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MANUFACTURE OF FERMENTED NUTRACEUTICAL MILK PRODUCTS HIGHLY IN SOLUBLE DIETARY FIBER

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ABSTRACT

This study was undertaken to evaluate the feasibility of using soluble dietary fiber and probiotic bacteria to produce nutraceutical fermented cow milk. Fresh cow milk without addition served as a control (T1). β- glucan (T2) or glucomannan (T3) was added to fresh cow milk (30mg/L). All treatments were heated at 85°C /10min, cooled to 45°C. Each treatment inoculated with yoghurt starter culture (Yo) or *Lb. acidophilus* (A) or *Lb. helveticus* (H) at a level of 3% and incubated at 42°C. After fermentation all samples were cooled and keep at 4°C for 1 day, yoghurt mixed with fermented milk contains *Lb. acidophilus* or/ and *Lb. helveticus* as well as *Lb. acidophilus* fermented milk mixed with *Lb. helveticus* at ratio 1:1 and 1:1:1. Titratable acidity, microbiological analysis and sensory evaluation were determined in all samples. Incubation time was different among all treatments to reach the titratable acidity 0.7%, where it was lower in control than other treatments. Titratable acidity of fermented milk was affected by adding soluble dietary fiber, where fermented milk with β- glucan (T2) or glucomannan (T3) had lower acidity than fermented milk without dietary fiber (T1). Types of starter cultures had no significant effect on the titratable acidity of fermented milk. Addition of β- glucan or glucomannan decreased total lactic acid bacterial counts (TLAB) as compared with control. Treatments of Yo, A and H resulted in significant lower TLAB count than mixed treatments. Spore forming counts in T2 and T3 treatments were significantly lower than control. Addition of β-glucan or glucomannan improved the body and texture, compared to the control, particularly, Yo+H and Yo+A+H.

Keywords*: soluble dietary fiber; starter cultures; β-glucans; Glucomannan*

INTRODUCTION

Fermented milk products containing probiotic bacteria are some of the most popular fermented food products in the world. It has numerous health benefits due to the functional properties of their viable microorganisms and a health claim for fermented milk which allowed towards improved lactose digestion for individuals with lactose maldigestion (EFSA, 2010). Probiotics have been defined as “Live microorganisms which when administered in adequate amounts confer a health benefit to the host” by improving their intestinal microbial balance. Probiotic bacteria have been shown to provide several therapeutic benefits such as modification of the

immune system, alleviation from lactose intolerance, maintained remission of Crohn's disease, faster relief from diarrhea, and prevention of urogenital infections (Reid, 1999; Reid et al., 2003 a,b), decrease in levels of blood lipids, nutrients synthesis and their bioavailability enhancement and prevention of cancer and mutation activities in the human gut (Noh et al., 1997; Gorbach, 2000; Kailasapathy and Chin, 2000; Cremonini et al., 2001; Kopp-Hoolihan, 2001; Femia et al., 2002; Rafter, 2002 and Kim et al., 2008). Soluble dietary fibers are present in small quantities in almost each and every commodity and in combination with insoluble dietary fiber contribute towards total dietary fiber. The beneficial properties of soluble dietary fibers have been associated with their significant role in human physiological function. It includes, reductions in cholesterol level and blood pressure, prevention of gastrointestinal problems, protection against onset of several cancers, which include colorectal, prostate, and breast cancer, increased mineral bioavailability, and many more are the salient features of their potential (Chawla and Patil 2010).

As active compounds of the soluble dietary fiber, β-glucans are naturally-occurring polysaccharides found in the cell walls of yeast, fungi, bacteria and cereal plants such as oat, barley (Akramiene et al. 2007, Chen and Seviour, 2007).β-Glucans are a diverse class of long-chain glucose polymers that have a backbone of β-(1-3)-linked β-D glucopyranosyl units with primarily β-(1,4)- or (1,6)- linked side chains. These naturally occurring substances have been shown to provide health benefits including enhancing the bio-defense activity against bacterial, viral, fungal and parasitic challenge, increasing hematopoiesis and radioprotection, stimulating the wound healing response, stimulating the immune mechanisms of the host and have also antimicrobial effects, decreasing blood lipid concentration, reduce the risk of coronary and ischemic heart diseases, protection against infection, inhibition of tumor development, and promotion of tumor regression (Delatte et al. 2001; Hong et al. 2004; Maki et al. 2007; Yoon et al. 2008; Driscoll et al. 2009; Shah et al. 2009 and Asano et al. 2012). *S. cerevisiae* β -glucan showed protective effects against genotoxicity and cytotoxicity of some drugs, such as cyclophosphamide, adriamycin and cisplatin. Such effects have been attributed to the ability of β -glucan to trap free radicals produced in the course of biotransformation of these drugs (Tohamy et al., 2003). In addition, it has been demonstrated that β -glucan-containing products are potent antioxidants, preventing damage caused by H_2O_2 and other reactive oxygen species (Laugier et al. 2012).

Mannan polysaccharides are widespread in nature. They are considered to be one of the major components of hemicellulose in the cell walls of plants (Moreira and Filho, 2008). Microbes are a rich source of mannans, so it called ‘mannan oligosaccharides’ which are derived from the outer layer of the yeast cell walls especially from *Saccharomyces cerevisiae*. Mannans represent carbohydrates, especially polysaccharides, that contain mannose (sugar) residues. They can be divided into four types; mannan, galactomannan, glucomannan and galactoglucomannan (Tester and Al-Gazzewi, 2013). Mannan oligosaccharides are often promoted as an alternative to antibiotics

in the animal feed industry, where mannose may prevent attachment of the intestinal pathogens such as *Salmonella* species and *Escherichia coli* to the gut mucosa (Benites et al. 2008). Also mannan oligosaccharides from yeast cells had a significant impact with respect to change the bacterial ecology in the gut by stimulating the immune system (Miguel et al. 2004; Bozkurt et al. 2009; Corrigan et al. 2011)).

The objective of this study is to measure and compare the sensory and microbiological characteristics of fermented nutraceutical milk products containing β-glucans and glucomannan.

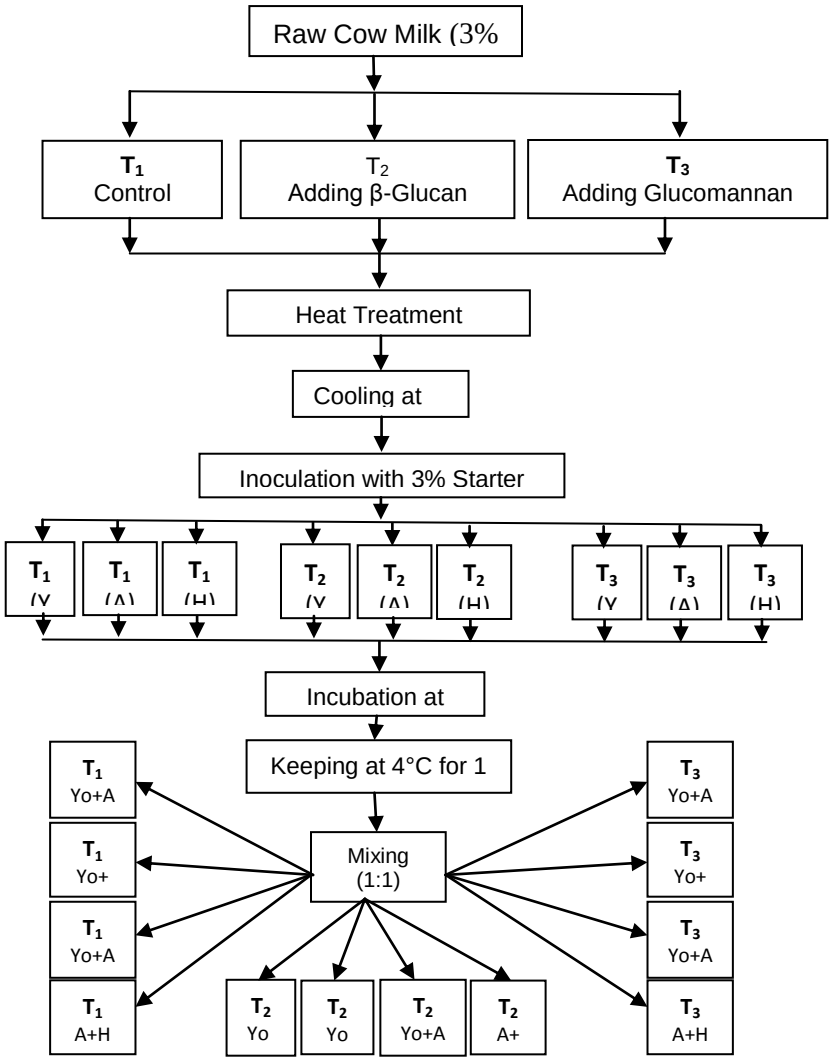
MATERIALS AND METHODS

Fresh raw cows milk was obtained from the herd of the Faculty of Agriculture, Cairo University. Giza,Egypt. Yoghurt cultures YC-fast-1(*Str.thermophilus, Lb.bulgaricus)*, *Lactobacillus acidophilus* La – 5 and *Lactobacillus helveticus* Lh. BO2 were obtained from Chr. Hansens Laboratories, Copenhagen Denmark. Violet red bile glucose agar, oxytetracycline – glucose -yeast extract agar (OGYE Agar), plate count agar (tryptone glucose yeast agar) medium were obtained from Oxoid Ltd., Basingstoke, Hampshire, England. β-glucan and glucomannan were obtained from Alltech Company, USA.

Fresh cow milk (18 kg, 3% fat) was divided into three equal parts. The first part of milk served as a control, while the β-glucan (30mg/L) was added to the second part and glucomannan (30mg/L) was added to the third part of milk. All parts were heated at 85^{o}C for 10 min, cooled to 45^{o}C. Each part of milk was subdivided into three equal portions, the first portion inoculated with the yoghurt starter culture. The second portion inoculated with the *L. acidophilus.* The third portion was inoculated with the *L. helveticus.* The inoculation rate was 3% (w/w) for all cultures. The inoculated milk was transferred into polystyrene plastic cups and incubated at 42^{o}C. Once fermentation was completed (acidity reached 0.7%) all samples were cooled and kept at 4^{o}C until the second day. Yoghurt was mixed with fermented milk containing *Lb. acidophilus* or/ and *Lb. helveticus* at ratio 1:1or 1:1:1. Also fermented milk containing *Lb. acidophilus* mixed with *L b. helveticus* at the ratio of 1:1 (Fig. 1). Titratable acidity, microbiological analysis and sensory evaluation were determined in all samples when fresh.

Titratable acidity, fat, total protein (T.P), ash and total solids (T.S) of fermented milk samples were determined according to AOAC (2012).

Fermented milk samples were kept in the refrigerator approximately 1 day before the microbiological analysis. Total lactic acid bacterial count (TLAB) were estimated using MRS agar medium as recommended by the American Public Health Association (APHA, 2004), plates were incubated at 37^{o}C for 48hr. The count of aerobic spore forming bacteria was carried out as described by Luck, 1981, plates were incubated at 32^{o}C for 48hr. Coliform counts were enumerated using violet red bile glucose agar medium, as reported by APHA, 2004, plates were incubated at 37^{o}C for 24hr. Moulds and yeasts were enumerated using oxytetracycline-glucose-yeast extract agar (OGYE Agar) medium according to IDF, 1990, plates were incubated at 25^{o}C for 5 days.



Fig(1):Schematic representation of the process used in making the fermented nutraceutical milk products

The fermented milk samples were judged by the staff members of the Dairy Science Department, Faculty of Agriculture, Cairo University and Dairy Science &Technology Department, Animal Production Research Institute, Agriculture Research Center, Ministry of Agriculture. The fermented milk samples were organoleptically scored for Flavor (45 points), body and texture (30 points), acidity (10 points) and appearance (15 points) with a total acceptance of 100 points, as mentioned by El-Senaity (1999).

The experiments were repeated in triplicates and each analysis duplicates, and the average results were recorded. All statistical analysis was

carried out using SPSS- 21. Overall effects of treatments were analyzed conducting a 2-ways ANOVA; statistically different groups were determined by Duncon test ($P \leq 0.05$).

RESULTS AND DISCUSSION

Chemical composition of all fermented milk samples was almost the same, where it contained 3.67% fat, 3.58% total protein, 0.77% ash and 12.50% total solids.

As can be observed from the results in Table (1) and Fig. (2), the incubation time needed to reach the titratable acidity of 0.7% was different among all treatments. The times were 3hr, 8hr and 5hr for yoghurt starter culture (Yo), *Lb. acidophilus* (A) and *Lb. helveticus* (H) in control treatment (T1), respectively. While the times were 4hr, 18hr and 6hr for Yo, A and H in β-glucan treatment (T2). The same results were obtained for glucomannan treatment (T3). These results might be due to β-glucan or glucomannan are considered as antibacterial agents (El-Dieb et al. 2014). After fermentation in all samples were kept at 4°C for 1 day then the treatments of Yo+A, Yo+H, Yo+A+H and A+H were prepared by mixing as shown in Fig (1). This technique was followed to avoid the competition of different starter cultures used. As can be observed from the results in Table (1) or Fig (2), titratable acidity of fermented milk was affected by adding soluble dietary fiber, where fermented milk with β- glucan (T2) or glucomannan (T3) had lower acidity than fermented milk without dietary fiber (T1). The same data in Table (1) or Fig. (2), showed that the starter cultures type had no significant effect on the development of the titratable acidity of fermented milk after 1 day of storage at 4°C.

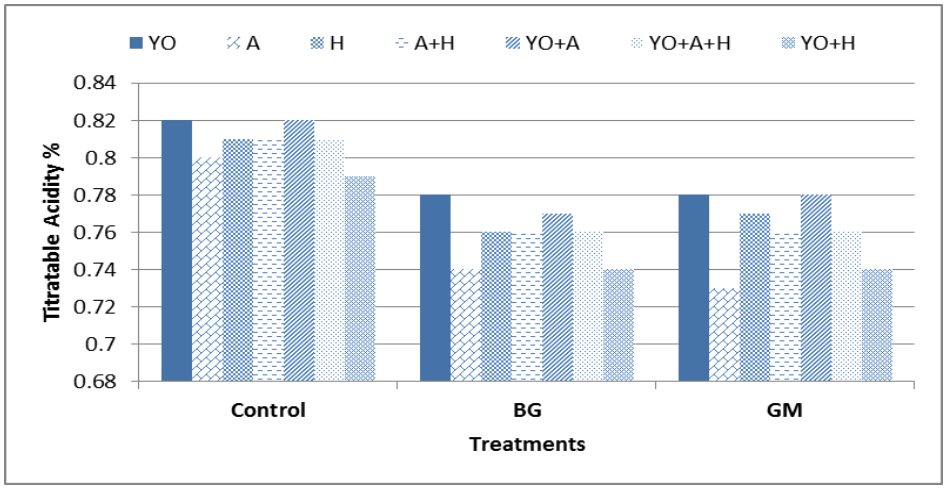


Fig. (2):Titratable acidity of fermented nutraceutical milk products made with soluble dietary fiber after 1 day keeping at 4°C.

The different capital letters have a significant effect between treatments at the level 0.05 and the different small letters have a significant effect for starter culture at the level 0.05**.**

Table(1):Incubation time and titratable acidity of fermented nutraceutical milk products made with soluble dietary fiber.

Treatments	Starter culture	Incubation time	*Acidity %
(T 1) Control	Yo	3	0.820 ^{Aa}
	A	8	0.800 ^{Aa}
	H	5	0.810 ^{Aa}
	**Yo+A	-	0.810 ^{Aa}
	**Yo+H	-	0.820 ^{Aa}
	**Yo+A+H	-	0.810 ^{Aa}
	**A+H	-	0.790 ^{Aa}
(T 2) With adding β-glucan	Yo	4	0.780 ^{Ba}
	A	18	0.740 ^{Ba}
	H	6	0.760 ^{Ba}
	**Yo+A	-	0.760 ^{Ba}
	**Yo+H	-	0.770 ^{Ba}
	**Yo+A+H	-	0.760 ^{Ba}
	**A+H	-	0.740 ^{Ba}
(T 3) With adding glucomannan	Yo	4	0.780 ^{Ba}
	A	18	0.730 ^{Ba}
	H	6	0.770 ^{Ba}
	**Yo+A	-	0.760 ^{Ba}
	**Yo+H	-	0.780 ^{Ba}
	**Yo+A+H	-	0.760 ^{Ba}
	**A+H	-	0.740 ^{Ba}

Yo: Yoghurt starter cultures, A: *Lb. acidophilus* H: *Lb. helveticus*
***Acidity was determined after 1 day kept at 4^{o}C.**
****these treatments were prepared by mixing treatment Yo, A or H after fermentation.**

The microbiological analysis were performed after fermentation and keeping at 4^{o}C for 1 day. Mould and yeast were not detected in all treatments (data not mentioned). This might be attributed to follow very good hygienic condition during experimental manufacture and handling. As shown in Table (2), a significant difference ($P < 0.05$) was observed for total lactic acid bacterial counts (TLAB) between all treatments. The addition of β-glucan or glucomannan decreased the TLAB counts, as compared with control. These results might be due to anti microbiological effect of β- glucan and glucomannan. Regarding the type of starter cultures, treatments of Yo, A and H resulted in a significant lower TLAB than other treatments. TLAB ranged from 6.102 - 7.816 $\log_{10}$ cfu/ml in all treatments. The obtained results from the same Table (2) showed that spore forming counts were significantly affected by adding dietary fiber. Spore forming counts in treatment T2 and T3 were significantly lower, where it ranged from 0.150 - 0.849 $\log_{10}$ cfu/ml than control (T1) ranged from 1.500- 1.977 $\log_{10}$ cfu/ml.

Table (2): Lactic acid bacteria and spore forming bacterial counts (Log $_{10}$ cfu/ml) of fermented nutraceutical milk products made with soluble dietary fiber.

Treatments	Starter culture	Total Lactic Acid Bacteria	Spore forming Bacterial Count
(T 1) Control	Yo	7.166^{Abc}	1.650^{Aa}
	A	6.845^{Ac}	1.977^{Aa}
	H	6.871^{Ac}	1.738^{Aa}
	A+H	7.397^{Abc}	1.650^{Aa}
	Yo+A	7.418^{Aab}	1.801^{Aa}
	Yo+H	7.626^{Aab}	1.500^{Aa}
	Yo+A+H	7.816^{Aa}	1.650^{Aa}
(T 2) With adding β-glucan	Yo	6.544^{Bbc}	0.500^{Ba}
	A	6.127^{Bc}	0.150^{Ba}
	H	6.161^{Bc}	0.588^{Ba}
	A+H	6.390^{Bbc}	0.588^{Ba}
	Yo+A	7.323^{Bab}	0.698^{Ba}
	Yo+H	7.544^{Bab}	0.500^{Ba}
	Yo+A+H	7.698^{Ba}	0.801^{Ba}
(T 3) With adding glucomannan	Yo	6.968^{Bbc}	0.849^{Ba}
	A	6.102^{Bc}	0.150^{Ba}
	H	6.306^{Bc}	0.650^{Ba}
	A+H	6.422^{Bbc}	0.650^{Ba}
	Yo+A	6.836^{Bab}	0.500^{Ba}
	Yo+H	7.067^{Bab}	0.738^{Ba}
	Yo+A+H	7.457^{Ba}	0.738^{Ba}

Yo: Yoghurt starter, A: *Lb. acidophilus*, H: *Lb. helveticus*
The different capital letters have a significant effect between treatments at the level 0.05
The different small letters have a significant effect for starter culture at the level 0.05.

The results of sensory evaluation of the different treatments are presented in Table (3). It appears that the addition of β- glucan or glucomannan didn't affect significantly flavor scores comparing with the control. On the other hand, the effect of starter cultures kind on flavor was more pronounced. The samples of Yo+H was the highest, while the samples of Yo+A, A and H were the lowest for flavor score. The flavor score for other treatments ranged between (36.200 - 38.750).

From the results in Table (3), it could be observed that using β-glucan and glucomannan improved significantly body and texture compared to the control. This might be attributed to the presence of β- glucan which is used as thickening, water holding, emulsifying stabilizer or oil- binding agent (Thammakiti et al. 2004). The sample Yo+H in all treatments had the highest score (27), while the sample Yo+A had the lowest (19) scores for body and texture than the other samples.

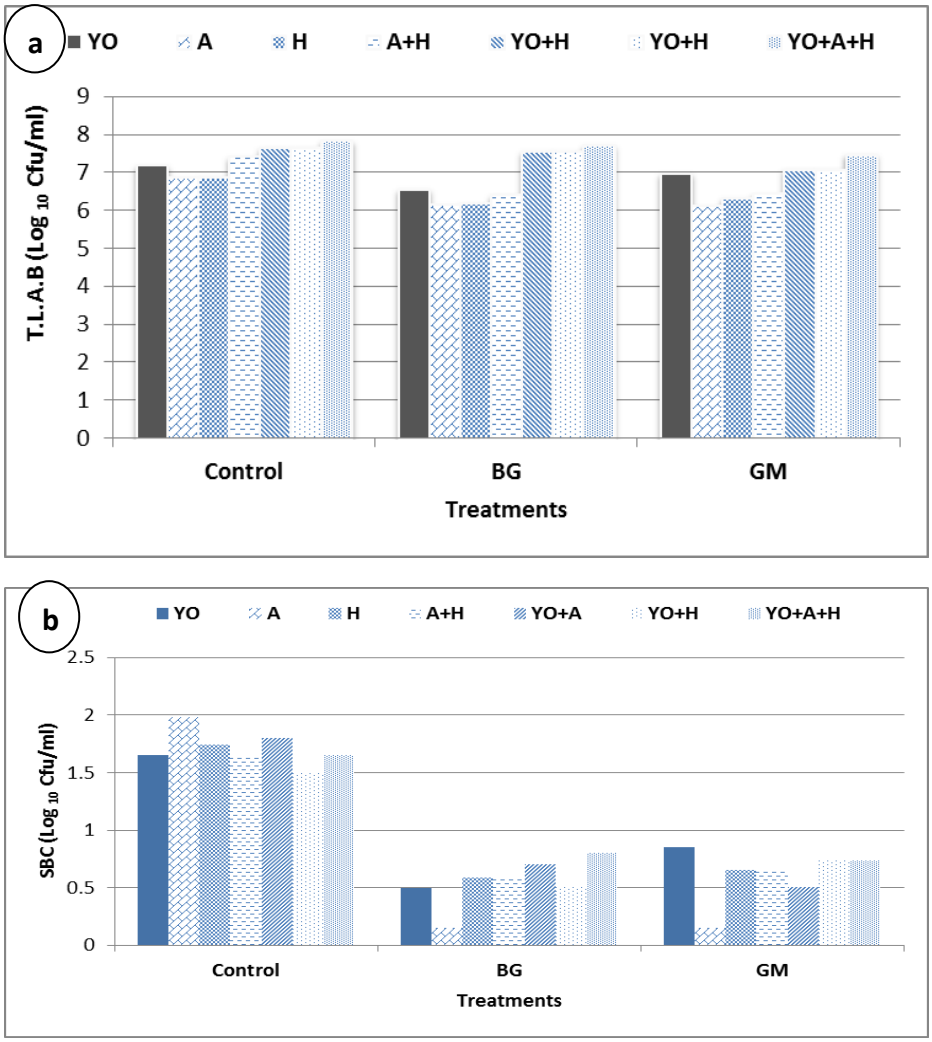


Fig. (3):Lactic acid bacteria (a) and spore forming bacterial counts (b)(Log $_{10}$ cfu/ml) of fermented nutraceutical milk products made with soluble dietary fiber.

All samples had almost similar scores for acidity, which ranged from (7.000 - 8.250). There were no significant differences ($P \leq 0.05$) in appearance values among treatments and starter cultures. Where all samples had almost similar scores ranging from 12.870 -13.800.

From the results of the same Table (3), treatments β- glucan were more superior, followed by control and glucomannan treatments depending on total scores. It related to starter cultures type, Yo+H gained the higher total scores than other samples. Fermented milk made using β- glucan and culture starter Yo+H gained the highest score (91.000), followed by Yo+A+H which gained 88.437.

Table (3): Sensory evaluation of fermented nutraceutical milk products made with soluble dietary fiber.

Treatments	Starter culture	Flavour (45)	Body and texture (30)	Acidity% (10)	Appearance (15)	Total score (100)
(T 1) Control	Yo	37.800 ^{Abc}	23.500 ^{Cab}	7.400 ^{Ab}	13.800 ^{Aa}	82.500 ^{Bbc}
	A	34.900 ^{Acd}	20.400 ^{Cb}	7.000 ^{Ac}	13.600 ^{Aa}	75.900 ^{Bcd}
	H	31.600 ^{Acd}	19.900 ^{Cb}	7.500 ^{Ab}	13.300 ^{Aa}	72.300 ^{Bd}
	Yo+A	34.600 ^{Ad}	20.400 ^{Cc}	7.100 ^{Ac}	13.000 ^{Aa}	75.100 ^{Be}
	Yo+H	40.900 ^{Aa}	25.200 ^{Ca}	7.400 ^{Ab}	13.000 ^{Aa}	86.500 ^{Ba}
	Yo+A+H	38.600 ^{Aab}	21.100 ^{Cab}	7.700 ^{Aa}	13.500 ^{Aa}	80.900 ^{Bab}
	A+H	36.200 ^{Acd}	22.400 ^{Cab}	7.300 ^{Ac}	13.200 ^{Aa}	79.100 ^{Bd}
(T 2) With adding β-glucan	Yo	37.800 ^{Abc}	26.750 ^{Aab}	7.500 ^{Ab}	13.625 ^{Aa}	85.675 ^{Abc}
	A	33.875 ^{Acd}	24.000 ^{Ab}	7.375 ^{Ac}	13.125 ^{Aa}	77.375 ^{Acd}
	H	36.875 ^{Acd}	24.875 ^{Ab}	7.000 ^{Ac}	12.870 ^{Aa}	81.620 ^{Ad}
	Yo+A	36.500 ^{Ad}	24.937 ^{Ac}	7.812 ^{Aa}	13.125 ^{Aa}	82.375 ^{Ae}
	Yo+H	41.875 ^{Aa}	27.000 ^{Aa}	8.250 ^{Aa}	13.875 ^{Aa}	91.000 ^{Aa}
	Yo+A+H	40.125 ^{Aab}	27.250 ^{Aab}	7.687 ^{Aab}	13.375 ^{Aa}	88.437 ^{Aab}
	A+H	38.625 ^{Acd}	27.000 ^{Aab}	7.750 ^{Aab}	13.125 ^{Aa}	86.500 ^{Ad}
(T 3) With adding glucomannan	Yo	37.100 ^{Abc}	23.100 ^{Bab}	7.150 ^{Ac}	13.600 ^{Aa}	80.950 ^{Bbc}
	A	34.600 ^{Acd}	26.600 ^{Bb}	8.000 ^{Aa}	13.100 ^{Aa}	82.300 ^{Bcd}
	H	32.200 ^{Acd}	26.2000 ^{Bb}	7.800 ^{Aa}	13.000 ^{Aa}	79.200 ^{Bd}
	Yo+A	33.875 ^{Ad}	19.0000 ^{Bc}	7.600 ^{Aab}	13.100 ^{Aa}	73.575 ^{Be}
	Yo+H	39.300 ^{Aa}	25.7000 ^{Ba}	8.1500 ^{Aa}	13.600 ^{Aa}	86.750 ^{Ba}
	Yo+A+H	38.750 ^{Aab}	27.750 ^{Bab}	7.5000 ^{Ab}	13.000 ^{Aa}	87.000 ^{Bab}
	A+H	36.900 ^{Acd}	25.00 ^{Bab}	7.200 ^{Ac}	13.00 ^{Aa}	83.100 ^{Bd}

Yo: Yoghurt starter, A: *Lb. acidophilus*, H: *Lb. helveticus*
The different capital letters have a significant effect between treatments at the level 0.05
The different small letters have a significant effect for starter culture at the level 0.05.

Conclusion

Regarding the forementioned results, it could be concluded that β-glucan addition extended the incubation time and decreased TLAB, enhanced the sensory properties of fermented milk, comparing with glucomannan as well as control. Also, by mixing different fermented milk containing probiotic cultures with yoghurt cultures, it could develop the desired texture, flavour, and aroma of final product.

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تصنيع ألبان متخمرة داعمة للحيوية مدعمة بالألياف الغذائية الذائبة

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تهدف الدراسة الى إمكانية إستخدام الألياف الغذائية الذائبة والبكتريا الداعمة للحيوية فى إنتاج ألبان متخمرة حيوية.

تم فى هذه الدراسة إستخدام لبن بقرى٣% دهن بدون إضافات (عينة مقارنة T1) وإضافة البيتاجلوكان (T2)أو الجلوكومنان (T3) بمعدل (30mg/L) .جميع المعاملات تم معاملتها حراريا على ٨٠°م لمدة ١٠ دقائق ثم التبريد إلى ٤٥°م. داخل كل معاملة تم التلقيح ببادىء الزبادى (Yo) أو *Lactobacillus acidophilus* (A) أو *Lb. helveticus* (H) بمعدل ٣% . ثم التحضين على ٤٢°م حتى تمام التجبن ثم التبريد على ٤°م. تم خلط معاملة Yo مع معاملة A (A + Yo) أو معاملة H (Yo+ H) أو الثلاثة معا (Yo+ A+ H) أيضا تم خلط معاملة A مع معاملة

H (A + H) بنسبة ١:١ أو ١:١:١ . تم تقدير كل من الحموضة والجودة الميكروبيولوجية والتقييم الحسى لكل المعاملات وهى طازجة.

وكانت أهم النتائج المتحصل عليها ما يلى:-

- زاد زمن التجبن بإضافة كل من البيتاجلوكان والجلوكومنان، كما تأثر زمن التجبن بنوع البادىء حيث كانت معاملة T1 Yo أسرع فى زمن التجبن (٣ ساعات) و T1 A (٨ ساعات) و T1 H (٥ ساعات) مقارنة ب T3،T2.
- إحتوت معاملات البيتاجلوكان والجلوكومنان على أعداد أقل معنويا من بكتريا حامض اللاكتيك الكلية مقارنة بعينة المقارنة.
- إحتوت معاملات اللبن المتخمر بالبادئات الفردية على أعداد أقل معنويا من بكتريا حامض اللاكتيك الكلية مقارنة بالعينات المختلطة.
- إحتوت معاملات البيتاجلوكان و الجلوكومنان على أعداد أقل معنويا من البكتريا المتجرثمة مقارنة بعينة المقارنة.
- إضافة البيتاجلوكان والجلوكومنان حسنت من قوام وتركيب اللبن المتخمر مقارنة بعينة المقارنة وكانت أفضل العينات من الناحية الحسية هى المحتوية على البادئات المختلطة (Yo+A+H ، Yo+H) .

لذلك توصى الدراسة بإمكانية إنتاج منتج لبنى متخمر يحتوى على الألياف الذائبة لرفع قيمته التغذوية والصحية .

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