

[27] Mohamed Doha A., Minar Mahmoud Hassanein, Tamer M. El-Messery, Mohamed T. Fouad, Marwa M. El-Said, KaremFouda and Adel Gabr Abdel-Razek. (2017). Amelioration of Type 2 Diabetes Mellitus by Yoghurt Supplemented with Probiotics and Olive Pomace Extract. J. Biol. Sci., 17:320-333.

[28] SAS (1994). Statistical Analyses System. SAS User's. Statistics. SAS institute Inc.1994. Editors, Cary, NC.

[29] Tamime, A .Y and R . K. Robinson (1999). Yoghurt science and technology .Cambridge , GB : CRC press, Woodhead publishing Ltd .

[30] Alroubaiya, K.A.S (2004). Studies on some factors affecting the rheological, structural and organoleptical properties of stirred yoghourt. M.Sc. Thesis, Dairy Science and Technology, Fac. Agric., Cairo Univ.

[31] Abou-Dawood, A. E. and S. M. Abdou. (1973). The effect of adding sodium citrate to skimmilk on quality, chemical changes and yield of the cheese (Kareish) during pickling. Egyptian J. Dairy Sci. 1:141-147.

[32] Ibrahim, S.A.; N.A. EL-Batawy and S.A. Fikry (1990).Utilization of butter milk in making Kareish cheese. J.Dairy Sci.18:95-105.

[33] Salem,O.M.; A.J.Hamed.; Y.K.M.K.Kebary and A.S. El Sisi (1997) Influence of attenuated lactococcion the quality of Kareish cheese made by direct acidification. Egyptain J. Dairy Sci., 25:253-268.

[34] Ghalab, H. M; N. M. Hanafy and A. A. EL-Ghandour (1998). Bacterial cholesterol assimilation .Proc 7 th Egyptian Conf. Dairy Sci and Techn., 250-260.

[35] Tamime, A. Y and H.C. Death (1980). Yoghurt: technology and biochemistry. J. Food Prot,. 43 (12): 939.

[36] Abdel Salam, M. H.; H. H. M. EL Etriby and N. M. Shabein. (1996). Influence of some stabilizers on some chemical and physical properties of yoghurt. J. Dairy Sci . 24 (1) 25-35.

[37] Rosic, J. and J.A. Kurmann (1978). Yoghurt. Published by the Authors. Denmark.

[38] Mehanna, A.S. and S.A. Hefnawy (1990). A study to follow the chemical changes during processing and storage of zabady. Egyptain J. Dairy Sci. 18(2):425-434.

[39] Rohm, H.; A. Kovac and W. Kneifel (1994). Effects of starter cultures on sensory properties of set-style yoghourt determined by quantitative descriptive analysis. Journal of Sensory Studies. 9(2):171-186.

[40] Salama, F. M.M. (2002). Production of therapeutic and diabetic stirred yoghurt-like fermented milk products. Egyptian J. Dairy Sci.; 30:177-190.

[41] Zedan, M.A.; F.M.M. Salama.; S.M.K Anis and A.M. Seiha (2003). The affect of stabilizers and sodium citrate on properties of Rayeb milk manufactured by combined culture of mesophilic/freeze shocked *lactobacillus helveticus* .Egyptain J. Dairy Sci 31 (2) 259-271.

[42] Tamime , A.Y and R . K. Robinson (1985). Yoghurt Science and Technology. Pergamon Press and Oxford, Engl.

[43] Youssef, Y.B; M.H. El-Senaity and N.S Abd-Rabou (1998).Manufactured of cultured buttermilk based on cows milk and mesophilic starters. J. Agric. Sci., Mansoura University, 23(5), 2119-2126.

[44] EL Senaity, M .H. (1999). The composition and quality of cultured buffalo butter milk fermented with mesophilic bacteria. Egyptian J. dairy Sci 27 (2): 269-280.

[45] Magdoub M.N.I., M.E. Fayed, A.A. Zidain and A.A.K. Abou-arab (1993). Milk preparedness for Bacterial prowing in relation to environmental contaminants. 4th Conf. Agric. Dev. Res, Ain-Shams Univ., Cairo, Feb.13-18. Annals Agric. Sci., issue, 1, 97-110.

[46] Ishibashi N. and S. Shimanura (1993). Bifidobacteria: research and development in Japan.Food Technol. 1993,47: 126

[47] Lopez de Felipe F., M. Kleerebezem, W.M. de Vos and J. Hugenholtz. (1998). Cofactor engineering: a novel approach to metabolic engineering in *Lactococcus lactis* by controlled expression of NADH oxidase. J. Bacteriol. 180: 3804–3808.