

Gum Arabic Uses as Prebiotic in Yogurt Production and Study Effects on Physical, Chemical Properties and Survivability of Probiotic Bacteria During Cold Storage

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Abstract: Five different concentrations of Gum Arabic (GA) added to yogurt product by probiotic culture starter, which contained *Lactobacillus acidophilus*, *Bifidobacterium bifidum* and *Streptococcus thermophilus*. Best concentration of GA was added 1%. The chemical composition of yogurt with 1% GA was (86.97, 3.88, 3.21, 0.79 and 5.15) % for moisture, protein, fat, ash and carbohydrates respectively. After one day of fermentation, pH values decreased to 4.52 while the total acidity was 0.91%. The WHC, STS and WD were 54%, 68% and 0 ml respectively. The cells viability of *Lactobacillus acidophilus* was 7.1 Log CFU/g, 7.15 Log CFU/g of *Bifidobacterium bifidum* and log 7.72 Log CFU/g of *Streptococcus thermophilus* after fermentation. At 21 days (the end of the storage period), the results of yogurt with 1% GA, pH values decreased to 4.05, while total acidity increased to 1.15%. The WHC, STS and WD were 50.5%, 71% and 1.2 ml. The cells viability of starter bacteria decreased with *Bifidobacterium bifidum* being affected. When GA added to the yogurt, the viability of probiotic bacteria was increased after production.

Key words: Gum Arabic • Yogurt product • Probiotic • Prebiotic

INTRODUCTION

Probiotic bacteria including some species of lactic acid bacteria (LAB) and other types of bacteria as bifidobacteria. It can grow in gut animals warm-blooded and Production metabolic materials beneficial such as organic acids, bacteriocins, active peptides and short-chain fatty acids [1].

Probiotic bacteria has used in many food products such as dairy product, meat product, pickles and fermented vegetables. The acidity and cold storage of products are decreasing the viability cells of probiotic bacteria [2].

Prebiotics are substances that induce the growth or activity of probiotic bacteria and non-digestible such as galactooligosaccharide [3], fructooligosaccharide [4], xylooligosaccharide, beta-glucans and inulin [5]. Gum Arabic (GA) is one member of prebiotic group when mix probiotic and prebiotic is symbiotic product [6].

Gum Arabic (GA), also known as acacia gum, char goond, chaar gund, or meska, is a natural gum made of the hardened plants juice of various species of the acacia tree. The acacia tree grow principally in Sudan and

Egyptian. Originally, gum Arabic was collected from *Acacia nilotica*, *Acacia senegal* and *Acacia seyal* which was named the Gum Arabic tree [7].

Gum Arabic (GA) contents variety of carbohydrates such as rhamnose, arabinose, galactose and glucuronic acid. The percentage of these carbohydrates differ according to the source of GA [8].

GA uses in pharmacy, clinical, cosmetic science and food industries as stabilizer and an emulsifier. In certain countries in traditional of patients who suffer from chronic kidney disease treatment [9]. It effects on cholesterol levels in blood of rates and reduce plasma cholesterol [10].

GA has found application in many foods, besides to as an emulsifier and stabilizer material GA uses as anti-oxidant, anti-microbial, anti-coagulant, anti-inflammatory and shelf-life enhancer of food products [11]. In dairy products, GA uses in ice cream and yogurt [12].

The aim of the present study was improved some chemical characteristics of yogurt, the viability cells of probiotic bacteria was maintained within the allowable level (10^6 - 10^8 CFU/g) during cold storage and symbiotic product.

MATERIALS AND METHODS

Chemicals: All chemicals, which have used in the study were analytical type. The nalidixic acid, neomycin sulphate and lithium chloride were obtained from BOH Company, UK. The Phenolphthalein indicator, NaOH, sorbitol, Gum Arabic (GA) from Sigma Company, Germany.

Probiotic Starter Culture: Probiotic starter was obtained from Chr. Hansen Middle East and Africa, UAE. It contains *Streptococcus thermophilus*, *Lactobacillus acidophilus* LA-5 and *Bifidobacterium bifidum* Bb-12 (1:1:1), which was grown in MRS broth (Hi-media, India) at 37°C for 24 h and used as a probiotic starter in yogurt production.

Yogurt Product: Caw's milk (Animals station of Agriculture College / Basrah University) was used in yogurt production [13]. The milk was heated at 90°C for 5 min. The heated milk was cooled to 40°C. Addition culture starter 2% and Gum Arabic (GA) concentrations (0.0, 0.2, 0.4, 0.6, 0.8 and 1.0) g respectively, they were mixed with 100 ml of milk. The mixture incubated at 40°C for 6 h. After fermentation, yogurt products were refrigerated at 4°C for 21 days.

Yogurt Tests

Chemical Composition: After one day of storage, yogurt samples were analyzed for chemical composition by using the methods of [14]. Moisture was determined by drying samples in a hot air oven at 105±2°C for until proven weight. Total protein was analyzed by Micro-Kjeldahl method. Ash was determined by lighting samples in the furnace oven at 621°C for 16-20 hours. Total lipid determination used method of [15]. Total carbohydrate was calculated by the equation:

$$100 - (\text{moisture} + \text{lipid} + \text{protein} + \text{ash}) \%$$

Total Acidity and pH Value: During 21 days of cold storage at 4°C, the yogurt samples were analyzed on 1, 7, 14 and 21 days for total acidity and pH value. Total acidity percentage determined by titrating yogurt samples. pH value of yogurt samples were estimated by using pH meter (SD-300 pH, Germany) [16].

Physical Analysis: Physical analysis of yogurt samples was determined during cold storage at 4°C for 1, 7, 14 and 21 days. It was included: Water holding capacity (WHC)

of yogurt samples was determined as described [17]. The WHC percentage value was calculated by the following equation: %WHC = $1 - [W_1/W_2] \times 100$. Where, W₁: The weight of whey after centrifugation; W₂: The weight of yogurt. Susceptibility to syneresis (STS) of yogurt sample was determined by putting 100 ml of yogurt sample on filter paper (Whatman No.1). After 6 hours of emptying, the volume of whey collected in the measuring cylinder reported by [12]. Used the following formula to calculate the STS percentage = $1 - [V1/V2] \times 100$. Where: V1: Whey volume collected; V2: Yogurt volume. Whey drainage (WD) was removed from yogurt samples, by a syringe during 24 hours after the test date. The amount of whey off (in ml per 100 ml of initial yogurt sample) was calculated as the whey drainage [18].

Microbial Tests: Microbial tests of yogurt samples were determined during cold storage at 4°C for 1, 7, 14 and 21 days. The viability cells of bacteria of yogurt samples was measured by pour plate method [19] and used selective culture media as M17 for *St. thermophilus*, MRS-Sorbitol agar for *Lb. acidophilus* and MRS-NNL agar for *B. bifidum* [20, 21].

All plates were incubated at 37°C for 24-48 h in anaerobic condition except *St. thermophilus* which had incubated at 37°C for 24 hour in aerobic condition [22].

Statistical Analysis: Statistical analysis of the results was performed using completely randomized design (CRD) and Least significant difference (L.s.ds.) when p≤0.05 by SPSS (version 16). The Data of yogurt chemical composition analyzes by standard deviations.

RESULTS AND DISCUSSION

Chemical Composition of Yogurt: Table 1 show, the chemical composition of yogurt produced after one day of fermentation. The percentage of components increased after the addition of GA except moisture. Increase the concentration of GA added increased solid components in yogurt production. The proteins, fat, ash and carbohydrates percentage was 3.88, 3.21, 0.79 and 5.15 respectively while the percentage of moisture was 86.97 after 1% addition of GA. The GA addition with milk led to the high concentrations of solid materials in yogurt produce. The GA contents 78-88% of solid materials and essential amino acids [8, 23]. The compositions of yogurt without GA were (87.80, 3.73, 3.18, 0.7 and 4.59) % of moisture, proteins, fat, ash and carbohydrates respectively.

Table 1: The chemical composition of yogurt produce with GA after one day of fermentation

Yogurt samples	Moisture	Proteins	Fat	Ash	Carbohydrates
0% gum Arabic	87.80±0.10	3.73±0.03	3.18±0.02	0.70±0.01	4.59±0.02
0.2% gum Arabic	87.62±0.08	3.76±0.01	3.18±0.02	0.72±0.00	4.71±0.03
0.4% gum Arabic	87.44±0.05	3.80±0.01	3.19±0.01	0.73±0.01	4.80±0.02
0.6% gum Arabic	87.26±0.06	3.82±0.04	3.19±0.01	0.76±0.02	4.92±0.04
0.8% gum Arabic	87.18±0.10	3.85±0.02	3.20±0.01	0.77±0.03	5.00±0.03
1% gum Arabic	86.97±0.05	3.88±0.01	3.21±0.01	0.79±0.00	5.15±0.02

SD±: Standard deviation

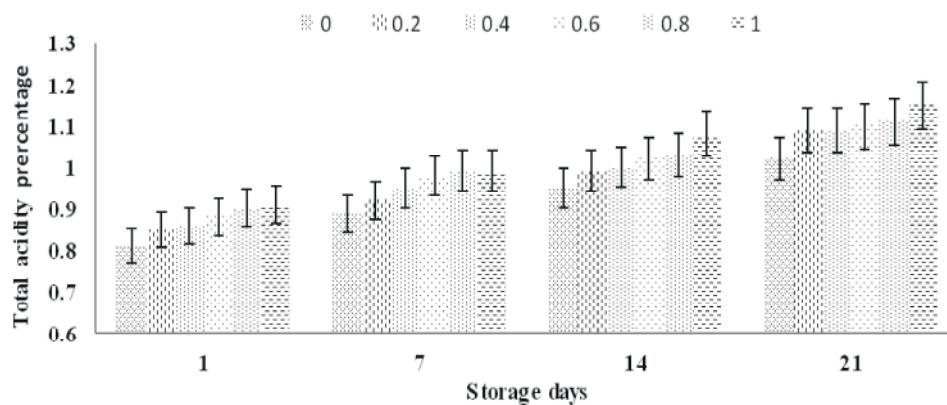


Fig. 1: Total acidity percentage values of yogurt with GA concentrations (0, 0.2, 0.4, 0.6, 0.8 and 1%) after fermentation and storage days

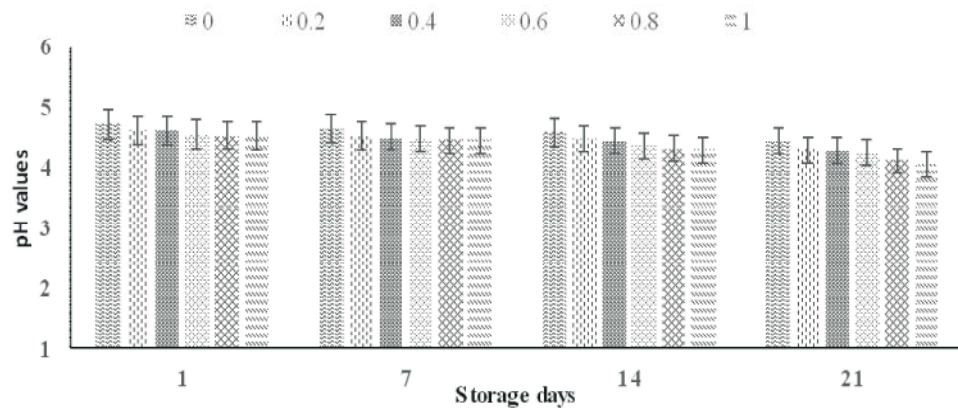


Fig. 2: pH values of yogurt with GA concentrations (0, 0.2, 0.4, 0.6, 0.8 and 1%) after fermentation and storage days

Total Acidity Percentage and pH Value of Yogurt: The total acidity percentage and pH value of yogurt without GA concentrations were 4.72 and 0.81 % respectively after fermentation. Low acidity in control yogurt caused by the weak growth of probiotic bacteria in the milk and lactose hydrolyze. The fermentation time for acid production should be increased [24]. In other samples of yogurt produce, the percentage of total acidity increased and the pH value decreased when increasing the concentration of added GA. The GA contains variety carbohydrates which probiotic bacteria can fermentation [25] and GA as a

natural prebiotic [26]. After fermentation, the total acidity and pH value were 0.85, 0.86, 0.89, 0.90, 0.91% and 4.6, 4.6, 4.55, 4.53, 4.52 for 0.2, 0.4, 0.6, 0.8, 1.0 % GA added, respectively (Fig.1 and Fig.2). The pH values further decreased during storage time. While percentage of total acidity increased during storage for yogurt samples without and within GA concentrations. Total acidity percentage of 1.02, 1.09, 1.09, 1.1, 1.11, 1.15 and namely pH 4.44, 4.3, 4.28, 4.25, 4.11, 4.05 for the different GA concentrations 0.0, 0.2, 0.4, 0.6, 0.8, 1.0 % respectively, after 21 days of storage (Fig. 1 and Fig. 2).

Table 2: The % WHC, % STS and WD values of yogurt with GA concentrations (0, 0.2, 0.4, 0.6, 0.8 and 1%) after fermentation and storage days

Yogurt samples	% WHC*				% STS*				WD* (ml)			
	1	7	14	21	1	7	14	21	1	7	14	21
0% gum Arabic	40.02 ^e	38.08 ^e	35.33 ^c	31.00 ^e	84.00 ^e	88.00 ^f	91.00 ^e	92.00 ^e	1.60 ^d	2.80 ^c	3.50 ^e	5.20 ^e
0.2% gum Arabic	43.10 ^d	40.00 ^d	39.05 ^d	37.55 ^d	82.00 ^e	85.00 ^e	87.00 ^d	90.00 ^e	1.00 ^c	1.90 ^{ab}	2.80 ^d	4.70 ^d
0.4% gum Arabic	44.00 ^d	42.80 ^d	40.20 ^d	38.61 ^d	78.00 ^d	80.00 ^d	81.00 ^c	83.00 ^d	1.00 ^c	1.50 ^b	2.50 ^c	4.30 ^d
0.6% gum Arabic	47.20 ^c	45.90 ^c	44.00 ^c	42.50 ^c	75.00 ^c	77.00 ^c	79.00 ^c	80.00 ^c	0.50 ^b	1.30 ^b	2.40 ^c	4.00 ^c
0.8% gum Arabic	50.11 ^b	49.00 ^b	48.03 ^b	46.30 ^b	71.00 ^b	74.00 ^b	75.00 ^b	77.00 ^b	0.00 ^a	0.60 ^a	1.40 ^b	2.00 ^b
1% gum Arabic	54.00 ^a	53.11 ^a	52.01 ^a	50.50 ^a	68.00 ^a	69.00 ^a	70.00 ^a	71.00 ^a	0.00 ^a	0.30 ^a	1.00 ^a	1.20 ^a

WHC: water holding capacity, STS: susceptibility to syneresis, WD: Whey drainage, ● : p= 0.05,

Physical Analysis of Yogurt: The WHC percentage of a protein gel is one of the important parameter in yogurt production. It is related to STS percentage and WD. This is due to instability and parcel of the proteins gel. The WHC percentage of yoghurt samples increased and susceptibility of yogurt samples to The STS percentage and WD decreased with increasing GA content. After fermentation, the WHC percentage of yogurt with added GA (43.10-54.00) % was significantly (P ≤ 0.05) than that of yogurt without GA while STS and WD values of yogurt with added GA (82.00- 68.00) % and (1.0-0.0) ml respectively (Table 2). In during storage time, the WHC percentage of yogurt samples was decreased and STS percentage and WD were increased (Table 2). The results were agreed with many of studies [12, 17, 27].

The GA uses in dairy products as stabilizer material. It have two basic functions in yogurt as the binding of water and improvement in texture. The stabilizer reduces water in matrix yogurt production. High GA concentrations in yogurt led to WHC percentage increased and STS percentage, WD decreased after fermentation and during 21 days of storage.

Viability of Bacteria Starter in Yogurt: The viability of *Lactobacillus acidophilus* LA-5 cells was Log 6.88 CFU/g after one day of fermentation and the numbers of viable cells decreased to Log 5.11 CFU/ g under storage conditions at 4°C for 21 days. GA addition to yogurt increased the cells viability of *Lb. acidophilus* after fermentation time compared to the yogurt control without GA., which was 6.96, 7.02, 7.03, 7.06, 7.10 Log CFU/ g for 0.2, 0.4, 0.6, 0.8, 1.0 % GA, respectively (Fig. 3). After 21 days of storage time, the viability of *Lb. acidophilus* LA-5 was 6.00, 6.24, 6.3, 6.39, 6.54 Log CFU/ g for 0.2, 0.4, 0.6, 0.8, 1.0 % GA, respectively (Fig. 3).

After one day of fermentation, the viability of *Bifidobacterium bifidum* Bb-12 cells in yogurt production

was 6.20, 6.86, 6.95, 7.01, 7.03, 7.15 Log CFU/ g for 0.0, 0.2, 0.4, 0.6, 0.8, 1.0 % GA, respectively. The storage decreased the viability of *B. bifidum* cells but has no effect on the viability of *Lb. acidophilus*. The viability of *B. bifidum* Bb-12 was 4.81, 5.45, 5.66, 5.81, 5.80, 5.95 Log CFU/ g for 0.0, 0.2, 0.4, 0.6, 0.8, 1.0 % GA, respectively after storage time (Fig. 4). GA one of the most important substances that act as prebiotic for Bifidobacteria and Lactobacilli [28]. It consists 97% of carbohydrates, which are often composed L-arabinose, D-galactose and Rhamnose units [29]. *Lb. acidophilus* LA-5 can ferment this carbohydrate as carbon source [30]. While *B. bifidum* Bb-12 cannot ferment it [31].

Figure 5 indicates the absence of a significant impact on viability of *Streptococcus thermophilus* in yogurt production within GA concentrations or yogurt without GA. The cells viability of *St. thermophilus* was 7.74, 7.73, 7.72, 7.71, 7.73, 7.72 Log CFU/ g for 0.0, 0.2, 0.4, 0.6, 0.8, 1.0 % GA, respectively after one day of fermentation. The viability of *St. thermophilus* was slightly decreased during storage to values of 7.39, 7.39, 7.42, 7.51, 7.44, 7.49 Log CFU/ g for the respective GA concentrations (0.0, 0.2, 0.4, 0.6, 0.8, 1.0) % GA, respectively after 21 days.

The loss of probiotic bacteria viability in yogurt production was attributed to low temperature and acidity during storage time [32, 33]. Prebiotic substances such as transgalactooligosaccharides, polydextrose, galactooligosaccharides, banana psyllium, wheat dextrin, whole grain wheat, acacia gum and whole grain corn significantly improved the cells viability of probiotic bacteria in dairy products [34]. The mix of probiotic bacteria with prebiotic give symbiotic product. In general, the cell numbers of probiotic bacteria in yogurt production containing GA concentrations are among the numbers allowed by FAO/WHO protocols that were reported to be 10⁶-10⁹ CFU/g.

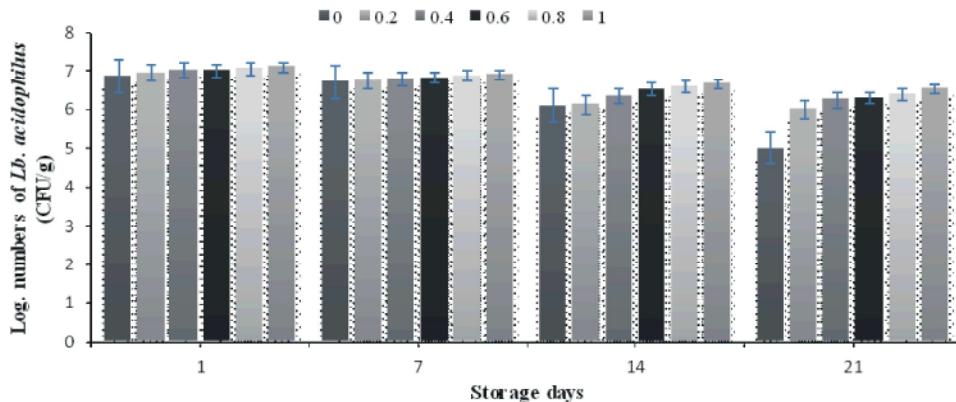


Fig. 3: Viability of *Lactobacillus acidophilus* LA-5 in yogurt with GA concentrations (0.0, 0.2, 0.4, 0.6, 0.8, 1.0%) after fermentation and storage days

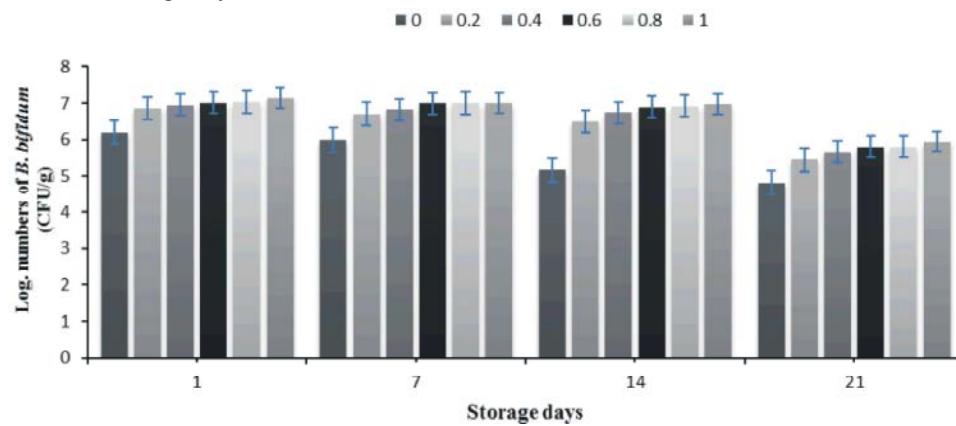


Fig. 4: Viability of *Bifidobacterium bifidum* Bb-12 in yogurt with GA concentrations (0.0, 0.2, 0.4, 0.6, 0.8, 1.0%) after fermentation and storage days

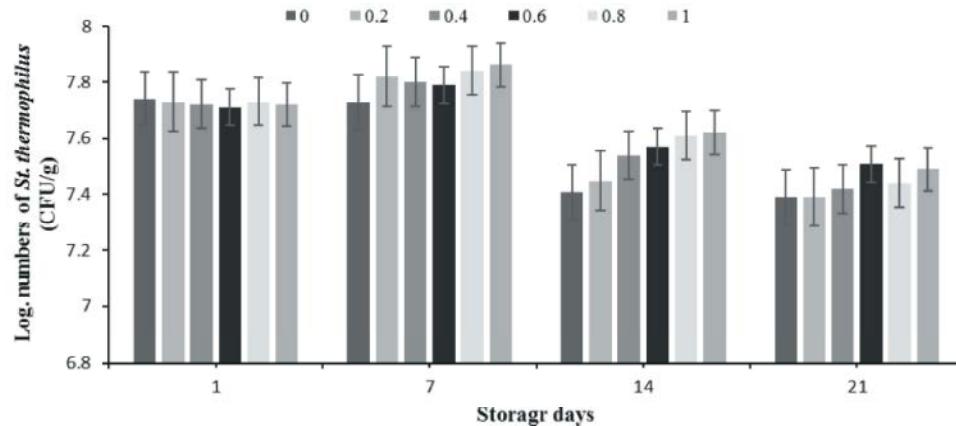


Fig. 5: Viability of *Streptococcus thermophilus* in yogurt with GA concentrations (0.0, 0.2, 0.4, 0.6, 0.8, 1.0%) after fermentation and storage days

CONCLUSIONS

The study revealed that yogurt product with GA added as prebiotic. GA has variety of properties and used in many fields such as medical, pharmaceutical and food

industries. It uses in dairy products as stabilizer and It do as prebiotic when added it in milk fermentation products. Add 1% GA to yogurt product led to improve the nutritional value through increasing the percentage of total solids, protein, fat and carbohydrates. Physical

properties of yogurt improved to increase WHC and decreasing STS, WD. GA added to yoghurt maintained survivability of *Lactobacillus acidophilus* LA-5 and *Streptococcus thermophilus* during storage time while the viability of *Bifidobacterium bifidum* Bb-12 decreased.

REFERENCES

1. Caramia, G. and S. Silvi, 2011. Probiotics: from the ancient wisdom to the actual therapeutical and nutraceutical perspective. In: Probiotic bacteria and enteric infections. Malago, J.J.; Koninkx, J. F. J. G. and Marinsek-Logar, R. (Eds). Springer, New York, USA. pp: 3-37.
2. Tamime, A.Y., M. Saarela, A.K. Sondergaard, V.V. Mistry and N.P. Shah, 2005. Production and maintenance of viability of probiotic microorganisms in dairy products. In: Probiotic dairy products. Tamime, A. (Ed.). Oxford, UK, pp: 39-56.
3. Nauta, A., A.M. Bakker-Zierikzee and M.H.C. Schoterman, 2010. Galacto-oligosaccharides. In: Handbook of prebiotics and probiotics ingredients: health benefits and food applications. Cho, S.S. and Finocchiaro, E.T. (Eds.). CRC Press, USA, pp: 75-94.
4. Birkett, A.M. and C.C. Francis, 2010. Short-chain fructo-oligosaccharide: A low molecular weight fructan. In: Handbook of prebiotics and probiotics ingredients: health benefits and food applications. Cho, S.S. and Finocchiaro, E.T. (Eds.). CRC Press, USA, pp: 13-42.
5. Paineau, D., F. Respondek and Y. Bouhnik, 2010. Inulin and oligosaccharides: a special focus on human studies. In: Handbook of prebiotics and probiotics ingredients: health benefits and food applications. Cho, S.S. and Finocchiaro, E.T. (Eds.). CRC Press, USA, pp: 43-74.
6. Clark, D.T., M.I. Gazi, S.W. Cox, B.M. Eley and G.F. Tinsley, 1993. The effects of Acacia arabica gum on the in vitro growth and protease activities of periodontopathic bacteria. Journal of Clinical Periodontology, 20(4): 238-43.
7. Al-Assaf, S., G.O. Phillips and P.A. Williams, 2005. Studies on acacia exudate gums. Part I: the molecular weight of Acacia Senegal gum exudate. Food Hydrocolloids, 19(4): 647-660.
8. Montenegro, M.A., M.L. Boiero, L.Valle and C.D. Borsarelli, 2012. Gum Arabic: more than an edible emulsifier. In: products and applications of biopolymers. Verbeek, J. (Ed.). InTech, Croatia, pp: 3-26.
9. Ali, B.H., S. Beegam, I. Al-lawati, M.I. Waly, M. Al-Zaabi and A. Nemmar, 2013. Comparative efficacy of three brands of gum acacia on adenine induced chronic renal failure in rats. Physiological Research, 62: 47-56.
10. Ali, B.H., A. Ziada and G. Blunden, 2009. Biological effects of gum arabic: A review of some recent research. Food and Chemical Toxicology, 47: 1-8.
11. Patel, S. and A. Goy, 2015. Applications of natural polymer gum Arabic: a review. International Journal of Food Properties, 18: 986-998.
12. Ibrahim, A.H. and S.A. Khalifa, 2015. The effects of various stabilizers on physicochemical properties of camel's milk yoghurt. Journal of American Science, 11(1): 15-24.
13. Tamime, A.Y. and R.K. Robinson, 1999. Yoghurt science and technology. CRC press, New York, pp: 129-131.
14. AOAC., 2000. Official Methods of Analysis of AOAC International. 17th ed. AOAC International, Arling, VA.
15. Folch, J., M. Lees and G.H.S.A. Stanley, 1957. Simple method for the isolation and purification of total lipids from animal tissues. Journal of Biological Chemistry, 226: 497-510.
16. Nielsen, S.S., 2010. Food analysis laboratory manual. Springer science and business media. New York, pp: 95-99.
17. Shori, A.B., A.S. Baba, M. Misran and H.W. Tan, 2014. Influence of *Allium sativum* or *Cinnamon verum* on physicochemical characteristics of yogurt. International Journal of Dairy Science, 9(2): 45-55.
18. Supavittipatana, P., T.I. Wirjantoro and P. Raviyani, 2010. Characteristics and shelf life of corn milk yogurt. CMU. Journal of Natural Sci., 9(1):133-149.
19. Harley, J.P. and L.M. Prescott, 2002. Laboratory exercises in microbiology .5th Ed. The McGraw – Hill companies, USA, pp: 449.
20. Dave, R.I. and N.P. Shah, 1996. Evaluation of media for selective enumeration of *Streptococcus thermophilus*, *Lactobacillus delbrueckii* ssp. *bulgaricus*, *Lactobacillus acidophilus* and bifidobacteria. Journal of Dairy Sci., 79: 1529-1536.
21. Vinderola, C.G. and J.A. Reinheimer, 1999. Culture media for the enumeration of *Bifidobacterium bifidum* and *Lactobacillus acidophilus* in the presence of yoghurt bacteria. International Dairy Journal, 9: 497-505.

22. Tabasco, R., T. Paarup, C. Janer, C. Peláez and T. Requena, 2007. Selective enumeration and identification of mixed cultures of *Streptococcus thermophilus*, *Lactobacillus delbrueckii* subsp. *bulgaricus*, *Lb. acidophilus*, *Lb. paracasei* subsp. *paracasei* and *Bifidobacterium lactis* in fermented milk. *International Dairy Journal*, 17:1107–1114.
23. Amid, B.T., H. Mirhosseini and S. Kostadinoviæ, 2012. Chemical composition and molecular structure of polysaccharide-protein biopolymer from *Durio zibethinus* seed: extraction and purification process. *Chemistry Central Journal*, 6(117): 1-14.
24. Lourens-Hattingh, A. and B.C. Viljoen, 2001. Yogurt as probiotic carrier food. *International Dairy Journal*, 11: 1-17.
25. Osman, M.E., A.R. Menzies, P.A. Williams, G.O. Phillips and T.C. Baldwin, 1993. The molecular characterization of the polysaccharide gum from Acacia Senegal. *Carbohydrate Research*, 246: 303-318.
26. Rayes, A.A.H., 2013. Comparative studies between Gum Arabic recognized as a natural prebiotic and *Bifidobacterium* as probiotic as potential cure for experimental bacterial infection in mice. *World Rural Observations*, 5(4): 128-135.
27. Kpodo, F.M.K., E.O. Afoakwa, B.B. Amoa, A.S. Budu and F.K. Saalia, 2014. Effect of ingredient variation on microbial acidification, susceptibility to syneresis, water holding capacity and viscosity of soy-peanut-cow milk yoghurt. *Journal of Nutritional Health & Food Engineering*, 1(2): 3-6.
28. Calame, W., A.R. Weseler, C. Viebke, C. Flynn and A.D. Siemensma, 2008. Gum Arabic establishes prebiotic functionality in healthy human volunteers in a dose-dependent manner. *British Journal of Nutrition*, 100: 1269-1275.
29. Islam, A.M., G.O. Phillips, A. Sljivo, M.J. Snowden and P.A. Williams, 1997. A review of recent developments on the regulatory, structural and functional aspects of gum Arabic. *Food Hydrocolloids*, 11(4): 493-505.
30. Hammes, W.P. and C. Hertel, 2009. Genus *Lactobacillus* in Bergey's manual of systematic bacteriology, 2nd Edition, Vol.3. Springer Dordrecht Heidelberg London New York, pp: 465-510.
31. Biavati, B., 2012. Genus *Bifidobacterium* in Bergey's manual of systematic bacteriology, 2nd Edition, Vol. 5. Springer Dordrecht Heidelberg, London, New York, pp: 171-205.
32. Ferdousi, R., M. Rouhi, R. Mohammadi, A.M. Mortazavian, K. Khosravi-Darani and A.H. Rad, 2013. Evaluation of probiotic survivability in yogurt exposed to cold chain interruption. *Iranian Journal of Pharmaceutical Research*, 12: 139-144.
33. Daneshi, M., M.R. Ehsani, S.H. Razavi and M. Labbafi, 2013. Effect of refrigerated storage on the probiotic survival and sensory properties of milk/carrot juice mix drink. *Electronic Journal of Biotechnology*, 16(5): 1-14.
34. Slavin, J., 2013. Fiber and prebiotics: mechanisms and health benefits. *Nutrients*, 5: 1417-1435.