International Food Research Journal 24(5): 2259-2264 (October 2017)

Journal homepage: *http://www.ifrj.upm.edu.my*

Mannan extract from *Saccharomyces cerevisiae* used as prebiotic in bio-yogurt production from buffalo milk

Al-Manhel, A. J. and *Niamah, A. K.

Department of Food Science, Agriculture College, Basrah University, Basra City, 61004, Iraq

Article history

Received: 19 July 2016
Received in revised form: 16 August 2016
Accepted: 1 September 2016

Keywords

Saccharomyces cerevisiae
Mannan
Prebiotic
Bio-yogurt

Abstract

Four strains of the dry yeast *Saccharomyces cerevisiae* were obtained from different sources. The identification of yeasts was confirmed by morphological and physiological properties. Mannan extracts from yeast cell walls ranged between 25.80-34.40% of the dry weight of yeast cells. Five different concentrations of mannan extract were mixed with buffalo milk, to be used for bio-yogurt production. Probiotic starter culture contained *Lactobacillus acidophilus, Bifidobacterium* sp. and *Streptococcus thermophilus* used. After fermentation, pH values decreased to 4.6-4.8, while total acidity was 0.89-1%. The cells viability of *Lactobacillus acidophilus* was 6.8-7.85 Log CFU/g, 5.8-7.15 Log CFU/g of *Bifidobacterium* sp. and 6.74-7.22 Log CFU/g of *Streptococcus thermophilus* after fermentation. At 28days (the end of the storage period), pH values decreased to 3.7-4.2, while total acidity increased to 1-1.3%, The cells viability of starter bacteria decreased with *Streptococcus thermophilus* being affected. Consequently mannan extract added to the yogurt increase the viability of probiotic bacteria after production.

Introduction

Yeast cell wall is a non-specific stimulator of the immune system in both human and animals which is generally composed of of 30-60% polysaccharides (beta-glucan and mannan sugar polymers), 15-30% proteins, 5 - 20% lipids and a small amount of chitin. Most of the protein is linked to the Mannan-OligoSaccharides (MOS) and is referred to as the Mannoprotein complex (Aguilar-Uscanga and Francois, 2003; Huang *et al*., 2005), (Klis *et al.*, 2006). The mannoproteins contain 80-90% mannose sugar which is linked with 5-20% proteins. The molecular weight of mannoproteins ranging from 20000 to 200000 Daltons. The backbone of complex chains in mannoproteins is composed of α-1,6-linked mannose which is 83% branched at O_2 within oligosaccharide side chains which is mostly found in the form of di-, tri- and tetramers (Izabela *et al*., 1974). The production of mannan is significantly affected by the mannan content in *Saccharomyces cerevisiae,* changes in the physiological state of yeast cells and interactions within the cells such as addition of alpha-factor (alos known as sex factor) or transfer to the non-permissive temperature (Diaz *et al*., 1992).

Prebiotics, known as non-digestible carbohydrate compounds, do not affect the host by reducing the activity and the number of bacteria in the colon, and thus improve the health of the host (Gibson and Roberfroid, 1995; Niamah *et al*.,2016). Short-chain carbohydrates including transgalactooligosaccharides, polydextrose, galactooligosaccharides, banana psyllium, wheat dextrin, whole grain wheat, acacia gum, and whole grain corn also have prebiotic effects (Slavin, 2013). Mannan oligosaccharide content is an important biological function of yeast cell walls and it's because of its prebiotic activity. The activity come from Prebiotics, known as non-digestible carbohydrate compounds, do not affect the host by reducing the activity and the number of bacteria in the colon, and thus improve the health of the host (Gibson and Roberfroid, 1995). Prebioticc activity which have a vital role that can serve as a nutrient source for the growth of beneficial bacteria (probiotic bacteria) in the colon of warm-blooded animals (Kneifel *et al*., 2000; Halas and Nochta, 2012) and fish (Akrami *et al*., 2013). The aim of the present work is to provide an appropriate approach to obtain mannan extract from yeast cell walls and study the effect of add on the acidity of yogurt and the viability of bacteria starter during storage time.

Materials and Methods

Saccharomyces cerevisiae *strains*

Many types of dry cultures of *Saccharomyces cerevisiae* were collected from the local markets of Basra city in Iraq which included Saf-instant

*Corresponding author.
Email: *alaakareem2002@hotmail.com*
Tel: +9647709042069

red (S) production coda 31150/Milwaukee, USA, Lallemand BRY-97, European (E)/ UK, Natu (N)/ arbin Fit Chemical Co., Ltd., China and Yuva-Maya (Y)/Yuva Company, Istanbul, Turkey. It was grow on yeast extract peptone dextrose medium (YPDA) agar composed of 20 g/L glucose, 20 g/L peptone, 10 g/L yeast extract and 20 g/L agar and kept at 4ºC before use. Identification tests of yeast types included morphological, microscopic and biochemical tests: carbon source fermentation, carbon source assimilation, decomposition of urea, acid production and growth at different temperatures (Lodder, 1970; Barnett *et al.*, 1990).

Chemicals

All chemicals used in the study were analytical grade. The glucose, yeast extract, peptone, phenol, agar, nalidixic acid, neomycin sulphate, lithium chloride LiCl and paromomycin sulphate were obtained from BDH Company, UK. Sodium hydroxide NaOH, Hydrochloric acid HCl fructose, sucrose, maltose, galactose, Lactose and raffanose from Sigma Company, Germany. Ethanol absolute, diethyl ether and sulfuric acid H_2SO_4 from Scharlau company, Spain.

Extraction of crude mannan oligosaccharides

The water-soluble mannan oligosaccharides were obtained from 5 g dry yeast by extraction with 1% NaOH (50 ml) at 100°C for 2h, cooling and neutralizing at pH7 with dilute HCl solution. After filtration, the mannan oligosaccharides were precipitated by adding 200 ml (4 volumes) of ethanol absolute. The precipitate was washed with ethanol absolute and diethyl ether redissolved in water, dialyzed against 2 changes of water and subsequent drying (Huang *et al.*, 2010).

Determination of mannan concentration and yield

The mannan concentration in the extracts (ME) was determined by the phenol-sulfuric acid method using mannose as standard method followed by (Dubois *et al.*, 1956). The yield value was estimated by dividing the weight of mannan obtained on total weight of the yeast.

Mannan production

Old yeast (age 18h) stimulated on the YPDA medium (Hi-media, India) was transferred to 100 ml conical flasks containing 50 ml of yeast extract peptone dextrose medium broth (YPDB) and incubated at 30ºC temperature for 72h in a shaking incubator at 150 rpm/min (Liu *et al.*, 2009). Thereafter, the cells were separated by centrifuge (Damon/IEC division, USA) at 6000 × g /min for 15 minutes after which, they were washed with distilled water and then estimated the biomass by the quantity of mannan extract.

Measurement of yeast biomass

The method described by DeSous *et al.* (2006) was followed for the measurement of the yeast biomass. Biomass was collected at the end of the fermentation period by centrifuging at 6000 × g / min at 4ºC and washed two times with distilled water. The precipitate was dried in aerobic oven at 105ºC temperature for 4h and the yeast biomass was measured with an analytical balance.

Probiotic starter culture

Probiotic starter was obtained from Chr. Hansen Middle East and Africa, UAE. It contains *Streptococcus thermophilus, Lactobacillus acidophilus* and *Bifidobacterium* sp. which was grown in de Man Rogosa Sharpe broth (Hi-media, India) at 37ºC for 24h and used as a probiotic starter in yogurt production.

Bio-yogurt production

Buffalo milk (Animals station of Agriculture College / Basrah University) was used in yogurt production (Tamime and Robinson, 1999). After heating of the milk at 90ºC temperature kept at 4ºC for 5min made it cold. The concentrations of mannan extract (0.5, 1.0, 1.5, 2.0 and 2.5%) (W/V) were mixed respectively with milk and then 3% probiotic starter was added. After that prepare mixture was incubated at 40ºC temperature for 4h. After fermentation, yogurt products were refrigerated at 4ºC temperature for 28 days.

Bio-yogurt tests

Total acidity and pH of yogurt were estimated by the method of Nielsen (2010) described as followed: Cells viability count of the probiotic starter cultures were done on selective media: M17 (Hi-media, India) for *S. thermophilus*, de Man Rogosa Sharpe (MRS)-raffanose for *L. acidophilus* and MRS-nalidixic acid-neomycin sulphate-LiCl-paromomycin sulphate (NNLP) for *Bifidobacterium* sp. (Shah, 2000; Ashraf and Shah, 2011). Tests were conducted at 0, 7, 14, 21 and 28 days of storage.

Results and Discussion

Saccharomyces cerevisiae *characteristics*

Morphological and biochemical analysis of *Saccharomyces cerevisiae* strains included white or

Table 1. Biochemical tests used to diagnose yeast

Kinds of yeast	Diagnostic tests																	
	Fermentable carbon Sources							Carbon Assimilation							Decomposition of urea	Growth at different temperatures		Acid production
	G	T	S	M	Ga	Ra	L	G	T	S	M	Ga	Ra	L		30℃	37℃	
S	+	-	+	+	+	+	-	+	-	+	+	+	+	-	-	+	±	+
E	+	-	+	+	+	+	-	+	-	+	+	+	+	-	-	+	±	+
N	+	-	+	+	+	+	-	+	-	+	+	+	+	-	-	+	±	+
Y	+	-	+	+	+	+	-	+	-	+	+	+	+	-	-	+	±	+

S: Saf-instant red, E: Lallemand European, N: Natu, Y: Yuva-Maya, G: Glucose, T: Trehalose , S:Sucrose , M:Maltose, Ga: Galactose, Ra: Raffinose, L: Lactose, ±: Weak growth, +: Good growth

Table 2. Yield of mannan extracted and mannan content in yeast isolates at the media of growth (YPD)

Yeast kinds	Mannan g /yeast 5g	Mannan yield %	Dry cell (g) /100 ml media	Mannan % of dry cell
S	1.58±0.01	31.60±0.05	0.56±0.00	27.87 ±0.10
E	1.72±0.01	34.40±0.03	0.73 ±0.03	36.51 ±0.06
N	1.42±0.02	28.40±0.05	0.53 ±0.01	30.44 ± 0.08
Y	1.29±0.03	25.80±0.10	0.47 ±0.05	24.43± 0.05

S: Saf-instant red, E: Lallemand European, N: Natu, Y: Yuva-Maya, SD±: standard deviation

cream colored colonies, circular or diagonal in shape colonies when grown on solid media. These colonies had regular and smooth edges and were elevated on the agar surface. Yeast cells appear blue in color when stained by blue methylene dye, spherical to cocci in shape with clear nucleus. The presence of buds were found more in all parts of the cells but the lack of mycelium was found under the microscopic examination.

The biochemical tests showed the ability of the yeast to ferment carbohydrates including glucose, fructose, sucrose, maltose, galactose and raffinose (Table 1). All strains were not able to ferment lactose. Ammonia was not produced from urea because it does not possess urease enzyme. These results also showed the ability of yeast strains to grow excellently at 30°C temperature while the growth was weak at 37°C. All tests confirm that the strains belong to Saccharomyces cerevisiae (Barnett *et al*., 1985).

The yield of mannan

The yields of mannan extracted were ranged between 25.80-34.40% of the dry weight of yeast cells (Table 2). Higher yield was attributed to the presence of a protein linked with mannan. All mannan in yeast cells is supposed to exist in the complex form with protein and were found in wall cell (Maier *et al*., 1993). The results were agreed with many studies which reported that mannan in the wall of yeast cells ranges between 30-50% (Aguilar-Uscanga and Francois, 2003; Klis *et al*., 2006).

The ability of yeast strains to grow on media culture was studied for the purpose of knowing the amount of biomass production, which ranged between 0.47 to 0.73g/100 ml, while mannan extracted ranged between 24.43-38.44% (Table 2). The higher yield of biomass and mannan production in the fermentation medium was attributed to carbon content and energy sources readily metabolized by *Saccharomyces cerevisiae* such as monosaccharides. In media culture these can be directly used for production (Dynesen et al., 1998). The highest amount of mannan extract (36.51%) was obtained from the European yeast which was found suitable for the mannan production as prebiotic in the present study.

pH and total acidity of yogurt

pH and total acidity value of control yogurt without mannan extract concentrations were 4.81 and 0.81% respectively after fermentation. Low acidity found in control yogurt because of weak growth probiotic bacteria in the milk and it need to increase of fermentation time suggested by (Lourens-Hattingh and Viljoen, 2001). With the addition of mannan extract to yogurt, the pH found to be decreased and the percentage of total acidity increased with increasing the concentration of added ME. The pH and percentage total acidity values were 4.6, 4.6, 4.55, 4.53, 4.45 and 0.89, 0.9, 0.95, 0.96, 1% for 0.5, 1, 1.5, 2, 2.5% mannan extract, respectively, after fermentation (Figure 1 and 2). The pH levels further decreased during storage, while percentage total acidity increased during storage for control sample and all mannan extract concentrations (namely pH 4.63, 4.2, 4.14, 4.1, 3.9, 3.7 and %total acidity of 0.9, 1, 1.2, 1.22, 1.28, 1.3% for the different mannan

Table 3. Viability of starter bacteria (Log CFU/g) in yogurt with mannan extract concentrations (0, 0.5, 1.0, 1.5, 2.0, 2.5%) after fermentation and storage days.

	Bifidobacterium sp.					***Lb. acidophilus***					***St. thermophilus***				
	Storage days														
ME	0	7	14	21	28	0	7	14	21	28	0	7	14	21	28
0.0%	5.80	5.6	5.1	4.8	4.6	6.8	6.6	6.1	5.0	4.9	6.7	6.9	5.0	4.9	4.5
0.5%	5.86	5.7	5.5	5.4	5.0	6.8	6.7	6.7	6.5	6.0	6.7	6.9	5.3	5.1	4.5
1.0%	6.10	6.0	5.7	5.4	5.1	6.9	6.8	6.7	6.3	6.0	6.8	7.0	5.7	5.6	4.5
1.5%	6.60	6.4	6.0	5.9	5.1	7.5	7.4	7.2	6.7	6.2	7.0	7.0	5.7	5.7	4.5
2.0%	7.00	6.5	6.3	6.1	5.5	7.7	7.6	7.5	7.0	6.6	7.1	7.0	5.8	5.7	4.5
2.5%	7.15	7.0	6.0	5.5	5.2	7.8	7.5	7.3	6.4	6.0	7.2	7.0	5.5	5.4	4.5

ME: Mannan extract

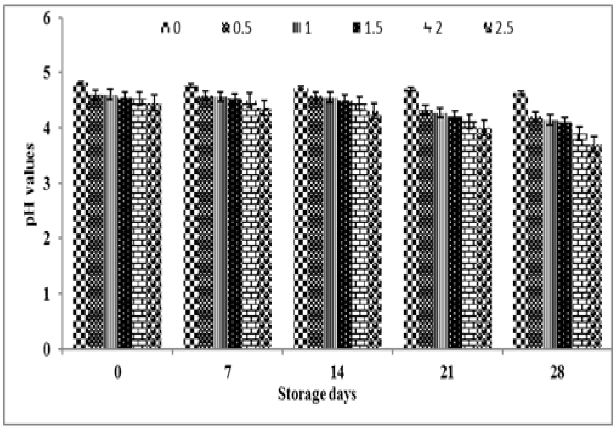

Figure 1. pH values of yogurt with mannan extract concentrations (0, 0.5, 1.0, 1.5, 2.0, 2.5%) after fermentation and storage days

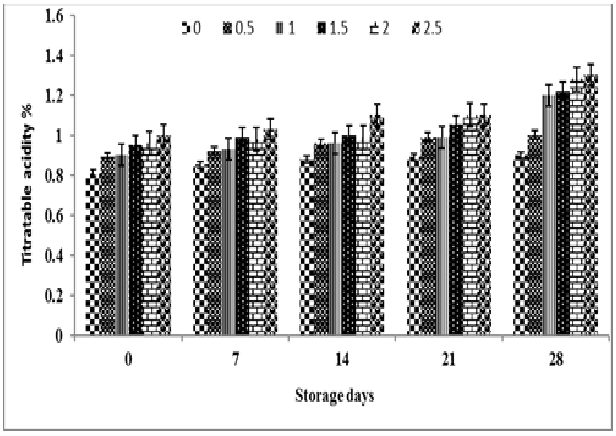

Figure 2. Titratable acidity %values of yogurt with mannan extract concentrations (0, 0.5, 1.0, 1.5, 2.0, 2.5%) after fermentation and storage days.

extracts 0, 0.5, 1, 1.5, 2, 2.5% respectively) after 28 days of storage (Figure 1 and 2).

Growth ratio and acidifying activity of the probiotic bacteria in products were variable depending on the type and concentration of the prebiotics. Mannan and other prebiotics bioavailability in media improve growth the of probiotic bacteria (Aryana *et al.*, 2007; Gustaw *et al.*, 2011). Prebiotics tend to increase viability and activity of probiotic bacteria (Lenoir-Wijnkoop *et al.*, 2007). Similar studies on results of the pH values of commercial yogurt containing probiotic bacteria during their storage were reported by (Shah *et al.*, 2000). The decline in pH was assumed because of the continuation growth of lactic acid bacteria during storage (Kailasapathy, 2006).

Survival of bacteria starter

The viability of *Lactobacillus acidophilus* was 6.8 Log CFU/g after fermentation time and the numbers of viable cells decreased to 4.9 Log CFU/g under storage conditions at 4ºC for 28 days. Added mannan extracts to yogurt increased the cells viability of *Lb. acidophilus* after fermentation time compared to the control without mannan extract (Fig. 3). After storage time, the viability of *Lb. acidophilus* was 6, 6.09, 6.21, 6.6, 6.09 Log CFU/g for 0.5, 1, 1.5, 2, 2.5% mannan extract, respectively (Table 3). After fermentation, the cells viability of *Bifidobacterium* sp. in yogurt production was 5.8, 5.86, 6.1, 6.6, 7, 7.1 Log CFU/g for 0, 0.5, 1, 1.5, 2, 2.5% mannan extract, respectively. The cells viability of *Bifidobacterium* sp. in yogurt without mannan extract (control) was 4.6 Log CFU/g while addition of mannan extract into yogurt lead to increased values of 5, 5.13, 5.11, 5.5, 5.2 Log CFU/g for the respective ME concentrations (0, 0.5, 1, 1.5, 2, 2.5% mannan extract) respectively after storage time (table 3). *Lb. acidophilus* and *Bifidobacterium* have β-mannanase (EC 3.2.1.78) enzyme which do mannan analysis into simple units and utilize it for growth (Hammes and Hertel, 2009; Kulcinskaja *et al.*, 2012).

Table 3 indicates the lack of a significant effect on the *Streptococcus thermophilus* cells viability in yogurt production within mannan containing extracts or without (control yogurt). The cells viability of St. thermophilus was 6.74, 6.79, 6.82, 7.01, 7.19, 7.22

Log CFU/g for the 0, 0.5, 1, 1.5, 2, 2.5% mannan extracts respectively after fermentation. The viability decreased during storage to values of 4.5, 4.51, 4.55, 4.53, 4.58, 4.5 Log CFU/g for the respective ME concentrations (0, 0.5, 1, 1.5, 2, 2.5%) mannan extract respectively after 28 days.

In previous studies, the loss of cells viability of probiotic bacteria in yogurt during storage, were attributed to the acidity and low temperature (Shah *et al*., 1995; Vinderola *et al.*, 1999). Prebiotics such as lactulose, inulin and β-glucan significantly improved the cells viability of probiotic bacteria (Ozer *et al*., 2005; Akalin and Erisir, 2008; Sahan *et al*., 2008).

In general, the cell numbers of probiotic bacteria in yogurt production containing mannan extract concentrations are among the numbers allowed by FAO/WHO protocols which were reported to be 10^6-10^9 CFU/g.

Conclusion

Mannan content of wall cell is different between strains of yeast. Mannan oligosaccharides work as prebiotic when it adds to milk fermentation products. The relationship between probiotics and prebiotics in food fermentation are called synbiotics. Add mannan extracted to yogurt product from buffalo milk led to increase the viability of probiotic bacteria in yogurt and enhancement of the survival of probiotic bacteria during storage. Viability of *Lb. acidophilus* and Bifidobacterium were within the allowable limits, despite low pH and increase the percentage of total acidity after storage time.

Acknowledgements

This research was supported by Biotechnology Lab., Basrah University, The Ministry of Higher Education and Scientific Research of Iraq.

References

Aguilar-Uscanga, B. and Francois, J.M. 2003. A study of the yeast cell wall composition and structure in response to growth conditions and mode of cultivation. Letters in Applied Microbiology 37:268–274.

Akalin, A.S., and Erisir, D. 2008. Effects of inulin and oligofructose on the rheological characteristics and probiotic culture survival in low-fat probiotic ice cream. Food Microbiology and Safety 00: M1-M5.

Akrami, R., Mansour, M. R., Ghobadi, Sh., Ahmadifar, E., Khoshroudi, M.S. and Moghimi Haji, M.S. 2013. Effect of prebiotic mannan oligosaccharide on hematological and blood serum biochemical parameters of cultured juvenile great sturgeon (*Huso huso* Linnaeus, 1754). Journal of Applied Ichthyology 29: 1214–1218.

Aryana, K.J., Plauche, S., Rao, R.M., McGrew, P. and Shah, N.P. 2007. Fat-free plain yogurt manufactured with inulins of various chain lengths and *Lactobacillus acidophilus*. Journal of Food Science 72:M79-M84.

Ashraf, R. and Shah, N.P. 2011. Selective and differential enumerations of *Lactobacillus delbrueckii* subsp. *bulgaricus, Streptococcus thermophilus, Lactobacillus acidophilus, Lactobacillus casei* and *Bifidobacterium* spp. in yoghurt- A review. International Journal of Food Microbiology 149: 194–208.

Barnett, J.A., Payne, R.W. and Yarrow, D. 1990. Yeasts: Characteristic and Identification. London: Cambridge University.

De Sous, S. R., Laluce, C. and Jafelicci, M. 2006. Effects of organic and inorganic additives on flotation recovery of washed cells of *Saccharomyces cerevisiae* suspended in water. Colloids and Surfaces B: Biointerfaces 48: 77-83.

Diaz, S., Zinker, S. and Ruiz, H.J. 1992. Alterations in the cell wall of *Saccharomyces cerevisiae* induced by the alpha sex factor or a mutation in the cell cycle. Antonie van Leeuwenhoek 61:269-276.

Dubois, N., Gilles, K.A., Hamilton, J.K., Rebers, P.A. and Smith, F. 1956. Colorimetric method for detection of sugars and related substances. Analytical Chemistry 28: 350-356.

Dynesen, J., Smits, H.P., Olsson, L. and Nielsen, J. 1998. Carbon catabolite repression of invertase during batch cultivations of *Saccharomyces cerevisiae*: the role of glucose, fructose, and mannose. Applied Microbiology and Biotechnology 50: 579-582.

Gibson, G.R. and Roberfroid, M.B. 1995. Dietary modulation of the human colonic microbiota: Introducing the concept of prebiotics. Journal of Nutrition 125: 1401–1412.

Gustaw, W., Kordowska-Wiater, M. and Kozioł, J. 2011. The Influence of selected prebiotics on the growth of lactic acid bacteria for bio-yoghurt production. Acta Scientiarum Polonorum Technologia Alimentaria 10:455-466.

Halas, V. and Nochta, I. 2012. Mannan oligosaccharides in nursery pig nutrition and their potential mode of action. Animals 2: 261-274.

Huang, G. L., Liu, M. X. and Mei, X. Y. 2005. Studies on the hydrolytic condition of β-1,3 glucan from yeast by fluorophore-assisted carbohydrate electrophoresis. Analytical Letters 38: 477 – 485.

Hammes, W.P. and Hertel, C. 2009. Genus *Lactobacillus.* In Whitman, W.B. and Parte, A.C. (Eds). Bergey's manual of systematic bacteriology, p.465-510. London: Springer.

Huang, G.L., Yang, Q. and Wang, Z.B. 2010. Extraction and deproteinization of mannan oligosaccharides. Zeitschrift für Naturforschung C 65: 387-390.

Izabela, L., Maria, M., Peter, C., Eva, M., Nakajima, T. and Ballou, C.E. 1974. Structure of the linkage region between the polysaccharide and protein parts of *Saccharomyces cerevisiae* mannan. The Journal of Biological Chemistry 249: 7685-7694.

Kailasapathy, K. 2006. Survival of free and encapsulated probiotic bacteria and their effect on the sensory properties of yogurt. LWT- Food Science and Technology 39: 1221-1227.

Klis, F.M., Boorsma, A. and De Groot, P.W.J. 2006. Cell wall construction in *Saccharomyces cerevisiae.* Yeast 23:185–202.

Kneifel, W., Rajal, A. and Kulbe, K. D. 2000. In vitro growth behavior of probiotic bacteria in culture media with carbohydrates of prebiotic Importance. Microbial Ecology in Health and Disease 12: 27-34.

Kulcinskaja, E., Rosengren, A., Ibrahim, R., Kolenová, K. and Stalbrand, H. 2012. Expression and characterization of a *Bifidobacterium adolescentis* beta-mannanase carrying mannan-binding and cell association motifs. Applied and Environmental Microbiology 79: 133-140.

Lenoir-Wijnkoop, I., Sanders, M.E., Cabana, M.D., Caglar, E., Corthier, G., Rayes, N., Sherman, PM., Timmerman, H.M., Vaneechoutte, M., Van Loo, J. and Wolvers, D.A. 2007. Probiotic and prebiotic influence beyond the intestinal tract. Nutrition Reviews 65:469-89.

Liu, H.Z., Wang, Q., Liu, X.Y. and Tan, S.S. 2008. Effects of spaceflight on polysaccharides of *Saccharomyces cerevisiae* cell wall. Applied Microbiology and Biotechnology 81: 543-550.

Liu, H.Z., Wang, Q., Liu, Y.Y. and Fang, F. 2009. Statistical optimization of culture media and conditions for production of mannan by *Saccharomyces cerevisiae.* Biotechnology and Bioprocess Engineering 14: 577-583.

Lodder, J. 1970. The Yeast a Taxonomic study. North-Holland Publishing Co., Amsterdam.

Lourens-Hattingh, A. and Viljoen, B. C. 2001. Yogurt as probiotic carrier food. International Dairy Journal 11:1-17.

Maier, H., Anderson, M., Karl, C., Magnuson, K. and Whistler, R. L. 1993. Guar, locust bean, tara, and fenugreek gums. In Whistler, R. L. and Be Mille, R. L. (Eds.) Industrial Gums. Polysaccharides and their derivatives, p. 181–226. San Diego: Academic Press.

Niamah, A. K., Al-Sahlany, S. T. G. and Al-Manhel, A. J. 2016. Gum arabic uses as prebiotic in yogurt production and study effects on physical, chemical properties and survivability of probiotic bacteria during cold storage. World Applied Sciences Journal 34: 1190-1196.

Nielsen, S.S. 2010. Food analysis laboratory manual. New York: Springer.

Ozer, D., Akin, S. and Ozer, B. 2005. Effect of inulin and lactulose on survival of *Lactobacillus acidopilus* LA-5 and *Bifidobacterium bifidum* BB-02 in *Acidophilus-Bifidus* yoghurt. Food Science and Technology International 11: 19-24.

Sahan, N., Yasar, K. and Hayaloglu, A.A. 2008. Physical, chemical and flavour quality of non-fat yogurt as affected by β-glucan hydro colloidal composite during storage. Food Hydrocolloid 22:1291-1297.

Shah, N.P. 2000. Probiotic bacteria: selective enumeration and survival in dairy foods. Journal of Dairy Science 83: 894–907.

Shah, N.P., Ali, J.F. and Ravula, R.R. 2000. Populations of *Lactobacillus acidophilus, Bifidobacterium* spp., and *Lactobacillus casei* in commercial fermented milk. Bioscience and Microflora 19: 35-39.

Shah, N.P., Lankaputhra, W.E.V., Britz, M.L. and Kyle, W.S.A. 1995. Survival of *Lactobacillus acidophilus* and *Bifidobacterium bifidum* in commercial yoghurt during refrigerated storage. International Dairy Journal 5: 515–521.

Slavin, J. 2013. Fiber and prebiotics: mechanisms and health benefits. Nutrients 5: 1417-1435.

Tamime, A. Y. and Robinson, R.K. 1999. Yoghurt science and technology. New York: CRC press.

Vinderola, C.G. and Reinheimer, J. A. 1999. Culture media for the enumeration of *Bifidobacterium bifidum* and *Lactobacillus acidophilus* in the presence of yoghurt bacteria. International Dairy Journal 9: 497–505.