PHYSICOCHEMICAL PROPERTIES OF ALOE VERA GEL AND ITS UTILIZATION IN PREPARING SYNBIOTIC FERMENTED MILK WITH *Bifidobacterium Lactis* BB-12

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ABSTRACT

This study was aimed to investigate the physicochemical structure of fresh and dried Aloe Vera gel (AVG) and its adequacy as a prebiotic in the preparation of Synbiotic fermented milk with *Bif. Lactis* BB-12, in addition to its effect on the survival of these bacteria during 28 days of cold storage. The results indicated that the yield of the gel was % 64.56±1.157, and the moisture content, total solids (TS) and total soluble solids (TSS) in the fresh gel were % 98.23±0.0816, %1.777±0.0103 and % 1.56±0.0816 respectively. The pH of fresh and dried gel was 4.48±0.0105 and 4.63±0.01, respectively, meanwhile the acidity was 0.083±0.00121 and 0.058±0.0083, respectively (expressed as malic acid). Addition of 0.03% , 0.05% of dried and 3% ,5% of fresh AVG to treatments under study which represented as B1, B2, B3, and B4 respectively, reduced the generation time (GT) to 71.91, 68.49, 80.14 and 95.17 min, respectively as compared to that of the control treatment B0 (AVG free), which was 112.52 min. The pH value decreased in treatments B0, B1, B2, B3, and B4 to 4.45, 4.41, 4.36, 4.39 and 4.38, respectively, as the acidity increased to 1.13, 1.18, 1.26, 1.20 and 1.21%, respectively.

Keywords: growth curve, prebiotic, Survival, generation time

الصفات الفيزيوكيميائية لهلام الاوليفيرا واستعماله في تحضير مخمر لبني بالتآزر الحيوي مع بكتريا *Bifidobacteriu Lactis* BB-12

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المستخلص

هدفت هذه الدراسة الى تقصي الصفات الفيزيوكيميائية لهلام الاوليفيرا الطازج والمجفف وإمكانية استعماله كمحفز حيوي في تحضير مخمر لبني بالتآزر الحيوي مع بكتريا المعزز الحيوي *Bif. Lactis* BB-12 بالإضافة الى دراسة تأثيره على عيوشيتها خلال 28 يوم من الخزن المبرد. أشارت النتائج إلى أن حاصل الهلام كان 64.56±1.157٪، أما المحتوى الرطوبي والمواد الصلبة الكلية (TS) والمواد الصلبة الذائبة الكلية (TSS) في الهلام الطازج كانت 98.23±0.0816٪، 1.777±0.103٪ و1.56±0.0816٪ على الترتيب. كان pH الهلام الطازج والمجفف 4.48±0.0105 و4.63±0.01 على الترتيب، بينما كانت الحموضة التسحيحية بواقع 0.083±0.00121 و0.058±0.0083 على الترتيب معبراً عنها كحامض الماليك. أدت إضافة هلام الاوليفيرا بتركيز 0.03% و0.05% مجفف و3% و5% طازج إلى المعاملات قيد الدراسة والتي تمثلت بـ B1 وB2 وB3 وB4 على الترتيب إلى خفض زمن الجيل (GT) الذي بلغ حوالي 71.91 و68.49 و80.14 و95.17 دقيقة على الترتيب، مقارنة مع معاملة السيطرة B0 اذ بلغ 112.52 دقيقة. لوحظ انخفاض pH في المعاملاتB0 وB1 وB2 وB3 وB4 الى حوالي 4.45 و 4.41 و 4.36 و 4.39 و 4.38 على الترتيب، رافق ذلك ارتفاع الحموضة التسحيحية اذ بلغت 1.13 و 1.18 و 1.26 و 1.20 و 1.21٪ على الترتيب.

الكلمات المفتاحية: منحني النمو، المحفزات الحيوية، العيوشية، زمن الجيل

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INTRODUCTION

Aloe vera is one of the oldest and most famous medicinal plants, it has been used for more than 5000 BC. It was used for medicinal purposes in most ancient civilizations such as Mexico, Greece, Egypt, India and China (23). The first documentation of its use by humans was in the Sumerian hieroglyphics inscribed on the clay tablets of the Mesopotamian civilization around 2200 BC, as it was used as a treatment for constipation and as a laxative (35). The Greeks described it as a healing balm, as Aristotle's explained its unique properties for treating burns, wounds and infections. The Pharaohs called it the "plant of immortality" It was mentioned in the ancient Egyptian medical records written on papyrus during the sixteenth century BC, as the queens of Egypt, Cleopatra and Nefertiti, used to acquire and use it in skin care recipes on a daily basis (10). During the workshop held on November 9, 2012 in Las Vegas, in conjunction with the Supply Side West trade fair, the International Aloe Science Council (IASC) indicated that the global market for Aloe vera products has reached $13 billion (16). At present, there is great interest in aloe vera as a potential source of functional nutritional supplements as the gel is used in many products such as fresh aloe vera gel, juice or other formulations used for health, medical and cosmetic purposes. This may be attributed to the fact that it contains interesting bioactive components, particularly acemannan, phenolic compounds, and anthrone C-glycosides (22). The Food and Drug Administration (FDA) has approved the oral consumption of aloe vera juice as a "dietary supplement" in the United States. It is also included among the "flavoring compounds" in the food industry within Annex I of Regulations to European Commission Law No. 1831/2003 (15). AVG appears to promote the growth of probiotic bacteria and has therefore been used for a long time in the treatment and prevention of malnutrition, chronic diarrhea and gastrointestinal disease in many countries. Nagpal *et al.* (33) reported that AVG prepared at 5% enhanced the growth and development of L. acidophilus, L. plantarum and L. casei. The fermented milks is one of the most widely distributed dairy products in the world and is widely consumed so it is popular food, It may be a modern food, but it has ancient origins (39). Fermented milks fortified with prebiotics and probiotics is one of the most important nutritionally and healthy synergistic functional foods. Many starters consisting of lactic acid bacteria strains are used in milk fermentation, for their high ability to hydrolyze milk proteins and produce vital peptides capable of inhibiting the activities of α-amylase, glucosidase and pancreatic lipase enzymes, thus reducing the chances of obesity or diabetes (26). Eating Synbiotic foods promotes the growth and activity of beneficial bacterial strains in the gut. In the same way, prebiotics selectively stimulate the growth of beneficial bacteria without harmful bacteria and give them protection during their passage in the upper part of the alimentary canal, as well as improving the sensory qualities of fermented milks and making it more acceptable to consumers. It was recently revealed that AVG can be used as a source of prebiotics. It has been observed that polysaccharides such as glucomannan known as acemannan promote the growth of beneficial microbial communities more than inulin (25). It is also able to increase the growth of *Bifidobacterium* spp. and produce more short chain fatty acids (SCFAs) than commercial prebiotic (FOS) (37). This leads to the regulation of the immune system and the control of the balance of inflammatory cytokines (6). The aim of this study was to estimate the chemical composition of AVG, and evaluate its effects on the physicochemical and microbiological of Synbiotic fermented milks produced with *Bifidobacterium Lactis* BB-12, as well as on the viability of these bacteria.

MATERIALS AND METHODS

Collection of samples: Aloe vera leaves (3 years old) were obtained from the Medicinal Plants Unit of the Faculty of Agricultural Engineering Sciences - University of Baghdad, after being diagnosed and classified as "*barbadensis miller*". Leaves were cleaned and washed with distilled water several times to get rid of dust and dirt. They were then disinfected by immersing them in a 100 ppm solution of sodium hypochlorite for 5 minutes and then washed with large volumes of

distilled water to remove the disinfectant solution residue. Next, the bottom part of the leaf base, the upper ends and the side spines were cut, and placed in vertically in water for 24 hours to leave out the latex (aloin). The outer green shell was removed and the inner gel was washed with large amounts of water to get rid of the remnants of latex, then the gel homogenized in a blender to obtain a homogeneous gel, then a filtered to exclude the residues and remaining crust particles. The gel yield ratio was calculated according to (2) based on the following equation: The resulting gel was dried in the air oven at 45˚ C for 24 hours. Then ground by an electric grinder and kept in dark glass containers at the temperature of the refrigerator until use.

$$\text{Gel Yield (\%)} = \frac{\text{Weight of gel}}{\text{Weight of leaves}} \times 100$$

Physicochemical analysis of AVG

Moisture content: The moisture content of AVG was estimated using air oven at 103 ± 5 °C until the weight is stable (5).

Total soluble solids (TSS)

A hand refractometer was used to measure the total soluble solids of gel as described by (5).

Fat content

The percentage of fat was determined according to the method described by (17)

Protein content

The protein content of dried AVG was determined according to (17)

Ash content

The ash content was determined by combustion of 2 g of dried gel powder in a crucible of a known weight in a muffle furnace at 600˚ C until a light white ash was formed, then cooled in a desecrator, (17).

Fibers content

The fiber content was determined according to (17) by digesting 3 g of defatted AVG powder in 200 ml of sulfuric acid solution (1.25%). The mixture stirred using Magnet stirrer at boiling temperature for half an hour, then filtered with a cloth and the precipitate was washed with hot distilled water to remove the acid. After that, the precipitate was re-digested with a (1.25%) sodium hydroxide solution in the same way mentioned and washed with hot distilled water to remove the base, then the precipitate was dried in a drying oven at 100 ° C until a constant weight was reached, which represents the weight of the fibers plus ash (A). The latter was placed in a muffle furnace at 550°C/4h, the weight of the obtained ash designated as (B). The following equation was adopted to calculate the fibers:

$$\text{Fibers content (\%)} = \frac{A - B}{\text{Sample weight}} \times 100$$

Determination of non-nitrogenous compounds (Nn%)

The non-nitrogenous compounds (including carbohydrates, vitamins and some water-soluble organic compounds) in the model were calculated according to (32) on the basis of dry weight as follows:

$$\text{Nn \%} = 100 - (\text{protein\%} + \text{fat\%} + \text{ash\%} + \text{moisture\%} + \text{fiber \%})$$

pH: The pH of AVG was estimated as reported by Silva-Vazquez *et al.* (40). Dissolve 2 g of dried aloe vera powder in 75 ml of distilled water (pH 7). The pH of the fresh gel was measured directly by immersing the electrode of a pH meter (model 211, type HANNA) in the gel.

Titration acidity

The titration acidity of fresh AVG was estimated according to Silva-Vazquez *et al.* (40). expressed as a percentage of malic acid. six g of gel was transferred to a 100 ml beaker and 50 ml of deionized water was added to it. Then titration was done with 0.1N NaOH until the end point was reached at pH 8.2 and the amount of base consumed (ml) was recorded. The acidity was calculated (g of malic acid / 100g of sample) as follows:

$$\text{Acidity \%} = \frac{\text{NaoH (ml) X 0.1 X 0.067}}{\text{Sample Weight (g)}} \times 100$$

Skim milk and Probiotic

Commercial skimmed milk (Slim), produced by the Omani Al-Modhesh Company, reconstituted (12%) using distilled water. *Bifidobacterium Lactis*-BB12 bacteria from PROBIOTIC QUEST, UK, in lyophilized form was used after reactivation for three times as it was incubated at 37°C until reaching the daily working starter.

Effect of AVG on the generation time (GT) and growth curve of *Bif. Lactis* BB-12

The generation time of *Bif. Lactis* BB-12 was estimated in milk models supplemented with AVG according to (4).

Bacteria were activated three times on MRS-broth at 37°C. AVG was added to the skimmed milk at (0.03%, 0.05% as dried) and (3%, 5% as fresh) represented as treatments B1, B2, B3, and B4, respectively, B0 was AVG-free control sample and sterilized at 121°C for 5 minutes. Then it cooled to 37° C and inoculated with 5% of the *Bif. Lactis* BB-12. Samples were taken every two hours for a period of 24 hours and the viable numbers of the probiotic were counted by pouring plates onto MRS-agar under anaerobic conditions. The generation time was determinated according to the following equation.

$$\text{Generation time (G.T)} = \frac{t \times 0.301}{\text{Log10 Nt - Log10 N0}}$$

Where, t: The time (minutes) required for the initial number to reach the final number; Nt: Log of the final number; N0: Log of the initial number.

Preparation of fermented milk

Skimmed milk (12%) was reconstituted, then aloe vera gel was added, pasteurized at (90-95°C for 5-10 min), then cooled to incubation temperature and starter culture (5%) was added, then packed and incubated at 37°C and store at 5±1°C

Microbial analysis

Viable numbers of *Bif. Lactis* BB-12

The method described by Speak (42) was used to find out the viable numbers of *Bif. Lactis* BB-12. As 1 ml of decadal dilutions prepared from bacterial cultures were transferred to sterile Petri dishes, then MRS agar was poured over them in homogeneous quantities with horizontal stirring from different directions. Then incubated at 37°C for 48 hours under anaerobic conditions. .Then, petri dishes with colonies number between 30-300 were counted using a colony-counting device, and the number of bacteria per milliliter was counted (average number of colonies for two petri dishes X inverted dilution).

Total coliform bacteria

Total coliforms bacteria of the product were determined as described in (42) using Mac-Conkey agar.

Molds and yeasts

Molds and yeasts were estimated using potato dextrose agar and incubated at 25˚ C for 5 days, according to (42).

Psychrotrophic bacteria

Psychrotrophic bacteria were estimated using Nutrient agar and incubated at 5˚ C for 10 days, according to (42).

pH and Total acidity of fermented milk

The pH was determined by placing the cathode of the Romanian HANNA Model 211 pH meter directly into the sample.

Total acidity was determined according to (17). 9 g of sample was transferred to a beaker and 1 ml of 1% phenolphthalein reagent was added and titrated with 0.1 N NaOH until pink color appeared.

Statistical analysis

The statistical analysis program GenStat V.12.1 was used. The statistical analysis of the data was carried out according to Completely Randomized Design (CRD). The averages for the different treatments were compared by Duncan's Multiple Range test) at the level ($p \leq 0.05$) in order to determine the significant differences between the means.

RESULTS A ND D ISCUSSION

Physicochemical analysis of AVG

Fresh AVG: Table (1) shows the yield and chemical composition of fresh and dried AVG. The gel yield was 64.56 ± 1.157%. This is consistent with Muñoz *et al.* (32), who reported that the gel yield was 64.03%, while the outer shell was 34.23%, with a loss of 1.74% during handling and preparation. In addition, the result was in agreement with Ahmad *et al*. (2), who found that the gel yield ranged between 62.45 - 65.66%. The moisture content, total solids and soluble solids of the gel before drying were about %98.23 ±0.0816, %1.777 ±0.0103 and %1.56 ± 0.0816, respectively. These results agree with the previous studies which mentioned that the AVG moisture content was 98% (1). While Hęś *et al.* (19) reported that AVG is mainly composed of 96% moisture and 4% solids including proteins, fats, dietary fiber and ash (6.86%, 2.91%, 73.35% and 16.88%) respectively. The pH of fresh AVG was 4.48±0.0105 and the acidity was 0.083±0.00121 g malic acid /100g. While for dried AVG was 4.63±0.01 and 0.058±0.0083g as malic acid/100g, respectively. These results are in agreement with Calderón-Oliver *et al.* (8) who reported that the pH for AVG ranging from 4.4 - 4.7. In general, the pH values of the experimental samples are within the range set

by the International Aloe Science Council, which ranged 3.5 - 4.7 (2).

Table 1. Yield and Physicochemical properties of AVG

Parameters	Results
Gel Yield	64.56 ± 1.157 %
Moisture % (fresh weight)	98.23 ±0.0816 %
Total Solids TS%	1.777 ± 0.0103 %
Total soluble solids TSS% (Brix)	1.56 ± 0.0816 %
pH of fresh AVG	4.48 ± 0.0105
Acidity for fresh AVG	0.083 ± 0.00121 g/100g
pH of Dried AVG	4.63±0.01
Acidity of dried AVG	0.058±0.0083 g/100g

**** Values are given as mean of three replicates ± SD**

The high acidity and low pH of the AVG may be due to the accumulation of organic acids such as malic acid, salicylic acid, citric acid and isocitric acid (45). In addition AVG has high content of ascorbic acid (vitamin C), which may reach to 40 mg/100g (13).

Composition of dried AVG

Figure (1) shows the total composition of the dried AVG. The moisture content was 3.096% ± 0.0966. This result is close to that reported by (1) who reported that the final moisture of the powder is often less than 4% in the dried aloe vera gel as determined by Qmatrix method, which is an innovative energy-saving technology used to dry aloe vera gel at low temperature/short time (LTST) with an average moisture of about 2.5% in the final powder. While Haque *et al.* (17) reported that the moisture content of whole aloe vera leaf powder that was dried by a solar dryer for 16 hours was 6.75%. The protein, fat and ash content of dried AVG were 7.092% ± 0.532, 3.81% ± 0.114 and 17.16% ± 0.386, respectively. These results partially agree with Luta and McAnalley (28), who reported that the protein, fat and ash content of AVG were 7%, 4% and 16%, respectively, calculated on the basis of dry weight. On the other hand, Muñoz *et al.* (32) reported that the protein, fat and ash content of dried AVG were 6.11% ±3.47, 2.07% ± 0.61 and 17.20% ±0.99, respectively. Ahmed and Hussain (3) also noted that the above components in the dried AVG were 6.86%±0.06, 2.91% ±0.09, and 16.88% ±0.04, respectively. The difference in the chemical composition of the Aloe vera plant is affected by many factors such as the geographical location of cultivation, soil quality, water availability, sunlight and temperature (32). Therefore, the discrepancy in results between different researches may be due to the environment in which these plants were grown, as well as the quality of the soil, fertilizers and the abundance of water (2). The methods of estimating the components and treatments used during the preparation processes, such as drying and extraction, may also have an impact on the variation and difference of these results (22).

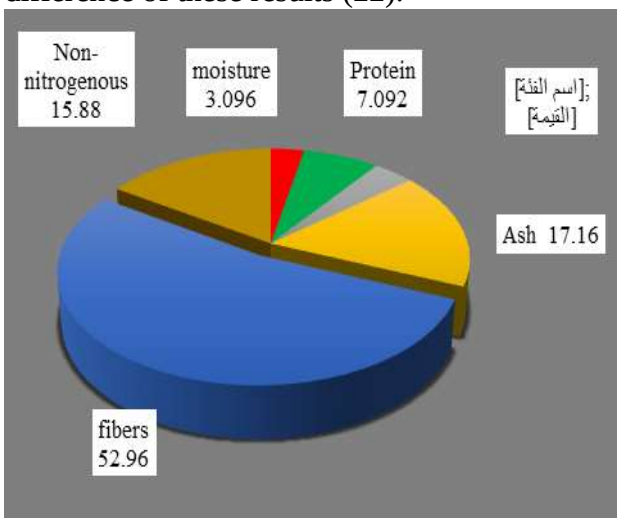

Figure 1. Total composition of dried AVG

Figure (1) also shows the total fiber content in the dried aloe vera gel, which was 52.96% ± 0.345. This result is close to what was reported by Luta and McAnalley (28) and Tornero-Martínez *et al*. (43) who reported that the crude fibers is about 55% of the dry weight of AVG. On the other hand, these results were lower than Hęś *et al*. (19) who reported that the fiber content of aloe vera leaves is 73.35% on a dry matter basis. Whereas, (45) showed that the gel samples separated from the soluble components were contained 45.8 g/100 g of crude fibers on a dry weight basis. He confirmed that the ratio of dry soluble parts in the inner gel of aloe vera leaves, which mostly represent polysaccharides to insoluble parts, ranged from 2:1 to 3:1. Polysaccharides are the main component responsible for most of the beneficial properties attributed to aloe vera gel, which can provide health benefits for patients with diabetes, obesity, inflammatory bowel disease and neurodegenerative diseases

when consumed on a regular basis (30). Figure (1) also shows the content of non-nitrogenous compounds in the dried aloe vera gel was 15.88% ± 0.483, which consisted mainly of available carbohydrate as well as some organic acids, phenolic compounds, and some vitamins and enzymes (32). This result is similar to what Ahlawat and Khatkar (1) reported that the available carbohydrates constitute was 17% of the dry weight of AVG. While it was lower than what (43) found, the non-nitrogenous components were 26.85±1.1 g/100g of dry gel. However, Ahmad *et al*. (2) noted that the average carbohydrate available in aloe vera gel is 84.70% ± 0.80 for different seasons, which include mannose, glucose, galactose, arabinose, and xylose, as well as starch and dietary fiber. Several researchers have stated that the chemical composition of the parts of the aloe vera plant may vary from place to place, emphasizing that the age of the plant, the soil in which it grows, and weather play major roles in the overall variance. In addition to the harvest season, processing methods and poor storage (30).

Effect of AVG on the generation time (GT) and growth curve of *Bif. Lactis* BB-12

Table (2) shows a decrease in the generation time of *Bif. Lactis* BB-12 in treatments with added AVG (B1, B2, B3 and B4), which were about 72, 68.5, 80 and 95 min, respectively, compared with control treatment B0, where the generation time was 112.5 min. Martínez-Villaluenga and Gómez (29) observed that *Bif. Lactis* BB-12 achieved the shortest generation time of 1.51±0.01 hours compared with six other *bifidobacteria* spp. when grown in fermented milk enriched with oligosaccharides isolated from lupin and used as a prebiotic. In another study, it was observed that the generation time of *Bif. Lactis* BB-12 in cow's milk was 2.11 ± 0.35 hours, compared with 2.13 ± 1.71 and 4.81 ± 1.35 hours in goat's milk and soy milk, respectively (41). In another study conducted by Cicvarek *et al.* (11), it was noted that the shortest generation time for this bacteria was 38 minutes in the medium supplemented with caseinomacro peptide (CMP), compared with two hours in the unsupported medium. Figure (2) shows the growth curve of *Bif. Lactis* BB-12 during its growth in skimmed milk supplemented with different percentages of aloe vera gel B0, B1, B2, B3 and B4 for 24 hours. It is observed that the lag-phase persists for all treatments between 8-10 hours, which is the phase in which the number of cells remains constant, but their size increases and their enzymes start to become active (11). It appears that the increased in the growth rates during the first six hours may be due to the presence of monosaccharides in the milk and the added prebiotic (41). In general, the lag-phase is similar to what was founded by Cicvarek *et al.* (11) who reported that the growth of *Bif. Lactis* BB-12 was weak in non-supplemented milk, but after adding a prebiotic, it was reduced to 8 hours. This is what was observe during the experiment, there was an increase in growth rates after adding AVG and the generation time became shorter, as B2 treatment achieved the maximum growth rate and the shortest generation time. This may be due to the presence of partially acetylated polysaccharides acemannan, which is a good precursor to support the growth of lactic acid bacteria (41).

Table 2. Generation time of Bif. Lactis BB-12 during its growth in skimmed milk supplemented with different concentrations of AVG during 24 hours

Treat.	*B0*	*B1*	*B2*	*B3*	*B4*
G.T (min)	**112.5226**	**71.91155**	**68.49893**	**80.14716**	**95.17017**

****Parameters B0, B1, B2, B3 and B4 represent skimmed milk supplemented with 0%, 0.03%, 0.05% dried and 3% , 5% fresh AVG, respectively**

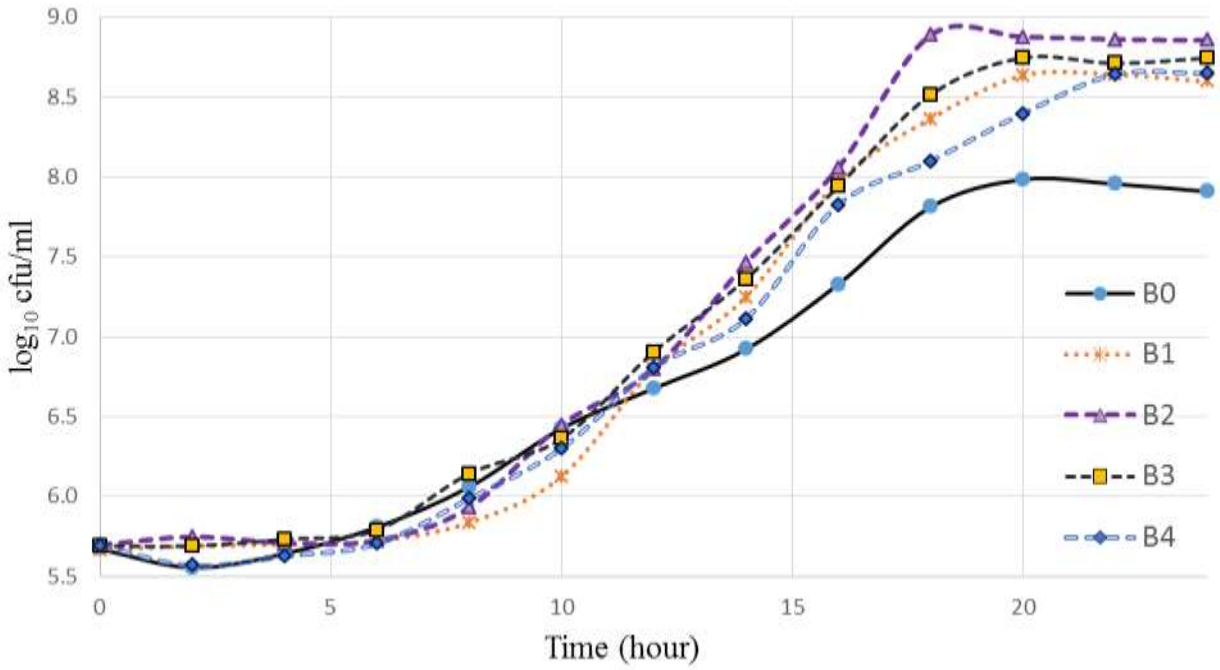

Figure 2. Growth curve of Bif Lactis BB-12 during its growth in skimmed milk supplemented with different percentages of AVG during 24 hours

The growth curve of the obtained *Bif. Lactis* BB-12 is similar to the growth curve of these bacteria in several previous studies in terms of Lag phase, log phase, growth rates, and stationary phase (29,41), However, it differs from what was found by Ali *et al*. (3). This may be due to the difference in the culture medium used, the type of bacteria and the method used in the bacterial count (4).

Effect of adding dried and fresh AVG and cold storage on the survival of *Bif. Lactis* BB-12: Table (3) shows the effect of adding different percentages of dried and fresh AVG to skimmed milk on the survival of *Bif. Lactis* BB-12 during cold storage at 5°C for 28 days. It was noticed on the first day after manufacturing a significant superiority at ($p \leq 0.05$) for treatment B2, which achieved the highest logarithm mean viable count of 9.821 ± 0.109 cfu/ml compared with the other treatments. Significant superiority of treatments B1, B3 and B4 was also observed, with their values reaching 9.509 ± 0.109, 9.493 ± 0.131 and 9.515 ± 0.147 cfu/ml, respectively, compared with the control treatment B0, which recorded the lowest viable numbers value of 9.012 ± 0.0917 cfu/ml. The results continued at the same rate until the end of the experiment on day 28. The viable numbers of treatment B2 were the highest 7.478 ± 0.192 cfu/ml with significant superiority over the other treatments B1, B3 and B4, as the differences among them were not significant. The viable numbers were 6.847 ± 0.0894, 6.983 ± 0.0730, and 7.015 ± 0.0741 cfu/ml, respectively. However, all treatments differed significantly with control treatment B0, which recorded the lowest value of viable numbers at the end of the experiment, which was 6.538 ± 0.112 cfu/ml. It is also noted that treatments B2 and B4 maintained viable bacterial numbers higher than 7 logarithmic cycles at the end of the storage period. This corresponds to the minimum acceptable number of probiotics in dairy products required to provide health benefits to the consumer, which is 10^7 cfu/ml (7). The decrease in the viability and numbers of bacteria with the age of the product may be attributed to the depletion of sugars in the fermented milks. As well as the low pH and the accumulation of organic acids resulting from growth and fermentation, which are among the most important reasons for its loss of viability (34) Adding AVG to skimmed milk had a positive effect on the survival of *Bif. Lactis* BB-12 bacteria and their viability during storage. This may be due to the gel's high content of polysaccharides such as acemannan and glucomannan, which are a good carbon source for lactic acid bacteria (9). The results obtained are consistent with

Nagpal *et al.* (33) findings who reported that the highest growth rate of probiotic bacteria was with adding 5% of AVG. Due to the special nutritional requirements and the ability to consume polysaccharides, a selective growth of *Bif. Lactis* BB-12 bacteria occurs, which leads to the production of good amounts of SCFA, which in turn inhibits the growth of other bacterial species (26).

Table 3. Log_{10} Total viable numbers of Bif. Lactis BB-12 in skimmed milk to which different amounts of AVG were added during cold storage for 28 days.

Starter culture	Treat.	Age of fermented milk (days)				
		1d	7d	14d	21d	28d
log_{10} cfu/ml For *Bif. Lactis* BB-12	B0	a	a	a	a	a
		9.012±0.0917	8.585±0.0892	7.809±0.0904	7.230±0.0760	6.538±0.112
	B1	b	a	b	b	b
		9.509±0.109	8.711±0.118	8.190±0.0573	7.582±0.0956	6.847±0.0894
	B2	c	b	c	c	c
		9.821±0.109	9.079±0.219	8.917±0.0883	8.088±0.10	7.478±0.192
	B3	b	a	b	b	b
		9.493±0.131	8.805±0.0873	8.096±0.0622	7.747±0.161	6.983±0.0730
	B4	b	a	b	b	b
		9.515±0.147	8.808±0.0810	8.061±0.0544	7.709±0.114	7.015±0.0741
L.S.D		0.2166	0.2357	0.1314	0.2058	0.2126

*** Values are given as mean of three replicates ± SD.**

****The averages followed by different small letters within the same column indicate a significant difference at ($p \leq 0.05$)**

Parameters B0, B1, B2, B3 and B4 represent skimmed milk supplemented with 0%, 0.03%, 0.05% dried and 3% , 5% fresh AVG, respectively

Hussain *et al.* (21) reported an inhibitory effect of aloe vera gel juice on lactic acid bacteria in functional fermented milks "lassi" with increased addition ratio, which may be due to the antagonistic action of some secondary metabolites present in AVG, such as anthraquinone (9). This may be similar to what happened in sample B4. Which started with slow growth and then began to show synergistic action with the age of fermented milks. Pellizzoni *et al.* (36) mentioned that the secondary metabolites in AVG are significantly affected by temperature and storage duration and this may underestimate the antagonistic effect of the gel, and with the presence of polysaccharides promotes the growth of lactic acid bacteria. Thus, it may offset the antimicrobial activity of AVG, allowing the growth of lactic acid bacteria and promoting their survival (44). Most of the *Bifidobacterium* spp., especially *Bif. Lactis* BB-12 are highly capable of consuming low-polymerized polysaccharides such as inulin. The addition of polysaccharides with milk significantly increases the number and survival of starter bacteria in fermented milks, and this increase in the number and survival of bacteria confirms the synbiotic action between polysaccharides that act as prebiotics and probiotic bacteria that may apply to the polysaccharides found in AVG such as acemannan (12).

Contamination tests

Regarding the contamination test, there were no growth of coliform bacteria and Psychrotrophic bacteria as well as yeasts and molds in the fermented milks produced by the probiotic *Bif. Lactis* BB-12, the product did not show any signs of spoilage during the 28 days of storage. This may be due to the proper laboratory fermented milks production method, during which it is exposed to high temperatures and sterile and controlled manufacturing conditions. In addition to the role of the used bacteria and their possession of many inhibitory mechanisms against pathogenic bacteria or causing food spoilage, as during the fermentation processes, substances with an inhibitory effect were produced, including organic acids, the most important of which is lactic acid. As well as the ability of lactic acid bacteria strains to inhibit types of bacteria spoiling milk by producing different types of bacteriocins such as bifidocin (38).

pH of fermented milk

Figure (3) shows the pH values of fermented milks for treatments to which aloe vera gel was added after processing and during the 28-day storage period. The control treatment B0

had the lowest pH value after one day of processing, being 4.89, which differed significantly as compared to treatments B1, B2 and B3, their pH values were 4.94, 4.93 and 4.93, respectively. While it did not differ significantly as compared to pH of treatment B4, which was 4.907. This result is higher than what was found by Ozturkoglu-Budak *et al.* (34), who reported that the pH value in the fermented milks of these bacteria was 4.79 using the 2% starter and the viable cell rate was 10^8 cfu/ml. During the cold storage period, a decrease in the pH values was observed for all treatments, as the lowest values were after 28 days of storage in treatments B2, B3, and B4, as their pH values were 4.36, 4.39 and 4.38,respectively which differed significantly compared with the control treatment B0, which was 4.45. While treatment B1 did not differ significantly in comparison to the rest, its pH value was 4.41.

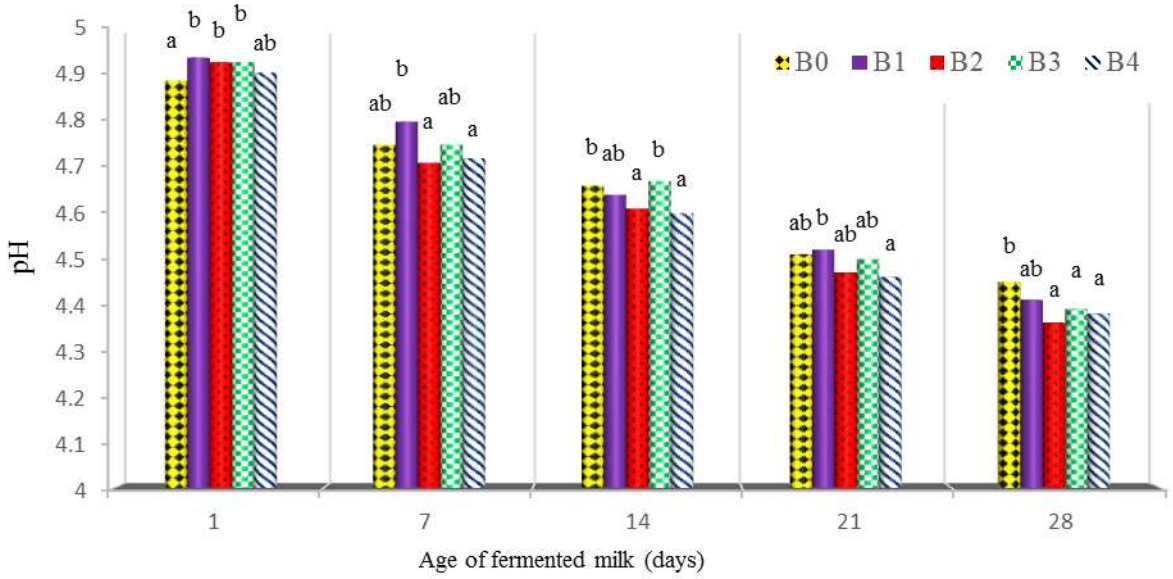

Figure 3. pH values of fermented milks after processing and during cold storage for 28 days

The decrease in pH during storage is related to the production of organic acids resulting from the fermentation of lactose by the probiotic starter bacteria, *Bif. Lactis* BB-12 ferments lactose into acetic acid and lactic acid in a ratio of up to 3:2 compared with the starter bacteria of yogurt (18). As for the decrease in the pH value of the treatments with added AVG compared to the control treatment, it may be due to the ability of BB-12 bacteria to metabolize and consume soluble fibers such as acemannan found in AVG. Which obviously contribute to increasing the number and survival of these bacteria cells during the storage period or protecting the bacterial cells from the effects of the extreme environment and secondary metabolites in the medium, which is often the reason for the low numbers of probiotics in the cold-stored fermented milks (14). Many studies reported that *Bif. Lactis* BB-12 bacteria can tolerate a low pH that ranges between 5-2 because its low leads to stimulating the activity of the enzyme H^+ - ATPase, an enzyme complex involved in maintaining the pH balance inside cells in bacteria (24).

Total acidity of fermented milk

Figure (4) shows the total acidity of fermented milks for the control and the treatments to which AVG was added after processing and during 28 days of cold storage. The control B0 had the highest acidity after one day of production, reaching 0.78%, which differed significantly at the probability level ($p \leq 0.05$) compared with treatments B1 and B3, which recorded the lowest acidity of 0.75%. While there were no significant differences compared with treatments B2 and B4, as the total acidity was 0.76% and 0.78%, respectively. This result is similar to Mukhekar and Desale (31), who reported that the acidity ranged between 0.64 - 0.79%, which increased with increase the amount of aloe vera gel fortification. The increase in acidity after one day of processing in control B0 compared to treatments B1 and B3 may be due to the increase in the buffering capacity of the milk resulting from the addition of dried or fresh AVG, which requires

additional development of the acid by the starter bacteria to reach the required pH (27). While there was no significant difference compared to treatments B2 and B4, it may be due to the increase in the number of starter bacteria with the increase in the amount of addition of the gel, which encouraged its growth and production of larger amounts of acid, and then contributed to overcoming the "buffering capacity" and developing the acidity of the product. It is also noted that the total acidity increased in all treatments with the age of the product, as well as the presence of significant differences between the treatments at ($p \leq 0.05$). The highest acidity was recorded after 28 days of cold storage in treatment B2, which was 1.26%, which differed significantly compared with control B0, which recorded the lowest acidity of 1.133% and treatment B1, which was 1.18%, while it did not differ significantly compared with treatments B3 and B4, as the their total acidity were 1.20% and 1.21%, respectively. These results are consistent with Houshang *et al*. (20), who reported that increasing the fortification of Aloe vera gel for the milk used in the preparation of fermented milks for *Bifidobacterium Bifidum* led to a faster increase in total acidity compared to the unfortified control or treatments fortified with a lower amount of gel, which it had a positive effect on the growth of these bacteria and contributed to reaching the preferred acidity level during the cold storage period. Studies indicate that Bifidobacterium is able to use polysaccharides and produce amounts of different organic acids that contribute to an increase in the total acidity of fermented milks during cold storage (14).

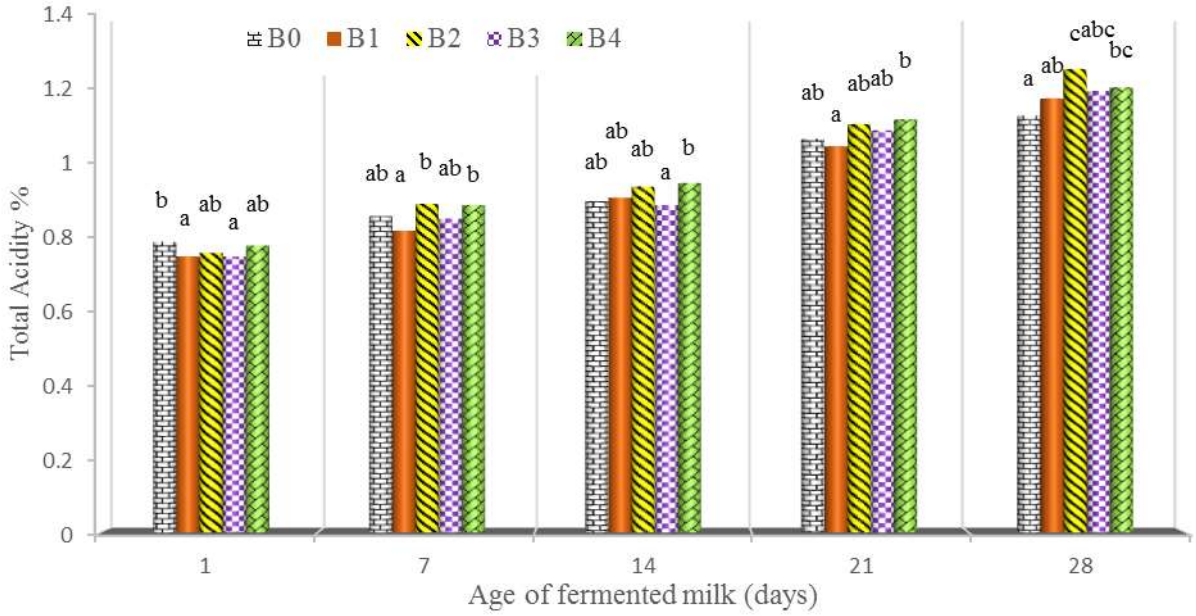

Figure 4. Total Acidity % of fermented milk after processing and during cold storage for 28 days

CONCLUSIONS

It is here by concluded that the use of aloe vera gel in the preparation of Synbiotic fermented milks encouraged the growth and survival of *Bif. Lactis* BB-12 bacteria and kept its numbers above 10^7 cfu/ml during 28 days of cold storage, especially the treatments B2 and B4. Hence, the using of aloe vera gel as a prebiotic is promising in the preparation of Synbiotic foods.

REFERENCES

1**.** Ahlawat, K. S., and B. S. Khatkar, 2011. Processing, food applications and safety of aloe vera products: a review. Journal of Food Science and Technology, 48(5), 525-533. doi: 10.1007/s13197-011-0229-z

2. Ahmad, N., M. Sarfraz, N. Akhtar, K. Hussain, W. Ahmad, and A. B. Sadique, 2020. Effect of seasonal variation on the recovery and composition of aloe vera (*Aloe Barbadensis*) Gel. J. Agric. Res, 58(4), 253-259.https://apply.jar.punjab.gov.pk/upload/160 9395880_142_6._1400_.pdf

3. Ahmed, M., and F. Hussain, 2013. Chemical composition and biochemical activity of Aloe vera (*Aloe barbadensis Miller*) leaves. Int. J.

Chem. Biochem. Sci, 3, 29-33. Semanticscholar.

4. Ali, M. A., W. Hui, G. Lin, F. F. Dang, S. Wu, C. Man, and Y. Jiang, 2019. Development of a standard curve to account for viable loads of *Bifidobacterium animalis subsp.lactis* HN019 using RNA by real-time PCR. Journal of AOAC International, 102(2) 532-538. DOI: 10.5740/jaoacint.18-0216

5. Association of Official Agricultural Chemists A.O.A.C 2012. Official methods of analysis, 18th ed.: AOAC International

6. Aziz, H. I., and, D. J. Al-Hawezy 2022. Effects of probiotic, prebiotic and symbiotic on broiler breeder performance, egg production at different stock density. Iraqi Journal of Agricultural Sciences, 53(3), 636-644. https://doi.org/10.36103/ijas.v53i3.1573

7. Boylston, T. D., C. G. Vinderola, H. B. Ghoddusi, and J. A. Reinheimer, 2004. Incorporation of bifidobacteria into cheeses: challenges and rewards. International Dairy Journal, 14(5),375-387.
https://doi.org/10.1016/j.idairyj.2003.08.008

8. Calderón-Oliver, M., M. A. Q. Peña, and J. Pedraza-Chaverrí, 2011. Beneficial effects of Aloe in health.Vertientes Revista Especializada en Ciencias de la Salud 14(2), 53-73.
doi.org/10.1111/1750./vientes3841.13568

9. Chiodelli, G., M. Pellizzoni, G. Ruzickova, and L. Lucini, 2017. Effect of different aloe fractions on the growth of lactic acid bacteria. Journal of food science, 82(1), 219-224. https://doi.org/10.1111/1750-3841.13568

10. Christaki, E. V., and P. C. Florou-Paneri, 2010. Aloe vera: a plant for many uses. J Food Agric Environ, 8(2), 245-249.
doi: 10.1111/**j**.1365-2621.1986.tb10861

11. Cicvarek, J., L., Čurda, O., Elich, E., Dvořáková, and M. Dvořák, 2011. Effect of caseinomacropeptide concentrate addition on the growth of bifidobacteria. Czech Journal of Food Sciences, 28 (6), 485-494.
DOI: 10.17221/269/2009-CJFS

12. Ehsani, A., E. K. Banihabib., M. Hashemi, M. Saravani, and E. Yarahmadi, 2016. Evaluation of various properties of symbiotic yoghurt of buffalo milk. Journal of Food Processing and Preservation, 40(6), 1466-1473. DOI: 10.1111/jfpp.12732

13. Elbandy, M. A., S. M. Abed, S. S. A. Gad, and M. G. Abdel-Fadeel, 2014. Aloe vera gel as a functional ingredient and natural preservative in mango nectar. World Journal of Dairy and Food Sciences, 9(2), 191-203. DOI: 10.5829/idosi.wjdfs.2014.9.2.1139

14. Faraki, A., N. Noori, H. Gandomi, S. A. H. Banuree, and F. Rahmani, 2020. Effect of auricularia auricula aqueous extract on survival of *Lactobacillus acidophilus* La-5 and *Bifidobacterium bifidum* Bb-12 and on sensorial and functional properties of synbiotic yogurt. Food science and Nutrition, 8(2), 1254-1263. DOI: 10.1002/fsn3.1414

15. Franz, C., R., R., Bauer, D., Carle, A. Tedesco, Tubaro, and K. Zitterl-Eglseer, 2005. cft/efsa/ feedap/2005/01.DOI: 10.1007/978-3-540-72546-6_3

16. Grace, O. M., S., Buerki, M. R. Symonds, F., Forest, A. E. van Wyk, G. F. Smith, and N. Rønsted, 2015. Evolutionary history and leaf succulence as explanations for medicinal use in aloes and the global popularity of Aloe vera. BMC Evolutionary Biology, 15(1), 1-12.DOI: 10.1186/s12862-015-0291-7

17. Haque, M. Z., M. B. Islam, M. A. Jalil, and M. Z. Shafique, 2014. Proximate analysis of aloe vera leaves. Journal of Applied Chemistry, 7(6), 36-40. DOI: 10.9790/5736-07613640

18. Hartayanie, L., and V. K. Ananingsih, 2013. Bifidobacteria as potential probiotic in yogurt. In international conference on environment and health "Integrating Research Community Outreach and Service Learning". Unika Soegijapranata.
doi:10.1016/j.jtrangeo.2005.10.003

19. Hęś, M., K. Dziedzic, D. Górecka, A. Jędrusek-Golińska, and E. Gujska, 2019. Aloe vera (L.) webb.:natural sources of antioxidants–a review. Plant Foods for Human Nutrition, 74(3), 255-265.
doi: 10.1007/s11130-019-00747-5

20. Houshang, J; M. M. Hossein, Y. Mohaddese, K. J. Zahra, and R. Elham, 2014. Studying the influence of aloe vera on the growth of the lactobacillus acidophilus and bifidobacterium bifidum probiotic bacterias in producing probiotic milk and yoghurt. Unique J Pharma. Boil. Sci, 2(02), 16-20. https://ujconline.net/wp-

content/uploads/2013/09/4-UJPBS-1485-Rs.pdf
21. Hussain, S. A., G. R. Patil, V. Yadav, and R. R. B. Singh, 2015. Effect of storage on sensory quality, pH, wheying-off and probiotic count of lassi supplemented with aloe barbadensis miller juice. Indian J Dairy Sci, 68, 105-110. http://epubs.icar.org.in/ejournal/index.php/IJDS/article/view/41545/pdf_67
22. Ibrahim, O. M. S., A. M. Abed, and N. Z Yahea, 2020. Evaluation of biological activity of greenly synthesized silver nanoparticles using aloe vera gel extract as antibacterial agent in-vitro and in-vivo. Iraqi J. of Agric. Sci, 51(6), 1706-1715. https://doi.org/10.36103/ijas.v51i6.1199
23. Jadhav, A. S., O. A. Patil; S. V. Kadam; and, M. A. Bhutkar 2020. Review on Aloe vera is used in medicinal plant. Asian Journal of Research in Pharmaceutical Science, 10(1) 26-30. DOI: 10.5958/2231-5659.2020.00006.5
24. Jungersen, M., A. Wind; E., JohansenJ. E. Christensen, B. Stuer-Lauridsen, and D. Eskesen, 2014. The science behind the probiotic strain *Bifidobacterium animalis subsp*. *lactis* BB-12® Microorganisms, 2(2), 92-110. doi.org/10.3390/microorganisms2020092
25. Khangwal, I., and P. Shukla, 2019. Potential prebiotics and their transmission mechanisms: Recent approaches. Journal of food and drug analysis, 27(3), 649-656. https://doi.org/10.1016/j.jfda.2019.02.003
26. Kim, S., E. Huang, S. Park, W. Holzapfel, and S. D. Lim, 2018. Physiological characteristics and anti-obesity effect of *Lactobacillus plantarum* K10. Korean journal for Food Science of Animal Resources, 38(3), 554. doi: 10.5851/kosfa.2018.38.3.554
27. Lee, W. J., and J. A. Lucey, 2010. Formation and physical properties of yogurt. Asian-Australasian Journal of Animal Sciences, 23(9), 1127-1136.https://doi.org/10.5713/ajas.2010.r.05
28. Luta, G., and B. McAnalley, 2005. Aloe vera: chemical composition and methods used to determine its presence in commercial products. GlycoScience and Nutrition, 6,1-12. Google Scholar
29. Martínez-Villaluenga, C., and R. Gómez, 2007. Characterization of bifidobacteria as starters in fermented milk containing raffinose family of oligosaccharides from lupinas prebiotic. International Dairy Journal, 17(2), 116-122.https://doi.org/10.1016/j.idairyj.2006.02.003
30. Minjares-Fuentes, R., A. Femenia, F. Comas-Serra, and V.M.Rodríguez-González, 2018. Compositional and structural features of the main bioactive polysaccharides present in the Aloe vera plant. Journal of AOAC International, 101(6), 1711-1719. https://doi.org/10.5740/jaoacint.18-0119
31. Mukhekar, A. and R. J. Desale, 2018. Studies on physico-chemical properties of yogurt fortified with aloe vera. Multilogic in Science 7(25), 140-142. Research gate
32. Muñoz, O. M., X. Leal, V. Quitral, and Cardemil,L.2015.Extraction,characterization and properties of the gel of Aloe vera (*Aloe barbadensis Miller*) Cultivated in Chile. Med Aromat Plants 4, (3) 2-7. DOI: 10.4172/2167-0412.1000199
33. Nagpal, R., V. Kaur, M., Kumar, and F. Marotta, 2012. Effect of Aloe vera juice on growth and activities of Lactobacilli in-vitro. Acta Biomed, 83(1), 183-8. https://www.researchgate.net/publication/261474886
34. Ozturkoglu-Budak, S., H. C. Akal, İ. Buran, and A. Yetişemiyen, 2019. Effect of inulin polymerization degree on various properties of synbiotic fermented milk including *Lactobacillus acidophilus* La-5 and *Bifidobacterium animalis* Bb-12. Journal of Dairy Science, 102(8), 6901-6913. DOI: 10.3168/jds.2019-16479
35. Park, Y. I., and T. H. Jo, 2006. Perspective of industrial application of Aloe vera. In new perspectives on aloe Springer, Boston, MA (pp. 191-200). https://link.springer.com/chapter/10.1007/0-387-34636-8_14#citeas
36. Pellizzoni, M., G. P. Molinari, and L. Lucini, 2011. Stability of the main aloe fractions and Aloe-based commercial products under different storage conditions. Agrochimica 55(5), 288-296. https://publicatt.unicat.it/handle/10807/9102
37. Quezada, M. P., C. Salinas, M. Gotteland, and L. Cardemil, 2017. Acemannan and fructans from aloe vera (*Aloe barbadensis*

miller) plants as novel prebiotics. Journal of Agricultural and Food Chemistry, 65(46), 10029-10039. https://doi.org/10.1021/acs.jafc.7b04100

38. Ravaei, A., Z. H. Poor, T. Z. Salehi, I. A. Tamai, M. Ghani, and J. D. Pour, 2013. Evaluation of antimicrobial activity of three-lactobacillus spp. against antibiotic resistance salmonella typhi murium. Advanced Studies in Biology, 5(2):61-70. https://www.researchgate.net/publication/257985399

39. Sadiq, I. H., and K. S. Doosh, 2019. Study of the physochemical, rheological and sensory properties of yoghurt fortified with micro encapsulation iron. Iraqi Journal of Agricultural Science, 50(4), 1345-1355. https://doi.org/10.36103/ijas.v50i5.799

40. Silva-Vazquez, R., E., Flores-Giron, A. Quintero-Ramos, M. E. Hume, and G. Mendez-Zamora, 2018. Effect of inulin and pectin on physicochemical characteristics and emulsion stability of meat batters. CyTA-Journal of Food, 16(1), 306-310.doi.org/10.1080/19476337.2017.1403490

41. Slačanac, V., M. Lučan, J., Hardi, K. Habschied, V. Krstanović, and G. Gagula, 2013. Predictive modeling of *Bifidobacterium animalis subsp*. *lactis* bb-12 growth in cow's, goat's and soymilk. mljekarstvo časopis za unaprjeđenje proizvodnje J. Prerade Mlijeka, 63(4), 220-227. https://doi/full/10.5555/20133418985

42. Speak, M. 1984. Compendium of methods for the microbiological examination for food 2nd Ed. Washington DC USA. https://doi.org/10.2105/MBEF.0222

43. Tornero-Martínez, A., R. Cruz-Ortiz, M. E. Jaramillo-Flores, P. Osorio-Díaz, S. V. Ávila-Reyes G. M. , Alvarado-Jasso, and R. Mora-Escobedo, 2019. In vitro fermentation of polysaccharides from Aloe vera and the evaluation of antioxidant activity and production of short chain fatty acids. Molecules, 24 (19), 3605. doi.org/10.3390/molecules24193605

44. Yadav, A. K., A. Singh, and K. C. Yadav, 2018. Efficacy of flavored Aloe vera cubes in probiotic yogurt. Journal of Pharmacognosy and Phytochemistry, 7(4), 1609-1614. DOI: 10.13140/RG.2.2.26103.06567

45. Zhang, Y., Z. Bao, X. Ye, Xie, Z., He, K., B. Mergens, and Q. Zheng, 2018. Chemical investigation of major constituents in Aloe vera leaves and several commercial Aloe juice powders. Journal of AOAC Internation-al 101(6), 1741-1751. DOI: 10.5740/jaoacint.18-0122