

## **The effect of adding Arabic gum in different ratios on some probiotics fermented milk and studying the inhibitory ability against some pathogenic bacteria**

**تأثير إضافة نسب مختلفة من الصمغ العربي في بعض المتخميرات اللبنية المعززة حيويًا ودراسة قابليته التثبيطية حيال بعض أنواع البكتيريا المرضية**

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### **Abstract**

This paper studies the usability of Arabic gum as prebiotic when adding it at two levels of 0.5, 1.0% to some fermented milk products and using two types of therapeutic bacteria *Lactobacillus plantarum* (LPB) and *Lactobacillus acidophilus* (LBA). It has been Observed that the logarithm of the number of bacteria LPB were higher than the logarithm numbers of bacteria LBA( cfu / ml)) at the treatment of 0.5, 1.0% Arabic gum, as it amounted at a concentration of 1.0% Arabic gum for bacteria LPB, LBA (9.86, 9.79) respectively which were compared to control( bacteria LPB, LBA only ) (9.73, 9.57), respectively. There has been a decrease in numbers of therapeutic bacterial products manufactured at the study of the effect of storage period, which lasted for a month , it decreased by quarters of the session to bacteria LPB, while LBA fell by a full cycle of the bacterium logarithm. the inhibitory ability of Arabic gum of 0.5, 1.0% concentration against the four types of bacteria test *Staphylococcus aureus* , *Bacillus subtilis* , *shigella dysenteriae*, *Salmonella typhimureum* the highest inhibitory ability for Arabic gum towards *Staphylococcus aureus* has been 24 mm .The manufactured products have been sensory Evaluated at three storage periods .It got treatment A2 (1.0% Arabic gum + bacteria LPB or LBA) in a period of zero time on high scores of sensory evaluation in terms of the qualities of flavor , Conception and textures, acidity and numbers of bacteria as it was (40, 34.5, 9.5, 9.0), respectively, for bacteria LPB, While amounted to (39.5, 34.5, 7.5, 9.0) for the treatment of bacterial LBA compared to control (A) .The storage period has not affect the sensory qualities of manufactured products, as the results were comparable to the results of sensory evaluation at the beginning of the manufacturing period (zero time).

### **المستخلص**

درست قابلية استخدام الصمغ العربي كمعزز حيوي عند اضافته بمستويين (0.5 ، 1.0)% الى بعض متخميرات الحليب وباستخدام نوعيين من البكتيريا العلاجية (*Lactobacillus plantarum* (LPB), *Lactobacillus acidophilus* (LBA). لوحظ ان لوغاريتم أعداد البكتيريا LPB كانت أعلى من لوغاريتم أعداد بكتيريا LBA (cfu/ml) عند معاملة (0.5 ، 1.0) % صمغ عربي، اذ بلغت عند تركيز 1.0% صمغ عربي لبكتيريا LBA، LPB (9.86، 9.79) على التوالي مقارنة بمعاملة السيطرة التي كانت لبكتيريا LBA، LPB (9.73، 9.57) على التوالي . لوحظ انخفاض بأعداد البكتيريا العلاجية للمنتجات اللبنية المصنعة عند دراسة تأثير فترة الخزن التي استمرت لمدة شهر حيث انخفضت بمقدار ثلاث ارباع الدورة لبكتيريا LPB بينما انخفضت بمقدار دورة لوغاريتمية كاملة لبكتيريا LBA. درست القابلية التثبيطية للصمغ العربي وتركيز (0.5 ، 1.0)% حيال اربع انواع من بكتيريا الاختبار *Staphylococcus aureus* , *Bacillus subtilis* , *shigella dysenteriae* , *Salmonella typhimureum* فكانت اعلى قابلية تثبيطية للصمغ العربي حيال بكتيريا *Staphylococcus aureus* اذ بلغت 24 mm . قيمت المنتجات المصنعة حسيًا وعلى ثلاث فترات خزنية. حصلت معاملة A<sub>2</sub> (1.0% صمغ عربي +بكتيريا LPB or LBA) في فترة zero time على أعلى نتائج للتقييم الحسي من حيث صفات النكهة ،القوام والنسجة ، الحموضة وأعداد البكتيريا اذ بلغت (40 ، 34.5 ، 9.5 ، 9.0) على التوالي لبكتيريا LPB ، بينما بلغت (39.5 ، 34.5 ، 7.5 ، 9.0) لبكتيريا LBA مقارنة بمعاملة السيطرة (A) . لم تؤثر الفترة الخزنية على الصفات الحسية للمنتجات المصنعة اذ كانت النتائج مقارنة لنتائج التقييم الحسي في فترة بداية التصنيع (zero time).

## **Introduction**

Acacia gum (known as Arabic gum) a type of fiber made of hardened sap taken from two species of the acacia tree : *Acacia Senegal* (L.) Willd. And *Acacia seyal* Delile. It is a soluble substance in water that dissolves in water in a concentration of 50%w/v (low viscosity) with acidic properties( pH4.5),.Acacia gum has been a complex mixture of polysaccharide and glycoproteins ,namely branched galactan composed of units of D-galactose and side chains of D-glucuronic acid with terminal L-rhamnose or L-arabinose units , the molecular weight had 200-600kDa (1,2, 3). It is the source of the sugars arabinose and ribose so it was used in the food industry as a stabilizer (E414),a thickener , an emulsifier and in encapsulation technique not only in food processing, but also in the medical and pharmaceutical industries and cosmetics(3). Arabic gum has played an important role in human health as it is not used only stabilizer in the food industry, but also is used in functional foods industry where it has been used as probiotics by increasing the beneficial bacteria in colon because it has been non-digestible in the human small intestine but it is fermentable by beneficial bacteria , this is due to a decrease in pathogenic gastrointestinal microorganisms might be a beneficial physiological effect. It works to regulate the digestion of food to improve intestinal conditions (pH, SCFA production) and intestinal functions (4,5) There are many studies conducted on the inhibitory ability of Arabic gum in vivo and in vitro was found to have a cooperative action between lactobacilli and Arabic gum that lead to a reduction of some types of pathogenic bacteria such as *Clostridium difficile*, *Bacillus cereus* ,*Salmonella typhi* and others (3,4).The prebiotic ability of gum studied by foreign and domestic scientists show the effect of Arabic gum as a nutrient medium for Lactobacilli and Bifidobacteria because it is a fermentable fiber (6).

In recent years , Arabic gum has been mixed well with all kinds of food and it does not affect the organoleptic properties of the finished product. So the range of functional foods that have potential benefits for health has grown tremendously. Example includes baby foods, bakery and cereals ,confectionery, dairy food ,ready meals , snacks, soft drinks such as energy and sport drinks ,meat products and spreads. These functional foods are associated with various types of benefit ; they are particularly rich with vitamins and fortifying minerals ,they reduce cholesterol , functions as antioxidant , antimicrobial , phytochemicals , dietary fiber , herbs and botanicals, and probiotics, prebiotics and symbiotics (7). Food ,particularly dairy products has been considered as an ideal vehicle for delivering probiotic bacteria to the human gastrointestinal tract(8).Some dairy products enhance microbial survival in gastric juice , most likely due to a buffering or protective effect (9).At present probiotic bacteria have been mainly incorporated into dairy product such as yoghurt ,fermented milks, milk powder ,ice cream and cheese (10).

Based on the above ,the aim of this study is to examine the effect of Arabic gum on increasing the number of therapeutic bacteria and decreasing the number of pathogenic bacteria .

## **Materials and Methods**

### ***1-Acacia Gum(Arabic Gum)***

Arabic gum that has been used for this study has been obtained from the local market ; the source is (Fibregum , Colloids Naturals International , Rouen , France ).

### ***2-Lactic Acid Bacteria Lactobacillus plantarum(LBP) , Lactobacillus acidophilus(LBA).***

*Lactobacilli* (LBP),(LBA) have been isolated from stools of breastfed children .They have been obtained from the laboratories of the Department of Food Science , Faculty of Agriculture , University of Baghdad .

### ***3-Culture media and solutions utilized under study***

- MRS Broth and MRS Agar**(oxid) has prepared the media as instructed by the manufacturing company.
- Mueller Hinton Broth and Mueller Hinton Agar** (Himedia) has prepared the media as instructed by the manufacturing company.

**-Peptone water**

0.1mg of peptone has been dissolved in 100 ml of distilled water then distributed in test tubes the size 9 ml of each tube then entered steam autoclave device at 121 C° , 15 min and 15 Joe .

**-Skim milk media**

12g of skimmed milk powder has been dissolved in 100 ml of distilled water and entered steam autoclave at 121C° , 5min and 15Joe. The purpose of the skim milk is to activate (LBP, LBA) for three consecutive times by inoculation of 5% and to incubate at 37C° , 24 h until the advent of coagulation (11).

**4- Addition of Arabic gum to skimmed milk media (preparation of prebiotic milk)**

Gum (powder)has been added to the skimmed milk media in different proportions ( 0.5 , 1.0)% has been sterilized in autoclave 121C° , 5 min ,15Joe then cooled at 37 C° and inoculated by 5% from LBP and LPA then incubated at 37 C° until the appearance of coagulation and it has been measured pH of treatments at (0.5 , 1.0) % Arabic gum .

**5- Estimate the total number of bacteria (LBP,LBA) in vitro**

A series of decimal dilutions have been prepared by dissolved 0.1 mg pepton / 100ml of distilled water) as mentioned previously in paragraph 2 and inoculated dilutions by 1 ml from starter prepared previously in paragraph 3 and followed by (Pour plate ) as a mentioned from (12) by using MRS Agar , dishes have been incubated at 37 C° for 48 hours under anaerobic conditions in a deflated container after the end of cuddling the number of developing colonies have been calculated using colony- counter device .

**6-Study antibacterial for Gum Arabic tendency of some types of pathogenic bacteria in vitro**

**-Source of pathogenic microorganism**

Strains of *Salmonella typhimureum* , *Shigella dysenteriae* , *Bacillus subtilis* , *Staphylococcus aureus* have been obtained from the laboratories of the Department of Food Science , Faculty of Agriculture , University of Baghdad .

**-The development of the test isolates:** Bacterial isolates have been cultured on the sloping surface of the media Mueller Hinton Agar and incubated at 37 ° for 24 hours.

**- Antibacterials from different concentrations of Arabic gum** by using the bacterial isolates diffusion method (well or cup assay) ( 13) Where the operation has been conducted centrifugal prebiotic milk prepared in paragraph 3 by 5000 rpm for 25 minutes at 2-4 C° then separated the precipitate and filtrated by using a sterility bacterial filter diameter of 0.45µm.

**7- Sensory evaluation of the product**

Evaluated treatments for the product depending on **the form** by Trout& Nelson and some modified ( 14 ) .

Features	Upper Class in the original form	Upper class in the modified form	Name of product	Notice
Flavor	45	40		
Conception and texture	30	35		
*The numbers of bacteria	-	15		
Acidity	10	10		
Appearance	10	10		
Package with Cover	5	5		

\*Evaluated according to the number of bacteria as follows:

\*The numbers of bacteria

Score

0-10 <sup>5</sup>	0
10 <sup>5</sup> -10 <sup>6</sup>	3
10 <sup>6</sup> -10 <sup>7</sup>	6
10 <sup>7</sup> -10 <sup>8</sup>	9
10 <sup>8</sup> -10 <sup>9</sup>	12
10 <sup>9</sup> -10 <sup>10</sup>	15

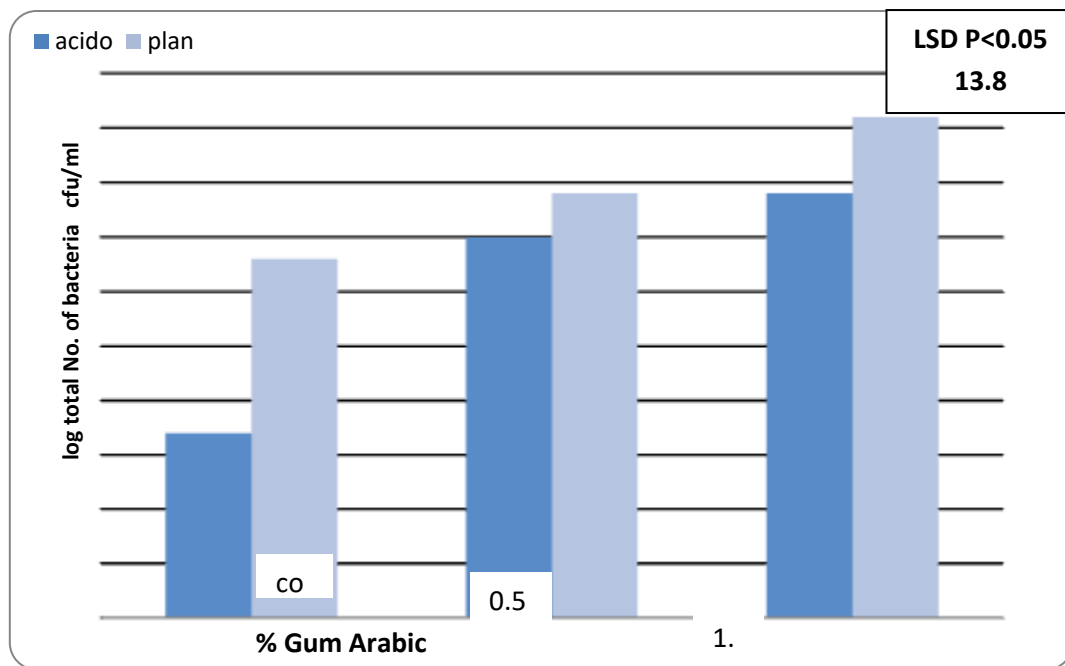


Fig 1. The effect of using different concentrations of Arabic gum in the number of live lactic acid bacteria in fermented milk product.

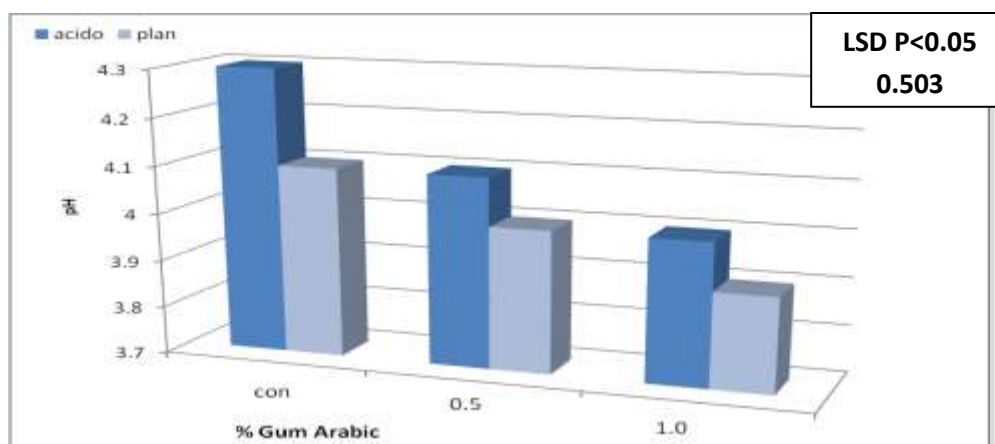


Fig 2. The effect of using different concentrations of Arabic gum in the pH in fermented milk product

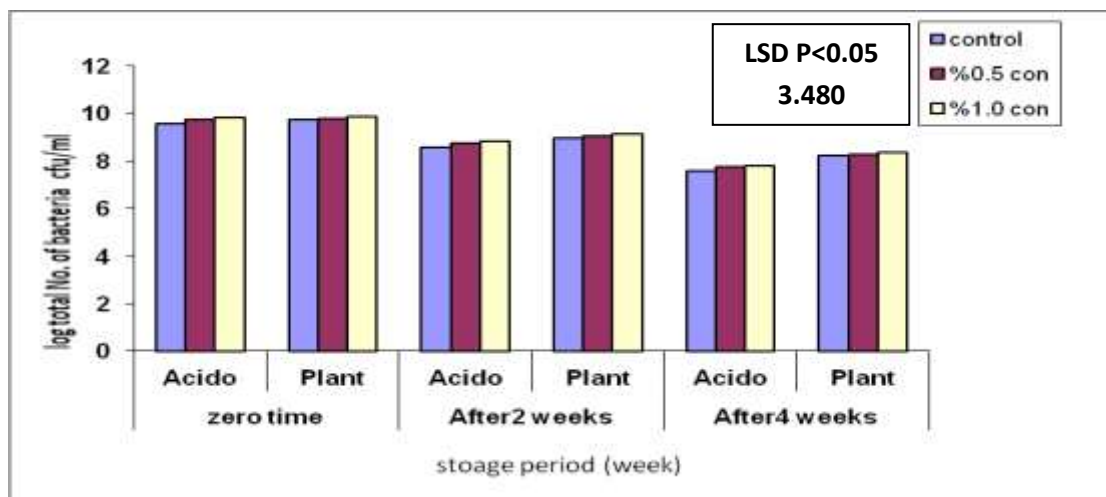


Fig 3. The Effect of different periods of storage on the numbers of lactic acid bacteria (LBP,LBA).

Table1. Inhibitory ability of Arabic gum with and without bacteria therapeutic tendency of some types of pathogenic bacteria.

Treatments	mm diameters halo of inhibition of bacterial test*			
	<i>Staphylococcus aureus</i>	<i>Bacillus subtilis</i>	<i>shigella dysenteriae.</i>	<i>Salmonella typhimureum</i>
** Arabic gum (AG) 0.5% w/v	2	2	0	0
Arabic gum (AG) 1 % w/v	4	3	2	2
<i>Lbs acidophilus</i>	15	13	11	9
<i>Lbs acidophilus</i> +0.5 %AG	18	16	13	12
<i>Lbs acidophilus</i> +1 %AG	22	20	16	14
<i>Lbs. plantarum</i>	20	19	15	13
<i>Lbs. plantarum</i> + 0.5%AG	21	20	17	15
<i>Lbs. plantarum</i> + 1 %AG	24	23	20	19
<i>LSD P&lt;0.05</i>	0.85			

\* A halo inhibition is not including the 4 mm diameter hole.\*\* (AG) an aqueous solution of Arabic gum w/v of the leave sterile filtration 0.45 µm.

Table 2. The sensory evaluation of the product fermented by form Trout Nelson with some modification.

** treatme nt	Period of storage (Week)	* <i>Lactobacillus acidophilus</i> (LBA)				<i>Lactobacillus plantarum</i> (LBP)			
		Flavor °40	Concepti on & textures °35	Acidit y °10	The numbe rs of bacteri a °15	Flav or °40	Concepti on & textures °35	Acidit y °10	The number s of bacteria °15
A	Zero time	38.5	30	6.5	9	39	33	8.5	9
	After 2 weeks	38	33	6.5	9	38.5	32.5	8.5	9
	After 4 weeks	37.5	33	6.5	6	38	32	8.5	6
A1	Zero time	39.5	33.5	7	9	39.5	34.5	9	9
	After 2 weeks	39.5	34.5	7	9	39	34.5	9	9
	After 4 weeks	38	33.5	7	6	38.5	34	9	6
A2	Zero time	39.5	34.5	7.5	9	40	34.5	9.5	9
	After 2 weeks	40	34.5	7.5	9	39.5	34.5	9.5	9
	After 4 weeks	39.5	34.5	7.5	6	39	34	9.5	6
LSD P<0.05		1.401							

\*Data have been represented as mean of 5 evaluator.\*\* A representes as control , A1 represents as ( 0.5% AG +LBP or LBA) , A2 representes as ( 1.0% AG+ LBP or LBA).

## Results and discussion

Figure 1. shows the log numbers of available bacteria ( *cfu \ ml* ) in the fermented milk products using different ratios of Arabic gum and both types of bacteria .It has been noted from the figure the numbers of bacteria increase one Cycle logarithmic for of LBA , while LBP bacteria increase only half to three quarters logarithmic cycle during manufacturing the fermented milk products by using two levels of AG( 0.5,1.0)% , for both of the treatments using two types of bacteria compared with control. The increasing of logarithm numbers for LBA bacteria rise from (9.57) to( 9.75, 9.79) respectively, while the increasing of logarithm number of LBP bacteria from ( 9.73) to (9.79, 9.86 ) when ( 0.5 , 1.0 )% AG has used respectively. According to the previous results Arabic gum has been a good prebiotic for the therapeutic bacteria in increasing their numbers .This is due to the ability of Arabic gum of the possession of property adsorption of water and returning it to its chemical composition because its glycoproteins has its distinct characteristics in food processing and this was consistent with (15); who found in his study a significant increase in the numbers of probiotics bacteria , *bifidobacteria* and *lactobacilli sp* when Arabic gum compares with Inulin .Similar findings have been reported by (16) Who found an increase in the number of lactic acid bacteria , *Bifidobacterium lactis* and *Lactobacillus bulgaricus* when 0.5, 1.0% AG were added . The results show also an increase in the logarithm numbers of bacteria LBP was higher than bacteria LBA and this may be due to the activity of the bacteria itself ( 14).

The results show in Figure 2 that the pH values of the treatments are decreased due to the increase of the concentration of Arabic gum for both kinds of starter bacteria, the pH value LBA bacteria treatments (0.5, 0.1)% AG are (4.1, 4.0) respectively, as compared to the control which is (4.3) while LBP bacteria pH value are (4.0, 3.8) respectively, for treatments (0.5, 1.0)% AG compared with control (4.2). It has been noted from the figure that the decrease in pH values inversely proportional to the increased in the number of starter bacteria and effectiveness of Arabic gum as prebiotics (16). The same findings have been reached when Arabic gum was added to MRS Agar, a decrease in pH values has been observed associated with an increase in the number of bacteria *Lactobacillus acidophilus*, *Lactobacillus plantarum* and other types of probiotic bacteria (6).

The results shown in Fig. 3 show that the effect of storage periods on the survival for both types of starter with (0, 0.5 and 1% AG) a clear reduction in logarithm number of bacteria during the storage period could be seen in Figure 3 there have been decline in logarithm number LBA. Bacteria has dropped by a full session of the logarithm during storage as it was (8.57, 8.75, 8.80) two weeks after storage while it was (7.57, 7.75, 7.80) four weeks after storage for treatments (0.0, 0.5 and 1.0)% AG respectively, compared to zero time as it was (9.57, 9.75, 9.79). While logarithm number of LBP bacteria declined up to three quarters session after storage it reached after two weeks of storage (8.98, 9.04, 9.11) while it was (8.23, 8.29, 8.36) four weeks after storage for treatments (0.0, 0.5, 1.0)% AG respectively, compared to zero time that was (9.73, 9.79, 9.86). Similar findings were reported by (14) who found in his study that the bacteria *Lactobacilli* suffer decline in their numbers during periods of storage, which lasted for 3 weeks, the shelf life of the product. Also (17) showed in her study that the bacteria were declining in numbers after a month of storage.

Table 1 shows rates diameters of clear zones of inhibition are caused by the Arabic gum water solution concentration 0.0, 0.5, 1.0% w/v for bacteria *Staphylococcus aureus*, *Bacillus subtilis*, *Shigella dysenteriae*, *Salmonella typhimureum* as it was (2, 2, 0, 0) respectively, at concentration 0.5% AG while at concentration 1.0% AG was (4, 3, 2, 2) respectively. (19) who has studied the antimicrobial effect of Arabic gum, against two strains of Gram positive *Bacillus cereus* *Staphylococcus aureus*, and three Gram negative, *Escherichia coli*, *Salmonella typhi* and *Pseudomonas aeruginosa*, this study mention that the methanol extract has shown the highest antibacterial activity, and that was due to the presence of hydrophilic components such as polyphenols, polysaccharides and tannins. The gram positive bacteria were more sensitive than gram negative bacteria towards the antimicrobial activity of AG.

Also table 1. shows the effective of Arabic gum concentration 0.5% with LBA towards *Staphylococcus aureus* as the diameter of clear zone of inhibition 18 mm while bacteria *Bacillus subtilis*, *Shigella dysenteriae*, *Salmonella typhimureum* as number rates were diameters inhibition zone (16, 13, 12) mm respectively. Other treatments of LBP starter with 0.5% AG for the bacteria *Staphylococcus aureus* 21 mm while the rest of the types were (20, 17, 15) mm respectively.

Table 1 shows that the concentration of 1% AG with the two types of probiotic bacteria LBA, LBP increase inhibitory action towards *Staphylococcus aureus*, *Bacillus subtilis*, *Shigella dysenteriae*, *Salmonella typhimureum* rates diameters inhibition zone were (22, 20, 16, 14) mm respectively, for the treatment of 1% AG with LBA bacteria while the diameters of inhibition zone for the treatment of 1% AG with LBP bacteria were (24, 23, 20, 19) respectively. It seems from the table that the bacteria *Staphylococcus aureus* are the most affected by the Arabic gum concentration of 1% with the two types of therapeutic bacteria. The results show that the gram positive bacteria are more susceptible to Arabic gum compared with bacteria gram negative bacteria and could explain the inhibitory act of Arabic gum toward testing bacteria to increase the solubility and viscosity of AG, which acts as a barrier surrounding wall cellular bacterial or its impact on the permeability of membranes and the lack of balance osmotic pressure on both sides of the cell wall Which lead to the leakage of water and components of the cell to the outside (18).

It has been noted that treatments LBA and LBP alone without adding Arabic gum to has portability inhibition direction bacteria test because of its production of Bactriocins during the storage period, which was characterized by susceptibility inhibitory bacterial pathogenesis This is consistent with (14) who found in his study susceptibility inhibitory of bacteria lactobacilli in therapeutic manufacturing

products . In another study it has found that Arabic gum inhibitory ability is in the direction of bacteria *Bacillus subtilis* , *Staphylococcus aureus* (6).

The main actions for the inhibitor toward the pathogenic bacteria was due to the fact that the probiotics bacteria has the ability to produce many kinds of inhibition substance such as lactic acid ,  $H_2O_2$  and bacteriocins LBA strains may produce bacteriocins such as acidolin, acidophilin and lactocidin. And they are very effective against pathogenic bacteria , also LBP probiotic bacteria produce more than 20 types of bacteriocins known as plantaricin(20) .

The results indicate in Table 2. that the degree of sensory evaluation of the lactic manufacturing products for three periods storage .It has been noted that at the beginning of manufacturing (zero time), the highest score for the recipe flavor are the treatments A1, A2 a bacterium LBP as it was 39.5° compared to the treatment control( A), as it was 39° while have been treated on the same degree of bacteria LBA , amounting to ( 39.5 ,40.0)° respectively, degree for A1,A2 compared to control as it was 38.5°. This result agrees with the result of ( 14) who found that studying The flavor of acidophilus milk is depended on the additives and chemical composition.

The recipe conception and textures at a zero time reached the highest score for the treatment of A2 as it got 34.5° of bacteria LBP and LBA while the lowest score for the Treatment of A1 as it was 33.5 ° for the treatment of bacteria LBA compared to control as it amounted to 30 °. This is similar to the results of (16), who found in his study of the clotting product lactic when using different ratios of Arabic gum is attributed to the viscosity of Arabic gum and act cooperatively with lactic acid bacteria .

The recipe acidity for treatments A,A1,A2 of bacteria LBP was higher than bacteria LBA, amounting ( 8.5,9.0,9.5 )° while the bacteria LBA( 6.5,7.0,7.5)° This was due to the bacteria LBP are more active than LBA. This was clear from the logarithm of the number of bacteria LBP compared to LBA ,in addition to Figure 2. which shows the pH as previously mentioned, showed a decrease in the pH of LBP are more than LBA this means that the numbers of bacteria LBP was higher . This has corresponded to ( 16) who found an increase in the number of lactic acid bacteria when increasing titrable acidity when different ratios of Arabic gum products are added to the manufactured products .

It can be noted from Table 2 that the number of bacteria LBP and LBA are within the  $10^7$ - $10^8$  cfu \ ml and gets 9° of the 15 ° for treatments A,A1,A2 in the zero time and after two weeks of storage. It has been noted that the number of bacteria within the limits of  $10^6$ - $10^7$  cfu \ ml four weeks after storage for each of the treatments A,A1,A2 and gets 6° of the 15 °. (14) who has found that the number of LBA bacteria he depended on in evaluation form matches the number mentioned in the modified form of Nelson and Trout.

It can be noted from the table that the sensory qualities of fermented manufactured products that flavor to the treatment after 2 weeks of storage for bacteria LBA was higher than LBP. As it was 40°, while the LBP was 39.5 compared to control that was 38° for LBA while 38.5° for LBP While has not affected the flavor after 4 weeks of storage.

While the recipe conception and texture for treatments A 1 and A2 do not change after 2 - 4 weeks of storage and was very close to the treatment compared to control . It has been noted from the table that the recipe was not affected by pH storage period for all treatments .This is similar to the findings of the study (14) to the effect of storage on the numbers of lactic acid bacteria during the storage period, which lasted for 3 weeks.



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