APPLICATION OF ENCAPSULATION TO IMPROVE VIABILITY OF SOME PROBIOTIC BACTERIA IN ZABADY CONTAINING SOME HERBS

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5. SUMMARY

Probiotic is a relatively new word meaning 'for life', which is used to name microorganisms that are associated with the beneficial effects for humans and animals. These microorganisms contribute to intestinal microbial balance and play a role in maintaining health. The probiotic microorganisms consist mostly of the strains of the genera Lactobacillus and Bifidobacterium . Together they play an important role in the protection of the organism against harmful microorganisms and also strengthen the host's immune system.

Probiotics are commonly known to have the beneficial influence on the host organism. They positively affect the gastrointestinal infections, possess antimicrobial activity, help with lactose metabolism, decrease serum cholesterol, stimulate immune system, possess antimutagenic properties, anti-carcinogenic properties, anti-diarrheal properties, alleviate the inflammatory bowel disease symptoms, suppress the infection caused by *Helicobacter pylori.*

Encapsulation is determined as a mechanical or physiochemical technique that traps materials that may be sensitive to the external conditions. It provides a protective barrier between the inner material and external conditions. This is different from simple immobilization, in which the core component may be exposed to the outside environment

On the other hand, Herbs are invaluable resources, useful in daily life as food additives, flavours, fragrances, pharmaceuticals, colours or directly in medicine. This use of plants has a long history all over the world, and over the centuries, humanity developed better methods for the extraction of essential oils from such materials. Essential oils are complex mixtures of volatile substances generally present at low concentrations. Herbs are important part of the human diet, which have been used for thousands of years in traditional medicine; also they used to enhance the flavor, color and aroma of foods. In addition to boosting flavor, herbs and spices are also known for their preservative, antioxidative and antimicrobial roles.

Therefore, the current investigation aimed to study :

1- To encapsulate ***Lactobacillus rhamnosus*** **ATCC7469** ***Lactobacillus acidophilus*** **DSM20079 and** ***Bifidobacterium bifidum*** **DSM20082** incorporate them into zabady and compare the survival of encapsulated cells with non encapsulated cells during the zabady shelf life.

2- Use of extrusion technique to encapsulated probiotic cultures with 3 different carrier material (k-carrageenan , xanthan gum and gelatin) in the presence of sodium alginate and inulin .

3- To evaluate the survival of free cells & encapsulated probiotic strains during exposure to low pH , bile salts and simulated gastric juice (S.G.J) & simulated intestinal juice (S.I.J).

4- To study the effect of clove and cinnamon extracts on the viability of probiotic strains .

5-The influence of these additives on the (chemical , microbiological , rheological and organoleptic) properties of zabady.

The obtained results could be summarized as follows :

1- Selection of tested strains for use as probiotic :

a- Acid tolerance :

Three tested ***Lactobacillus rhamnosus*** **ATCC**7469 , ***Lactobacillus*** **acidophilus DSM20079 and** ***Bifidobacterium bifidum*** **DSM20082** strains were examined for their tolerance to low PH (2 and 3) in MRS broth**.** From obtained results it could be stated that pH 2.0 seemed to be more effect on the tested strains.

Results also indicated :
1- Growth inhibition percent was 8.17 % of free cells of ***Lactobacillus rhamnosus*** **ATCC7469** after 3 h incubation at pH 2 compared to 5.89% at pH 3 while, at pH 2 growth inhibition percent of encapsulated cells was ranged (from 5.54 to 6.08 %) compared to (3.40 to 3.80 %) at pH 3. It was evident from the obtained data that the percentage of survivors as viable free cells was (97.5% after 60 min. incubation time and 91.83% after 180 min.) while, at pH 3 it was (98.85 after 60 min. % and 94.11 % after 180 min.). Encapsulated strains showed viability (98.58 to 99.23 % after 60 min.) and (93.92 to 94.46 % after 180 min.) survival at pH2 compared to (99.12 to 99.38% after 60 min.) and (96.20 to 96.60% after 180 min.) at pH3.

2- Free cells of ***Lactobacillus acidophilus*** **DSM 20079** was more affected tolerance to pH , where growth inhibition percent was 9.97 % after 3 h incubation at pH 2 compared to 6.17 % at pH 3 while, at pH 2 growth inhibition percent of encapsulated cells was ranged (from 5.83 to 6.93 %) compared to (3.92 to 4.89 %) at pH 3. Also, the percentage of survivors as viable free cells at pH2 varied (97.88% after 60 min. incubation time to 90.03% after 180 min.) while, at pH 3 it was (98.87 % after 60 min. and 93.83 % after 180 min.). Encapsulated strains showed viability (98.61 to 98.74 % after 60 min.) and (93.07 to 94.17 % after 180 min.) survival at pH2 compared to (99.27 to 99.39 % after 60 min.) and (95.11 to 96.08% after 180 min.) at pH3.

3- Growth inhibition percent of free cells of was 6.63 % after 3 h incubation at pH 2 compared to 3.71 % at pH 3 while, at pH 2 growth inhibition percent of encapsulated cells was ranged (from 4.57 to 4.84 %) compared to (2.49 to 2.74 %) at pH 3. The percentage of survivors as viable free cells was (93.37 % after 180 min.) while, at PH 3 it was (96.29 % after 180 min.). Encapsulated strains showed viability (95.16 to 95.43 % after 180 min.) survival at pH2 compared to (97.26 to 97.51 % after 180 min.) at pH3.

Generally , the obtained results strongly suggested that microencapsulation successfully improved the survival of ***Lactobacillus rhamnosus*** **ATCC**7469 , ***Lactobacillus acidophilus*** **DSM20079** and ***Bifidobacterium bifidum*** **DSM20082** strains

in low pH 2 and pH 3 as compared to non encapsulated . In addition to that ***Bifidobacterium bifidum*** **DSM20082** had a higher resistant tolerance to pH2 & pH3 and survived than the ***Lactobacillus rhamnosus*** **ATCC**7469 and ***Lactobacillus acidophilus*** **DSM20079.** On the other hand the encapsulation with xanthan gum and gelatin have the highest viable cells , followed by those encapsulated with kappa carrageenan .

b- Bile salt :

Three tested ***Lactobacillus rhamnosus*** **ATCC**7469 , ***Lactobacillus*** **acidophilus DSM20079 and** ***Bifidobacterium bifidum*** **DSM20082,**strains were examined the ability to grow in the presence of different bile salt concentrations (0.5 ,1 and 2 %) at 37 ^{o}c for 3h**.**

1- It could be noticed from obtained results that show sharply decrease in viable cell numbers of ***Lactobacillus rhamnosus*** **ATCC**7469 even after 3h incubation at 1 and 2% bile salt concentrations ,where a (0.45 , 1.6 and 2.70) – $\log_{10}$ decrease in viable cells of non encapsulated ***Lactobacillus rhamnosus*** **ATCC**7469 after 3 h incubation at concentrations (0.5 , 1 and 2 % bile salt) , respectively. While, (0.42 to 0.44 , 1.20 to 1.25 and 2.09 to 2.17) - $\log_{10}$ decrease in viable cells of encapsulated ***Lactobacillus rhamnosus*** **ATCC**7469 with different carrier material after 3 h incubation at concentrations (0.5 , 1 and 2 % bile salt) , respectively.

2- Also, showed sharply decrease in viable cell numbers of ***Lactobacillus acidophilus*** **DSM20079** even after 3h incubation at 1 and 2% bile salt concentrations ,where a (0.46 , 1.63 and 2.98) – $\log_{10}$ decrease in viable cells of non encapsulated ***Lactobacillus acidophilus*** **DSM20079** after 3 h incubation at concentrations (0.5 , 1 and 2 % bile salt) , respectively. While, (0.42 to 0.46 , 1.32 to 1.35 and 2.31 to 2.42) - $\log_{10}$ decrease in viable cells of encapsulated ***Lactobacillus acidophilus*** **DSM20079** with different carrier material after 3 h incubation at concentrations (0.5 , 1 and 2 % bile salt) , respectively.

3- Also, showed sharply decrease in viable cell numbers even after 3h incubation at 1 and 2% bile salt concentrations ,where a (0.59 , 1.77 and 2.74)– $\log_{10}$decrease in viable cells of non encapsulated ***Bifidobacterium bifidum*** **DSM20082** after 3 h incubation at concentrations (0.5 , 1 and 2 % bile salt) , respectively. While, (0.58 to 0.60 , 1.64 to 1.67 and 2.16 to 2.19) - $\log_{10}$ decrease in viable cells of encapsulated ***Bifidobacterium bifidum*** **DSM20082** with different carrier material after 3 h incubation at concentrations (0.5 , 1 and 2 % bile salt) , respectively.

Generally, the growth of ***Lactobacillus rhamnosus*** **ATCC**7469 , ***Lactobacillus*** **acidophilus DSM20079 and** ***Bifidobacterium bifidum*** **DSM20082** at different concentrations of bile salt decreased dramatically after 2h to 3h with increasing the bile salt concentrate. The encapsulation method used in this study proved to be very efficient in increasing the viability of ***Lactobacillus rhamnosus*** **ATCC**7469 , ***Lactobacillus*** **acidophilus DSM20079 and** ***Bifidobacterium bifidum*** **DSM20082** during exposure to

different concentrations of bile salts as compared to free cells , also the encapsulation with xanthan gum and gelatin have the highest viable cells compare with kappa carrageenan .

C- **Survival in simulated gastric juice (S.G.J):**

Three tested ***Lactobacillus rhamnosus*** **ATCC**7469 , ***Lactobacillus acidophilus*** **DSM20079 and** ***Bifidobacterium bifidum*** **DSM20082** strains were examined for tolerant gastric juice.

From obtained results, it could be noticed that gastric juice exerted a slight influence on the growth of probiotic strains (free and encapsulated cells).

1- The growth inhibition percent was 6.27 % of free cells ***Lactobacillus rhamnosus*** **ATCC**7469 after 3 h incubation while, growth inhibition percent of encapsulated cells was ranged (from 4.50 to 4.65 %) . It was evident from the obtained data that the percentage of survivors as viable free cells was (93.27 % after 180 min.) while, encapsulated strains showed viability from 95.35 to 95.50 % % after 180 min. incubation at 37 ^{o} c.

2- The growth inhibition percent was 6.03 % of free cells ***Lactobacillus acidophilus*** **DSM20079** after 3 h incubation while, growth inhibition percent of encapsulated cells was ranged (from 3.61 to 4.24 %) . It was evident from the obtained data that the percentage of survivors as viable free cells was (93.97 % after 180 min.) while, encapsulated strains showed viability from 95.76 to 96.39 % after 180 min. incubation at 37 ^{o} c.

3- The growth inhibition percent was 5.81 % of free cells after 3 h incubation while, growth inhibition percent of encapsulated cells was ranged (from 4.48 to 5.19 %) . It was evident from the obtained data that the percentage of survivors as viable free cells was (94.19 % after 180 min.) while, encapsulated strains showed viability from 94.81 to 95.52 % after 180 min. incubation at 37 ^{o} c.

Generally, as shown from results obtained , it could be pointed out that the microencapsulation technique used in this study proved to be very efficient in increasing the viability of all tested cultures compared to non encapsulated free cells when exposed for 180 min. to gastric juice.

d- Survival in simulated intestinal juice (S.I.J).

Three tested ***Lactobacillus rhamnosus*** **ATCC7469** , ***Lactobacillus acidophilus*** **DSM20079** and ***Bifidobacterium bifidum*** **DSM20082** strains were examined for tolerant intestinal juice (S.I.J).
The obtained results declared that probiotic strains retained viability during growth in simulated intestinal juice.

1- Counts of ***Lactobacillus rhamnosus*** **ATCC7469** (free cells) increased by 0.47 % and 0.23 % after 120 and 240 min of exposure ,respectively .But after 360 min of exposure to simulated intestinal juice the counts decreased from 8.42 to 8.37 log_{10} cfu/ ml,(growth inhibition 0.59 %) .In addition , counts of encapsulated ***Lactobacillus rhamnosus ATCC7469*** increased range (0.58 - 0.92 %) and (0.11 - 0.46 %) after 120 and 240 min of exposure ,respectively .But after 360 min of exposure to simulated intestinal juice the counts decreased range from (8.57- 8.64) to (8.54 - 8.63 log_{10} cfu/ ml.

2- Counts of ***Lactobacillus*** **acidophilus DSM20079** (free cells) increased by 0.33 % and 0.11 % after 120 and 240 min of exposure ,respectively .But after 360 min of exposure to simulated intestinal juice the counts decreased from 8.85 to 8.78 log_{10} cfu/ ml,(growth inhibition 0.79 %) . In addition , counts of encapsulated ***Lactobacillus*** **acidophilus DSM20079** with gelatin and xanthan gum increased by (0.65 % & 0.54 %) and (0.32 % & 0.32 %) after 120 and 240 min. of exposure ,respectively .But counts of encapsulated ***Lactobacillus acidophilus*** **DSM20079** with k-carrageenan increased by 0.43 % after 120 min. and then decreased by 0.10 % after 240 min of exposure to simulated intestinal juice.

3-Counts of ***Bifidobacterium bifidum*** **DSM20082** (free cells) increased by 0.42 % and 0.21 % after 120 and 240 min of exposure ,respectively .But after 360 min of exposure to simulated intestinal juice the counts decreased from 9.32 to 9.26 log_{10} cfu/ ml,(growth inhibition 0.64 %) .In addition , counts of encapsulated ***Bifidobacterium bifidum*** **DSM20082** with gelatin and xanthan gum increased by (0.73 % & 0.95 %) , (0.95 % & 1.16 %) and (0.42 % & 0.62 %) after 120, 240 and 360 min. of exposure ,respectively . But counts of encapsulated ***Bifidobacterium bifidum*** **DSM20082** with k-carrageenan increased by 0.73 and 0.31 % after 120 and 240 min. of exposure ,respectively and then decreased by 0.21 % after 360 min of exposure to simulated intestinal juice.

2- <u>Effect of different concentrations of clove and cinnamon extracts on the growth probiotic strains</u>.

Three tested ***Lactobacillus rhamnosus*** **ATCC**7469 , ***Lactobacillus*** **acidophilus DSM20079 and** ***Bifidobacterium bifidum*** **DSM20082.**Strains were examined for their survival at different concentrations of clove and cinnamon extracts (0, 2 , 6 and 10 %) .

- The obtained results showed that the viable counts slightly decreased at 6 % but strongly decreased at 10% compared to 2 %**.**

-Concentration 10 % of cold or hot water clove and cinnamon extract have negative effect on viability of probiotics .While, adding a 2% concentration led to a significant increase in the bacterial counts of ***Lactobacillus rhamnosus*** **ATCC**7469 , ***Lactobacillus*** **acidophilus DSM20079 and** ***Bifidobacterium bifidum*** **DSM20082** compared to control

-Also , the encapsulation with gelatin and xanthan gum have the highest viable cells compare with kappa carrageenan .

3- Incorporation of *Lactobacillus rhamnosus* ATCC7469 in zabady.

a- Chemical analysis :

1-Titratable acidity (%):

The titratable acidity of control and the examined treatments of zabady increased during the storage period at 6 ± 2.0°C for 15 days with simultaneous decreasing trend of the pH values**.** Titratable acidity ranged between (0.79 - 0.91 %) at zero time while , after 15 days of storage period was ranged between (0.97 - 1.13 %) . In addition , samples containing hot water clove or hot water cinnamon extracts had the highest acidity values along the storage period compared with other treatments .

2- pH values:

pH values ranged between (4.89 – 5.06) at zero time while , after 15 days of storage period was ranged between (4.69 - 4.85) . Simple significant differences were found between some treatments.

b- Microbiological tests:

1 -Total bacterial count:

The total bacterial count $\log_{10}$ cfu/ g gradually decreased in the control and other treatments containing clove and cinnamon extracts along the storage period. It can be noticed that the control had higher total bacterial count at all intervals of storage as compared with that of the other treatments also, total bacterial count decreased in the presence of the concentration of clove extract. There were significant differences between the control and all other treatments containing herbal extracts of clove and cinnamon either at cold or hot throughout the storage period.

In addition , the treatments (**F1, X1, K1 and G1**) non containing herbal extracts (control) and non encapsulated *Lactobacillus rhamnosus* ATCC7469 were the highest of (total bacterial count) after 15 days of storage period while , the treatments (**X3, K3 and G3**) containing encapsulated *Lactobacillus rhamnosus* ATCC7469 with(0.05 % xanthan gum , 1% k-carrageenan and 2% gelatin , respectively) and 2% hot water extract of clove were the lowest (total bacterial count) at the same time.

2-Coliform bacteria counts :

Coliform group bacteria were not detecting in all samples of zabady **.**

3- *Streptococcus thermophilus* counts :

Counts of *Streptococcus thermophilus* $\log_{10}$ cfu/ g was gradually decreased till the end of storage period . *Streptococcus thermophiles* count was considerably lower in samples (F3 , X3 and k3) supplemented with hot water extract of clove after 15 days of storage period . On the contrary , samples (F2, X2, K2 and G2) manufactured with cold water extract of clove , and samples (F4, K4 and G4) manufactured with cold water extract of cinnamon gave the highest of *Streptococcus thermophiles* count after 15 days of storage period .

In addition, *Streptococcus thermophilus* counts in all treatments were higher than *Lactobacillus bulgaricus* and they remained stable during storage at levels ranging between 10^7 and 10^9 cfu/ml throughout the storage period .

4- *Lactobacillus delbrueckii* subsp. *bulgaricus* counts:

The counts of *Lactobacillus bulgaricus* log_{10} cfu/ g was gradually decreased till the end of storage period .There were no significant difference between samples (F3,X3,k3 and G3) supplemented with hot water extract of clove and (F5, X5 , K5and G5) supplemented with hot water extract of cinnamon also, the lowest *Lactobacillus bulgaricus* count was recorded in these sample after 15 days of storage period . On the contrary , samples (F2, X2 and G2) supplemented with cold water extract of clove , and samples (F4, X4, K4 and G4) containing cold water extract of cinnamon gave the highest of *Lactobacillus bulgaricus* count after 15 days of storage period .

5- *Lactobacillus rhamnosus* ATCC7469 counts:

***Lactobacillus rhamnosus* ATCC7469** counts decreased ~ (1.35 – 1.50) log_{10} cfu/ g after 15 days of storage. Viability of ***Lactobacillus rhamnosus* ATCC7469** in all treatments remained above 10^6 cfu/ ml till the end of storage period. (**FAO/WHO, 2001**). Adding a 2% concentration of cold water extract of clove or cinnamon led to a significant increase in the bacterial counts of ***Lactobacillus rhamnosus* ATCC7469** compared to control. Encapsulation of ***Lactobacillus rhamnosus* ATCC7469** with xanthan gum and gelatin significantly improved the viability of the bacteria in zabady samples compared to free (non-encapsulated).

c- Rheological properties:

The instrumental textural attributes of different zabady samples contained free & encapsulated ***Lactobacillus rhamnosus* ATCC7469** and different herbal extracts was evaluated after one week of storage period .The obtained results showed the treatments (**F5, X5,** and **G5**) containing hot water extract of cinnamon and **k1(** control **)** were found to have higher hardness , gumminess and chewiness values than other treatments . There are no clear differences in resilience between all treatments

d- Organoleptic properties:

The total scoring point gradually decreased in all treatments till at the end of storage period. The sample (**G5**) containing hot water extract of cinnamon and encapsulated cells with gelatin got the highest scoring points than other treatments after 5 and 15 days of storage period which gained (95.36 and 91.67) ,respectively followed by sample (**X5**) made with xanthan gum encapsulated ***Lactobacillus rhamnosus* ATCC7469** and hot water extract of cinnamon which gained (94.84 and 90.60)respectively, at the same period.
Also , the treatment (**K3**) containing hot water extract of clove and encapsulated cells with k-carrageenan was the lowest scoring points after 15 days of storage period which gained (82.95).

4-Incorporation of *Lactobacillus acidophilus* DSM20079 in zabady.

a- Chemical analysis :

1-Titratable acidity (%):

The titratable acidity of control and the examined treatments of zabady increased during the storage period at 6 ± 2.0°C for 15 days with simultaneous decreasing trend of the pH values**.** Titratable acidity ranged between (0.78 - 0.92 %) at zero time while , after 15 days of storage period was ranged between (1.01 - 1.14 %) . In addition , The samples containing hot water clove and hot water cinnamon extracts had the highest acidity values along the storage period compared with other treatments .

2- pH values:

pH values ranged between (4.88 – 5.01) at zero time while , after 15 days of storage period was ranged between (4.65 - 4.77) . Simple significant differences were found between some treatments**.**

b- Microbiological tests:

1 -Total bacterial count:

The control had higher total bacterial count $\log_{10}$ cfu/ g at all intervals of storage as compared with that of the other treatments also, total bacterial count decreased in the presence of the concentration of clove extract. In addition , the treatments (**F1 and X1**) non containing herbal extracts (control) and non encapsulated *Lactobacillus acidophilus DSM20079* were the highest of (total bacterial count) after 15 days of storage period while , the treatments (**K3 and G3**) containing encapsulated *Lactobacillus acidophilus* DSM20079 with (1% k-carrageenan and 2% gelatin , respectively) and 2% hot water extract of clove were the lowest (total bacterial count) at the same time.

2-Coliform bacteria counts :

Coliform bacteria have not been detected in all treatments till the end of the storage period **.**

3- *Streptococcus thermophilus* counts :

From the foregoing results it could be stated that adding a 2% concentration of cold water extract of clove and cinnamon led to a significant increase in the bacterial counts of *Streptococcus thermophilus* $\log_{10}$ cfu/ g compared to control. Also, the results showed that the hot water extract of clove and cinnamon had significant effects on the viability of *Streptococcus thermophilus* .

4- *Lactobacillus delbrueckii* subsp*. bulgaricus* counts:

From the foregoing results it could be stated that *Lactobacillus bulgaricus* counts decreased ~ (1.85 – 2.15) $\log_{10}$ cfu/ g after 15 days of storage. Adding a 2% concentration of cold water extract of clove and cinnamon led to a significant increase in the bacterial counts of *Lactobacillus bulgaricus* compared to control. Also, the results

showed that the hot water extract of clove and cinnamon had significant effects on the viability of *Lactobacillus bulgaricus.*

5- *Lactobacillus acidophilus DSM20079counts*:

The samples (X2 , X4, K4, , G1 ,G2, and G4) gave the highest of ***Lactobacillus acidophilus DSM20079*** count after 15 days of storage period ranged from(8.75) to (8.81) $\log_{10}$ cfu/ g . While , samples (F1, F4 and F5) gained the lowest viability after 15 days of storage period which ranged from (8.27) to (8. 32) $\log_{10}$ cfu/ g . In addition, encapsulated ***Lactobacillus acidophilus*** **DSM20079** with different materials in all treatments was higher than non encapsulated (free cells) throughout the storage period , and there were significant difference between encapsulated ***Lactobacillus acidophilus*** **DSM20079** and non encapsulated

c- Rheological properties:

The obtained results showed the treatments (**F5, X5, k5** and **G5**) containing hot water extract of cinnamon and were found to have higher hardness , gumminess and chewiness values than other treatments .

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d- Organoleptic properties:

The total scoring point gradually decreased in all treatments till at the end of storage period. The sample (**G4**) containing cold water extract of cinnamon and encapsulated cells with gelatin got the highest scoring points than other treatments after 5 and 15 days of storage period which gained (95.22 and 92.84) ,respectively followed by sample (**X4**) made with xanthan gum encapsulated ***Lactobacillus acidophilus*** **DSM20079** and cold water extract of cinnamon which gained (94.98 and 91.92) respectively, at the same period.

In addition , the treatments (**K3**) containing hot water extract of clove and encapsulated cells with k-carrageenan , and (**K5)** containing hot water extract of cinnamon and encapsulated cells with k-carrageenan were the lowest scoring points after 15 days of storage period which gained (83.22 and 83.16) ,respectively .

5-Incorporation of *Bifidobacterium bifidum* DSM20082 in zabady.

a- Chemical analysis :

1-Titratable acidity (%):

The titratable acidity of the control and the examined treatments of zabady increased during the storage period at 6 ± 2.0°C for 15 days with simultaneous decreasing trend of the pH values**.** Titratable acidity ranged between (0.83 - 0.90 %) at zero time while , after 15 days of storage period was ranged between (1.01 - 1.12 %) . In addition , The treatment (**F3**) containing non encapsulated ***Bifidobacterium bifidum*** **DSM20082** and 2% hot water extract of clove had the highest acidity values along the storage period compared with other treatments .

2- pH values:

pH values for the different treatments of zabady containing free and encapsulated ***Bifidobacterium bifidum*** **DSM20082** decreased during the storage. pH values ranged between (4.92 – 5.09) at zero time while , after 15 days of storage period was ranged between (4.68 - 4.82) . Simple significant differences were found between some treatments.

b- Microbiological tests:

1 -Total bacterial count:

The total bacterial count $\log_{10}$ cfu/ g gradually decreased in the control and other treatments containing clove and cinnamon extracts along the storage period. Also, the control had higher total bacterial count at all intervals of storage as compared with that of the other treatments . Total bacterial count decreased in the presence of the concentration of clove extract. In addition , the treatments (**F1 and K1**) non containing herbal extracts (control) and non encapsulated ***Bifidobacterium bifidum*** **DSM20082** were the highest of (total bacterial count) after 15 days of storage period while , the treatments (**X5, K5 and G5**) containing encapsulated ***Bifidobacterium bifidum*** **DSM20082** with (.05 % , 1 % k-carrageenan and 2% gelatin , respectively) and 2% hot water extract of cinnamon and the treatment (**G3)** containing encapsulated ***Bifidobacterium bifidum*** **DSM20082** with 2% gelatin & 2% hot water extract of clove were the lowest (total bacterial count) at the same time.

2-Coliform bacteria counts :

Coliform bacteria was not detected in all of treatments whether in fresh or stored**.**

3- ***Streptococcus thermophilus*** **counts :**

The counts of *Streptococcus thermophilus* $\log_{10}$ cfu/ g was gradually decreased till the end of storage period . *Streptococcus thermophiles* count was considerably higher in samples (X4 , K4 and G4) supplemented with cold water extract of cinnamon after 15 days of storage period .There were no significant difference between samples (F1,F2,F3, F5 ,X3, X5, K3 , K5, G3andG5) also, the lowest *Streptococcus thermophilus* count was recorded in these sample after 15 days of storage period . In addition, *Streptococcus thermophilus* counts in all treatments were higher than *Lactobacillus bulgaricus* and they remained stable during storage at levels ranging between 10^7 and 10^9 cfu/ml throughout the storage period .

4- ***Lactobacillus delbrueckii*** **subsp.** ***bulgaricus*** **counts:**

The counts of *Lactobacillus bulgaricus* $\log_{10}$ cfu/ g was gradually decreased till the end of storage period . The samples (F4 , X4, K4, G2, G3 and G4) gave the highest of *Lactobacillus bulgaricus* count after 15 days of storage period while, other treatments gave the lowest viability and there were no significant difference between them. In addition, *Lactobacillus bulgaricus* counts in all treatments was lower than *Streptococcus thermophilus* . Adding a 2% concentration of cold water extract of clove

and cinnamon led to a slightly increase in the bacterial counts of *Lactobacillus bulgaricus* compared to control.

5- *Bifidobacterium bifidum* DSM20082 counts:

The counts of ***Bifidobacterium bifidum*** **DSM20082** $\log_{10}$ cfu/ g was gradually decreased till the end of storage period .The samples (X2 , X4, and G4) gave the highest of ***Bifidobacterium bifidum*** **DSM20082** count after 15 days of storage period ranged from(8.64) to (8.70) $\log_{10}$ cfu/ g . While , samples (F1 and F3) gained the lowest viability after 15 days of storage period which ranged from (7.86) to (7.92) $\log_{10}$ cfu/ g . In addition, encapsulated ***Bifidobacterium bifidum*** **DSM20082** with different materials in all treatments was higher than non encapsulated (free cells) throughout the storage period , and there were significant difference between encapsulated ***Bifidobacterium bifidum*** **DSM20082** and non encapsulated . The addition of cold water extract of clove and cinnamon led to improve the viability of ***Bifidobacterium bifidum*** **DSM20082** and also, significant increase in the bacterial counts of compared to control.

c- Rheological properties:

The treatments (**F5, X5, K5** and **G5**) containing hot water extract of cinnamon were found to have higher hardness , gumminess and chewiness values than other treatments.

d- Organoleptic properties:

The total scoring point gradually decreased in all treatments till at the end of storage period. The sample (**X4**) containing cold water extract of cinnamon and encapsulated cells with xanthan gum got the highest scoring points than other treatments after 5 and 15 days of storage period which gained (95.81 and 92.49) , respectively followed by sample **F 4** (containing free cells & cold water extract of cinnamon) and **K4** (made with kappa carrgeenan encapsulated ***Bifidobacterium bifidum*** **DSM20082** and cold water extract of cinnamon) which gained (94.64 & 90.68) and (94.66 & 91.85) respectively, at the same period.

In addition , the treatment (**F3**) containing hot water extract of clove and non encapsulated cells with was the lowest scoring points after 15 days of storage period which gained (84.45).