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QUALITY CHARACTERISTICS OF MAYONNAISE ENRICHED BY PROBIOTIC BACTERIA

BY

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ABSTRACT

The effect of free and encapsulated cells of *Lactobacillus casei* DSMZ 20011, *Lactobacillus acidophilus* ATCC 4321 and *Bifidobacterium longum* ATCC 15707, on mayonnaise quality characteristics were studied during subsequent refrigerated storage for six months. Bifidobacteria and Lactobacillus were added during mayonnaise preparation as either free or encapsulated cells. No viable cells were recovered after three months of storage in mayonnaise prepared using free cells of the studied bacteria. While, it was noticed such decreases in viability of encapsulated *L. casei*, *L. acidophilus* and *Bif. longum* these decreases not reached the viable counts to minimum levels (10^5 to 10^6/g) needed at consumption of the product to achieve the beneficial effects of bifidobacteria. The total bacterial count in all treatments were slightly increased during storage period, these increase were associated with the reduction in viable counts of Lactobacillus and Bifidobacteria. All prepared mayonnaise samples were free from salmonella during storage period, while, after one month of storage the staphylococcal disappeared in all treated and control samples. pH, acid, peroxide, anisidine, total oxidation (Totox) values and sensory attributes were determined during storage period. pH values decreased with prolonging the storage period till reached to their maximal values at the end of storage period ranging between 4.58 in control sample and 4.05 in treated samples. At the same time, mayonnaise samples prepared by encapsulated probiotic bacteria were more significantly ($p<0.05$) stable against lipid oxidation during storage period in comparison to samples prepared using free cells. Scores of sensory characteristics of untreated mayonnaise and mayonnaise treated with encapsulated cells were not significantly ($p>0.05$) affected during storage period.

Key words: Lactobacillus, Encapsulated, Bifidobacteria, Mayonnaise, Quality, Sensory, Oxidation.

INTRODUCTION

The food industry is directing new product development towards the area of functional foods due to consumers demand for healthier foods. The current terminology, probiotics, is used to describe foods that are produced by or contain live microorganisms that possess therapeutic or health benefits (Farnsworth, 2001). The most recent consensus requires that probiotics are live

and capable of surviving passage through the digestive tract and have the capability to proliferate in the gut (FAO/WHO, 2001), where they have been redefined as "live microorganisms when administrated in adequate amount confer a health benefit on the host" *lactobacillus and Bifidobacterium* are the principal bacterial genera centered to both probiotics and prebiotics (no digestible food ingredients that stimulate the growth and/or activity of certain colonic bacteria) approaches to dietary modulation of the intestinal microflora (Schrezenmeir and de Vrese, 2001).

For probiotic microorganisms to exert the health potential benefits, large numbers of viable cells must survive passage through the harsh environment of the gastrointestinal tract (GIT), hence the need to select strains that are acid, salt and bile salts tolerant and possess the capability to adhere intestinal cells (Rasic and Kurmann, 1983). Furthermore, probiotic bacteria must be a normal intestinal organism that can colonize the intestine and have to be metabolically active to give beneficial effects and remain viable in the food carrier up to consumption. Viable probiotic bacteria count should be $\geq 10^6$ CFU/g to be effective and should be consumed regularly (Guyot, 1990; Kurmann, 1988 and Rasic and Kurmann, 1983).

Probiotic supplements contain viable bacteria that beneficially influence health and nutrition when consumed (Salminen, *et al.*, 1998). Most commonly they contain *Lactobacillus acidophilus* and *Bifidobacterium*, both of which are part of the normal intestinal microbiota (Dali and Davis, 1998).

Lactobacillus acidophilus, L. casei, Bifidobacterium bifidum, B. longum and *Saccharomyces boulardii* are frequently used as probiotics in products for human consumption (Playne, 1994), although other species are also recognized as probiotics foods containing these microorganisms are sold in many countries, although their survival in foods is doubtful, since some of the strains are extremely sensitive to a series of factors. Also methods of counting these organisms have not yet been well established, which is an essential requirement to determine their survival in commercial products (Kailasapathy and Rybka, 1997).

Commercial mayonnaise and salad dressings are microbiologically shelf stable, and extremely safe processed foods. The safety of these products is directly associated with synergistic formulation components of which aqueous phase acetic acid and total formula pH level (< 4.1) are considered the most essential in inactivating food borne pathogens such as *Salmonella spp.* and *Staphylococcus aureus* (Miller and Martin, 1990).

The growth of bifidobacteria is considerably retarded at pH< 5.0 (Lankaputhra *et al.* 1996). Bifidobacteria must be alive in mayonnaise to provide benefits in the intestinal tract. Encapsulation may enhance the survival of bifidobacteria (Khalil and Mansour, 1998). Therefore, our study was undertaken to incorporate *Bifidobacteria* and *Lactobacillus* in mayonnaise as encapsulated cells.

The objectives of this work were to study the viability of encapsulated and free cells of *Bifidobacteria* and *Lactobacillus* in mayonnaise during refrigerated storage (~ 5°C) and to evaluate their effects on other microbiological changes and mayonnaise quality attributes.

MATEREIALS AND METHODS

Sources of bacterial strains

Lactobacillus casei DSMZ 20011 15707, *Lactobacillus acidophilus* ATCC 4321 and *Bifidobacterium longum* ATCC were obtained from the Egyptian Microbial Cultures Collection (EMCC) at Cairo Microbiological Resources Center (Cairo MIRCEN), Faculty of Agriculture, Ain Shams University.

Cultivation and harvesting of the bacterial cells

L. casei is grown at 37 °C for 24 h in MRS broth and *L. acidophilus* is grown in MRS broth supplemented with 0.2 % oxalgal at 37 °C for 24 h. Culture cells were harvested by centrifugation (5000 xg for 5 min at 4 °C).

Bifidobacterium longum is grown at 37 °C for 24 h in modified MRS (MRS+0.05 % L-cystein and 0.3 % lithium chloride.

The cell pellets were washed twice with 0.01 M. potassium phosphate buffer, pH 7. The washed bacterial cell pellets were resuspended in a volume of 0.01 M potassium phosphate buffer, pH 7.0 equivalent to 0.2 from the volume of the growth medium.

Preparation of gel beads

Cell pellets bacteria were washed twice with cold (4 °C) sterilized physiological peptone water (0.1 %). All immobilization steps were conducted under aseptic conditions and sterilized solutions.

Gel beads of reservoir type microcapsules were prepared by suspending 20 g cells (wet weight) in 1000 ml of sterile solution of 2 % sodium alginate, then the mixture was added drop wise using a micropipette into a flask containing sterile solution of $CaCl_2$ (2 % W/V) under agitation (30 rpm). The single layer gel beads of approximately 4 mm diameter were formed and left in the $CaCl_2$ solution for 60 min to allow the gel to harden. The gel beads were rinsed with sterile physiological peptone water (0.1 %) to remove the excess calcium ions and entrapped cells.

Preparation of mayonnaise

Mayonnaise sample was prepared using the suggested formula (%): corn oil, 70; whole egg, 19.1; salt, 1.0; sugar, 0.6; lemon juice 1.6; vinegar, 5.6; mustard, 1.8 and white pepper 0.3. Salt, sugar, mustard and white pepper were first mixed with whole egg, vinegar and lemon juice using electric mixer on liquefy velocity for 5 sec. The oil was then added to the system on puree velocity and more rapidly after the mass begins to thicken, with raising gradually the

velocity from puree to liquefy during 50 sec. All the ingredients were then mixed on liquefy velocity for 20 sec.

The prepared mayonnaise was divided into seven portions for treatments. One portion was left without bacterial inoculation as a control. The six other portions were inoculated separately with free cells (final concentrations of about $\sim 10^{11}$) and encapsulated cells (final concentrations of about $\sim 10^{10}$) of each of *L. casei.*, *L. acidophilus* and *Bif. longum.*

The prepared mayonnaise samples were packed in 100 g size glass jars with screw cap and stored at 5 °C. Samples were taken at the next day and after one month intervals in three replications tell 6 months for measuring pH, and analysis for their lipid oxidative rancidity; microbiological and sensory evaluations.

Microbiological analysis

L. Casei count was determined using MRS agar according to De Man *et al.*, (1960) at 37 °C for 48 h. *L. acidophillus* count was determined using modified MRS agar supplemented with 0.2 % oxagal according to Gilliland and Walker (1990). The plates were incubated at 37 °C for 48 h.

Bifidobacterium longum was enumerated according to Dave and Shah (1996) using modified MRS agar supplemented with 0.05 % L-cystein and 0.3 % lithium chloride. The plates were anaerobically incubated at 37 °C for 48 h.

Mayonnaise with encapsulated cells samples were released from the beads according to the procedure of Sheu *et al.*, (1993) by mixing mayonnaise samples with phosphate buffer (1 M., pH 7.5).

Total bacterial counts were enumerated on standard plate count agar (Marth, 1978) and incubated at 30 °C for 72 h.

Salmonella count was determined on bismuth sulphite agar and incubated at 35 °C for 48 h according to Marvin (2001).

Staphylococcal count was detected on Baird-parker medium and incubated at 32 °C for 48 h according to Blair *et al.*, (1967).

pH value

The pH values of mayonnaise samples were measured according to the procedure of Zaika *et al.* (1976).

Lipid analyses

Lipids were extracted from mayonnaise samples according to the method described in AOAC (2000). Progress of oxidation was monitored by determination of Acid value (AV) and peroxide value (PV) according to AOAC (2000), *p*-anisidine value (AnV) by the method described by IUPAC (1987) and

total oxidation value (Totox value) calculated by the formula as reviewed by Rossell (1983):

$$\text{Totox value} = 2\,PV \times AnV$$

Sensory evaluation of mayonnaise

Mayonnaise samples prepared using different type of Lactobacillus and Bifidobacterium as well as the control sample were asked for their quality attributes by ten members preference taste panel, from staff of the Department of Food Science, Faculty of Agriculture, Ain Shams University. The panelists were asked to score appearance, color, consistency, mouth feel, taste, overall acceptability by giving a degree up ten using the report sheet according to Worrasinchai *et al.*, (2006).

Statistical analysis

The obtained data were exposed to analysis of variance. Duncan multiple range at 5% level of significance was used to compare between means. The analysis was carried out using the PROC ANOVA procedure of Statistical Analysis System (SAS, 1996).

RESULT AND DESCUSION

Survival of Lactobacillus and bifidobacteria

The survival of Lactobacillus and bifidobacteria in prepared mayonnaise samples is presented in Figure (1). The sharp decline was observed in the population of *L. casei., L. acidophilus and Bif. longum*, respectively in the treatments containing free cells compared to those containing encapsulated cells. Microencapsulation was used successfully by (Rao, *et al.*, 1989) to increase the viability of bifdobacteria in simulated gastric and intestinal juice and by (Dinakar and Mistry, 1994) in cheddar cheese.

As shown in Figure (1) the viable count of the free cells of *L. casei., L. acidophilus and Bif. longum* decreased markedly after one month of refrigerated storage reaching 8.88, 8.91 and 7.41 (log cfu/g), respectively, then continued to declined reaching 4.25, 4.67 and 4.74 (log cfu/g), respectively after two months. No viable cells were recovered after three months; these decreases might be attributed to the bactericidal activity of acetic acid in mayonnaise samples (Collins, 1985; Lock and Board, 1994).

The viability of encapsulated *L. casei., L. acidophilus and Bif. longum* slightly decreased after one month of storage to 9.30, 10.27 and 10.2 (log cfu/g), respectively. At the third month, the viable counts of encapsulated *L. casei., L. acidophilus and Bif. longum* reached to 8.07, 8.49 and 8.04 log cfu/g, respectively. The high counts of survival of the encapsulated cells resulted from the protection by the calcium alginate.

Sheu, *et al.*, 1993 reported that calcium alginate could provide good protection (90 %) for lactobacilli in frozen ice milk.

The viability of encapsulated *L. casei., L. acidophilus and Bif. longum* decreased until the end of refrigerated storage reaching 5.85, 5.43 and 5.62 (log cfu/g) respectively. These counts were higher than the minimum levels (10^5 to 10^6/g) needed at consumption of the product to achieve the beneficial effects of bifidobacteria (Ishibashi and Shimamura, 1993). This reduction might be due to the release of lactobacillus and bifidobacteria into mayonnaise when calcium alginate beads are partially degraded and consequently the free cells were influenced by the bactericidal effect of acetic acid (Khalil and Mansour, 1998).

Total bacterial counts

From Table 1, the total bacterial counts of mayonnaise containing encapsulated *L. casei, L. acidophilus* and *Bif. longum* and the control were higher than mayonnaise containing free cells of *Lactobacillus* and *Bifidobacteria* at the initial of storage. These counts slightly increased in all mayonnaise samples as the storage period prolonged to six months.

Table (1): Total bacterial counts (log cfu/g) of control mayonnaise sample and the samples prepared using free and encapsulated Lactobacillus and Bifidobacteria during storage at 5°C.

Storage period (month)	Control	Free cells			Encapsulated cells		
		L. casei	*L. acidophilus*	*Bif. longum*	*L. casei*	*L. acidophilus*	*Bif. longum*
0	4.93	3.04	3.23	3.41	4.43	4.79	4.63
1	4.63	3.43	3.53	3.61	4.70	4.94	4.83
2	5.20	3.83	3.70	3.81	4.99	4.97	4.91
3	5.79	5.27	5.77	5.63	5.04	5.27	5.36
4	5.94	5.51	5.91	5.88	5.54	5.61	5.56
5	6.43	6.20	6.04	6.76	6.27	6.43	6.38
6	6.85	6.83	6.74	6.91	6.69	6.85	6.79

It was noticed that the total bacterial counts in mayonnaise containing the free cells were still lower than in those containing encapsulated cells and the control are by ~1 log cycle till the two months of storage. Thereafter, the counts in all samples were practically the same at all the storage period till the end of storage. The presence of viable bifidobacteria in mayonnaise and thus there ability to produce antimicrobial substances (Kang *et al.*, 1989; Kebary, 1995) might explain decline of total bacterial count in mayonnaise containing free cells of *L. casei., L. acidophilus and Bif. longum*. The reduction in viable lactobacillus and bifidobacteria at late storage was associated with the increase in total bacterial counts.

Salmonella and staphylococcal count

Figure (2) indicated that all prepared mayonnaise samples were free from Salmonella. While, they had detectable staphylococcus in zero time; Their counts

were lower in mayonnaise containing lactobacillus and bifidobacteria than the control. After one month of storage period, the staphylococcal disappeared in all treated and control samples. In this respect, Weagant, *et al.* 1994 indicated that mayonnaise with its inherent acidic nature, is not usually considered to be a significant risk as a vehicle for transmission of enteric pathogens (Wea gant , *et al.*, 1994).

pH value

The pH value in untreated mayonnaise sample was not affected by storage period (Table, 2). However, the addition of free and encapsulated cells of *L. casei., L. acidophilus and Bif. longum* in prepared mayonnaises lead to decreases in pH values than in the control sample.

Table (2): pH values of control mayonnaise sample and the samples prepared by using free and encapsulated Lactobacillus and Bifidobacteria during storage at 5°C.

Storage period (month)	Control	Free cells			Encapsulated cells		
		L. casei	*L. acidophilus*	*Bif. longum*	*L. casei*	*L. acidophilus*	*Bif. longum*
0	4.51	4.51	4.51	4.51	4.51	4.51	4.51
1	4.5	4.49	4.50	4.47	4.50	4.50	4.50
2	4.54	4.45	4.47	4.47	4.47	4.47	4.49
3	4.56	4.37	4.39	4.42	4.45	4.46	4.43
4	4.56	4.23	4.26	4.34	4.39	4.37	4.36
5	4.58	4.11	4.15	4.23	4.26	4.22	4.30
6	4.58	4.06	4.09	4.12	4.08	4.05	4.23

The decreases in pH values could be attributing to acid production by surviving *lactobacillus* and *bifidobacteria.* Kebary, (1996) reported that the acidity increased in frozen yogurt which manufactured using *Bifidobacterium bifidum.* Another indication that the metabolically activity *of bifidobacteria* during refrigerated storage generated acetic acid and raised the acid content was reported by (Adhikari, *et al.*, 2000). Khalil and Mansour (1998) also reported that the increase in titratable acidity could be attributed to acid production by surviving bifidobacteria. Therefore, the pH values were decreased in all mayonnaise samples prepared using the bacterial strains in this investigation.

Characteristics of extracted lipids from mayonnaise samples

The stability of mayonnaise lipids in different mayonnaises prepared by inoculation of free or encapsulated *L. casei., L. acidophilus and Bif. longum* cells were determined periodically after one month intervals till six months at 5°C. Lipids of stored mayonnaise were extracted and analyzed for their acid value (AV), peroxide value (PV), *p*-anisidine value (AnV), and total oxidation value (Totox V).

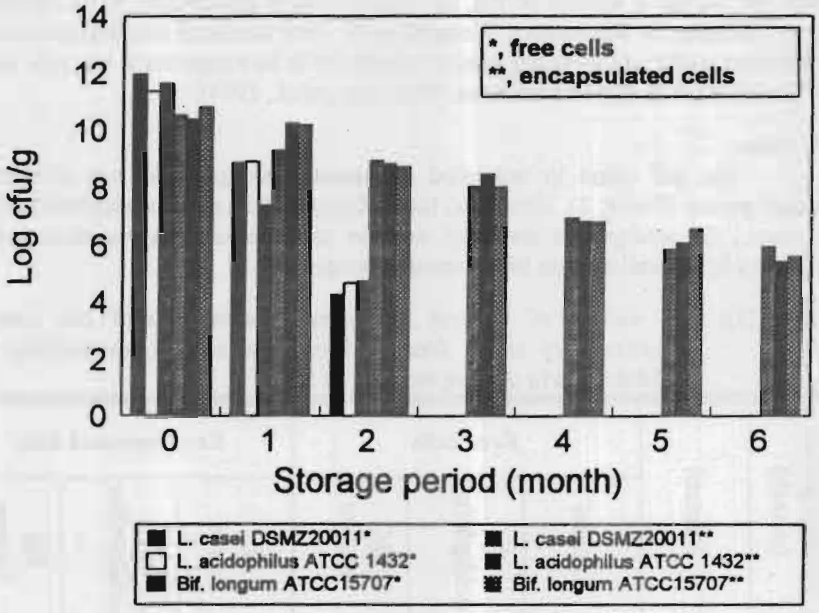

Fig.(1): Survival of free and encapsulated Lactobacillus and Bifidobacteria (log cfu/g) in mayonnaise samples during storage at 5°C.

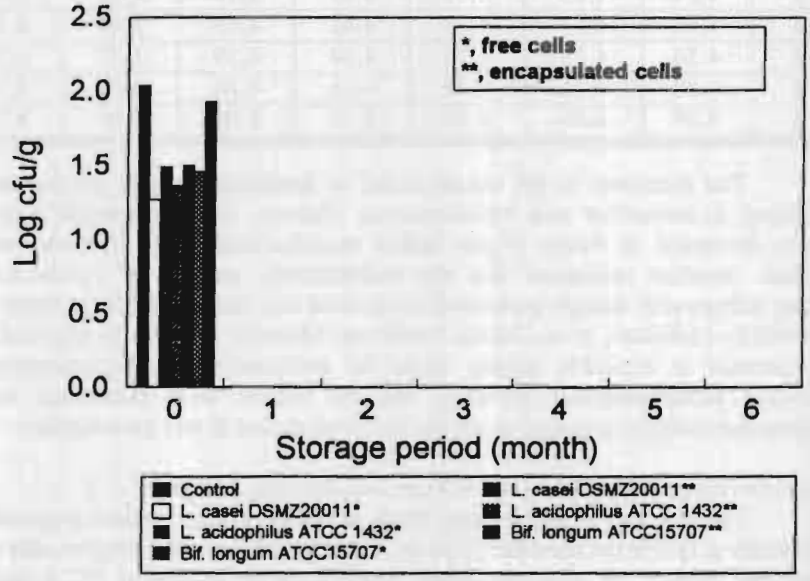

Fig.(2): Staphylococcal count (log cfu/g) of control mayonnaise sample and the samples prepared using free and encapsulated Lactobacillus and Bifidobacteria during storage at 5°C.

Acid value

The AV of extracted lipids was used as a measure of lipid hydrolysis that lead to formation of free fatty acids. From the presented data in Table (3) it could be noticed that, the AV increased after two months of refrigerated storage in all treated and control samples. It was significantly ($p<0.05$) increased during the storage period in all prepared mayonnaise samples. These increases might be due to the increase of lipolytic effect as a result of bacterial activity which encourages lipid oxidation. The significant ($p<0.05$) lowest AV was observed in control mayonnaise sample compared to treated mayonnaise samples. The increases in AV in treated samples was due to lipolysis activity of lipase enzyme which, produced by the active lactobacillus and Bifidobacterium used in preparation of mayonnaise samples (Rossi *et al.*, 1998). This enzyme, promote the hydrolysis of triglycerides formed di-mono-glycerides and/or glycerol and free fatty acids. El-shafie, *et al.*, 2003 reported that fat hydrolysis and accumulation of free fatty acids increased along the storage period in some emulsified foods containing probiotic culture. Other mechanism may be occurred according to Frankel *et al* (1994) who reported that prediction of antioxidant efficacy in emulsions is further complicated by what has been described as the "polar paradox": polar antioxidants such as organic acid is more effective.

Table (3): Acid values of extracted lipid from control mayonnaise sample and the samples prepared by using free and encapsulated Lactobacillus and Bifidobacteria during storage at 5°C.

Storage period (month)	Control	Free cells			Encapsulated cells		
		L. casei	*L. acidophilus*	*Bif. longum*	*L. casei*	*L. acidophilus*	*Bif. longum*
0	0.06^{aC}	0.06^{aD}	0.06^{aD}	0.06^{aB}	0.06^{aD}	0.06^{aD}	0.06^{aD}
1	0.07^{aC}	0.07^{aD}	0.07^{aD}	0.06^{aB}	0.06^{aD}	0.07^{aD}	0.07^{aD}
2	0.07^{aC}	0.08^{aD}	0.09^{aD}	0.08^{aB}	0.09^{aD}	0.10^{aD}	0.10^{aD}
3	0.36^{aBC}	0.50^{aC}	0.51^{aC}	0.49^{aD}	0.54^{aC}	0.58^{aC}	0.58^{aC}
4	0.70^{bAB}	1.06^{abB}	0.94^{abB}	0.98^{abC}	1.09^{abB}	1.17^{abB}	1.25^{aB}
5	0.87^{bAB}	1.56^{aA}	1.59^{aA}	1.54^{aB}	1.71^{aA}	1.67^{aA}	1.67^{aA}
6	1.01^{bA}	1.84^{aA}	1.88^{aA}	1.82^{aA}	1.71^{aA}	1.83^{aA}	1.83^{aA}

Different capital alphabets within the same column are significantly ($P<0.05$).
Different small alphabets within the same raw are significantly ($P<0.05$).

Peroxide value

PV is one of the most widely used tests for determination of the primary oxidation products. PV is a measure of the concentration of peroxides and hydroperoxides formed with initial stages of lipid oxidation (Goli *et al.*, 2005). Results in Table (4) showed that, the PV in extracted lipids from all prepared mayonnaise samples was periodically increased till reached its maximal levels at the end of storage period after six month. Its value ranged between 2.50 and 3.28; 2.22 and 2.38 meq/kg in extracted lipids from mayonnaise prepared using the free

and encapsulated cells *L. casei., L. acidophilus and Bif. longum*, respectively compared to 7.99 meq/kg in extracted lipids from control sample at the end of storage period. It could be noticed that preparation of mayonnaise using encapsulated cells lead to increase the lipid oxidation stability.

Table (4): Peroxide values (meq./kg) of extracted lipid from control mayonnaise sample and the samples prepared by using free and encapsulated Lactobacillus and Bifidobacteria during storage at 5°C.

Storage period (month)	Control	Free cells			Encapsulated cells		
		L. casei	*L. acidophilus*	*Bif. longum*	*L. casei*	*L. acidophilus*	*Bif. longum*
0	0.39^{aD}	0.39^{aC}	0.39^{aC}	0.39^{aC}	0.39^{aC}	0.39^{aC}	0.39^{aC}
1	0.39^{aD}	0.39^{aC}	0.39^{aC}	0.39^{aC}	0.42^{aC}	0.40^{aC}	0.45^{aC}
2	1.29^{aD}	0.39^{bC}	0.40^{bC}	0.39^{bC}	0.42^{bC}	0.41^{bC}	0.45^{bC}
3	4.89^{aC}	1.26^{bB}	1.29^{bB}	1.25^{bB}	0.42^{cC}	0.42^{c}	0.45^{cC}
4	5.93^{aB}	1.77^{bB}	1.80^{bB}	1.75^{bB}	0.84^{bBC}	0.89^{bC}	0.89^{bBC}
5	6.71^{aB}	2.56^{bA}	2.71^{bA}	2.63^{bA}	1.39^{cB}	1.49^{cB}	1.49^{cB}
6	7.99^{aA}	3.28^{bA}	3.09^{bA}	2.50^{cA}	2.22^{cA}	2.38^{cA}	2.38^{cA}
Slope	1.43	0.52	0.51	0.43	0.28	0.31	0.30
PF	1.00	2.75	2.83	3.29	5.10	4.64	4.72

Different capital alphabets within the same column are significantly ($P<0.05$).
Different small alphabets within the same raw are significantly ($P<0.05$).
PF, protective factor.

The high PV in extracted lipid from control mayonnaise sample due to adding of acetic acid which, lead to decrease pH value. Jacobsen *et al.*, (2001) reported that the low pH induced oxidation. Thus, it seems reasonable to suggest that the low pH activates metal ions present in the egg yolk, and that these metal ions subsequently are able to induce oxidation by peroxide cleavage reactions at interfacial surface in emulsion. On the other hand, mayonnaise samples prepared using *L. casei., L. acidophilus and Bif. longum* appeared a higher significant ($p<0.05$) retarding of lipid per-oxidation than of control sample. This effect may be due to microbial activation which produces some compounds cleavage peroxidation. Lin *et al.* (1999) found that *L. acidophilus* and *Bif. longum* strains were able to protect biological lipids (linoleic acid per-oxidation) from oxidation. They added that the antioxidative effect of each ml of intracellular cell free extract of *L. acidophilus* and *Bif. longum* was equivalent to 104-172 ppm of butylated hydroxy toluene.

The slopes of the rise in peroxide values of extracted lipids from mayonnaise samples were calculated as an indicator of the rate of oxidation (Malecka, 2002). The higher slope value means the high oxidation. The calculated slope of PV for control sample was the highest one being 1.43 with the

lowest protective factor of 1 while, samples prepared using encapsulated of *L. casei., L. acidophilus and Bif. longum* had the lowest slope values ranging between 0.28 and 0.31 having the highest protective factor ranging between 4.64 and 5.1, respectively. The experiments carried out proved that all treated mayonnaise samples inhibit lipid per-oxidation, the highest protective effect being observed in samples containing encapsulated cells followed by those containing free cells..

Anisidine value

The primary oxidative compounds mainly peroxides and hydroperoxides are transitory intermediates which decompose into various carbonyle and other compounds. The AnV test determines the level of aldehydes, principally 2-alkenals, present in the oil as an indication of the formation of secondary oxidative compounds. Consequently, Li Hsieh and Regenstein (1992) Predicated that peroxide value was the best test for early oxidation of mayonnaise oil, however, for later stages of oxidation the AnV was recommended. The AnV was evaluated periodically during storage of mayonnaise samples at refrigerated temperature as an indication of secondary oxidative compounds formation. AnV of extracted lipids of all prepared mayonnaise samples were significantly ($p<0.05$) increased as the storage period progressed (Table, 5).

Table (5): Anisidine values of extracted lipid from control mayonnaise sample and the samples prepared by using free and encapsulated Lactobacillus and Bifidobacteria during storage at 5°C.

Storage period (month)	Control	Free cells			Encapsulated cells		
		L. casei	*L. acidophilus*	*Bif. longum*	*L. casei*	*L. acidophilus*	*Bif. longum*
0	0.86^{aE}	0.86^{aA}	0.86^{aA}	0.86^{aA}	0.86^{aA}	0.86^{aA}	0.86^{aC}
1	0.89^{aE}	0.87^{aA}	0.90^{aA}	0.90^{aA}	0.94^{aA}	0.94^{aA}	0.88^{aC}
2	0.96^{aE}	0.87^{aA}	0.93^{aA}	0.94^{aA}	0.97^{aA}	0.94^{aA}	0.91^{aBC}
3	1.27^{aD}	0.91^{aA}	0.96^{aA}	0.96^{aA}	0.98^{aA}	0.94^{aA}	0.96^{aAB}
4	1.75^{aC}	1.02^{aA}	1.27^{aA}	1.20^{aA}	1.11^{aA}	0.98^{aA}	0.99^{aAB}
5	2.44^{aB}	1.38^{abA}	1.58^{abA}	1.50^{abA}	1.14^{bA}	1.12^{bA}	0.99^{bAB}
6	3.64^{aA}	1.66^{bA}	1.92^{abA}	1.82^{abA}	1.14^{bA}	1.36^{bA}	1.02^{bA}
Slope	0.44	0.13	0.17	0.15	0.05	0.07	0.03
PF	1.00	3.45	2.52	2.88	8.93	6.48	14.67

Different capital alphabets within the same column are significantly ($P<0.05$).
Different small alphabets within the same raw are significantly ($P<0.05$).
PF, protective factor.

Mayonnaise containing encapsulated *L. casei., L. acidophilus and Bif. longum* cells had significant lower ($p<0.05$) AnV than those containing free cells at any corresponding time. The AnV reflect the lack of production of primary oxidation products (hydroperoxides). Without them secondary oxidation products would not be produced. The AnV of extracted lipids from control mayonnaise sample reached to 3.64 mmol/kg after six month. This high AnV indicated that oil has been oxidized. Lipid oxidation is one of the most serious causes of quality deterioration in many foods because it leads to the generation of undesirable off-flavors and off-odors (McClements 1999).

Mayonnaise samples prepared using free cells of *L. casei., L. acidophilus and Bif. longum* had AnV ranging between 1.92 and 1.66 mmol/kg at the same storage period. The lowest AnV were noticed in lipids extracted from storage samples prepared using encapsulated cells ranging between 1.36 and 1.02 mmol/kg. The obtained data were in good relationships with those of primary products of lipid oxidation determined as peroxide value. It means that the using of encapsulated cells in mayonnaise preparation inhibited the autoxidation of unsaturated double bonds and the formation of peroxides, hydroperoxides and their decomposed products, principally 2-alkanol. This fact agrees with the results reported by Lin *et al.* (1999) who reported that the *Bif. longum* and *L. acidophilus* produce compounds that had best anti-oxidative effects. According to the hypothesis suggested by Mei *et al.* (1998), the production of lactic acid in mayonnaise samples prepared using encapsulated of *L. casei., L. acidophilus and Bif. longum* lead to decrease in pH values, it cause released free egg yolk iron reacts with lipid hydroperoxides located in the aqueous phase or at the oil-water interface. This fact retard formation of secondary oxidation products. The encapsulated of *L. casei., L. acidophilus and Bif. longum* appeared their highest protective factor effect against oxidation with maximal values ranging between 14.67 and 6.48.

Total oxidation value

Totox value represents total description of the extracted lipids quality, oxidation status and the presence of degradation production products formed from previous oxidation. The Totox value was calculated and the obtained data are presented in Table (6).

It could be noticed that, the Totox value increased significantly ($p<0.05$) as affected by refrigerated storage. The greater increase in Totox value was observed in untreated mayonnaise samples. The differences in means of AV, PV, AnV and Totox value between experimental and control mayonnaise were significant ($p<0.05$) from the zero time until the 6 months of storage.

In conclusion, mayonnaise samples prepared using encapsulated of *L. casei., L. acidophilus and Bif. longum* were more stable against oxidation at all storage period followed by samples prepared using free cells. It means that the use of encapsulated cells improved the oxidative stability of mayonnaise especially when stored for long periods.

Table (6): Totox values of extracted lipid from control mayonnaise sample and the samples prepared by using free and encapsulated Lactobacillus and Bifidobacteria during storage at 5°C.

Storage period (month)	Control	Free cells			Encapsulated cells		
		L. casei	*L. acidophilus*	*Bif. longum*	*L. casei*	*L. acidophilus*	*Bif. longum*
0	1.63^{aF}	1.63^{aC}	1.63^{aE}	1.63^{aC}	1.63^{aC}	1.63^{aC}	1.63^{aC}
1	1.66^{aF}	1.63^{aC}	1.67^{aE}	1.65^{aC}	1.77^{aC}	1.74^{aC}	1.74^{aC}
2	3.53^{aE}	1.63^{bC}	1.73^{bE}	1.68^{bC}	1.80^{bC}	1.75^{bC}	1.81^{bC}
3	11.06^{aD}	3.43^{bBC}	3.54^{bD}	3.46^{bBC}	1.81^{bC}	1.81^{bC}	1.85^{bC}
4	13.61^{aC}	4.55^{bcB}	4.88^{bC}	4.70^{bcB}	2.78^{cBC}	2.76^{cC}	2.78^{cBC}
5	15.85^{aB}	6.69^{bA}	6.99^{bB}	6.75^{bA}	3.92^{cAB}	4.10^{cB}	3.97^{cB}
6	19.62^{aA}	8.23^{bA}	8.10^{bA}	6.80^{bcA}	5.58^{cA}	6.12^{bcA}	5.78^{cA}

Different capital alphabets within the same column are significantly ($P<0.05$).
Different small alphabets within the same raw are significantly ($P<0.05$).

Sensory quality of different mayonnaise samples

The results of statistical analysis of sensory evaluation of prepared mayonnaise using free and encapsulated *L. casei., L. acidophilus and Bif. longum* cells are listed in Table (7). Significant differences were observed in the quality attributes of different mayonnaise samples. Scores of sensory properties of untreated mayonnaise and mayonnaise treated with encapsulated cells were not significantly ($p>0.05$) affected by storage period. While, the scores of treated samples with free cells were significantly ($p<0.05$) affected by storage period.

Untreated mayonnaise and mayonnaise containing encapsulated cells had higher ($p<0.05$) scores for all tested sensory properties than mayonnaise containing free cells. Kebary (1996) reported that the flavor of frozen yogurt made with *Bibifidum* was improved due to the increase in diacetyl and acetyl methyl carbinol. The low color scores of mayonnaise containing free cells might be due to the color changes associated with oxidative rancidity of the oil during storage. Li Hsieh and Regenstein (1991) observed a brown discoloration in fish oil mayonnaise due to the formation of secondary oxidation products such as aldehydes which presumably participated in browning reaction. The decrease of consistency scores in prepared mayonnaise samples using free cells due to the increases in acidity in the emulsion system according to decrease of pH as shown in Table (2). This decreases in pH caused to coagulate the egg protein and affect in the consistency. The improved texture in mayonnaise containing encapsulated cells could be attributed to the production of exopolysaccharides by lactobacillus and bifidobacteria and/or to the presence of calcium alginate. These findings are in accordance with (Khalil and Mansour , 1998).

Table (7): Mean values of sensory evaluation of control mayonnaise sample and the samples prepared by using free and encapsulated Lactobacillus and Bifidobacteria during storage at 5°C.

Storage period (month)	Control	Free cells			Encapsulated cells		
		L. casei	*L. acidophilus*	*Bif. longum*	*L. casei*	*L. acidophilus*	*Bif. longum*
Appearance							
0	9.6^{a}	9.6^{a}	9.6^{a}	9.6^{a}	9.6^{a}	9.6^{a}	9.6^{a}
2	9.6^{a}	8.3^{b}	8.6^{ab}	8.6^{ab}	9.0^{ab}	9.3^{ab}	9.6^{a}
4	10^{a}	7.6^{c}	7.6^{c}	8.0^{bc}	9.0^{ab}	9.6^{a}	9.6^{a}
6	9.6^{a}	7.0^{b}	6.6^{b}	7.0^{b}	9.3^{a}	9.3^{a}	9.6^{a}
Consistency							
0	9.6^{a}	9.6^{a}	9.6^{a}	9.6^{a}	9.6^{a}	9.6^{a}	9.6^{a}
2	9.6^{a}	7.6^{b}	7.6^{b}	7.6^{b}	9.6^{a}	9.3^{a}	9.3^{a}
4	9.0^{a}	7.3^{b}	7.3^{b}	7.3^{b}	9.0^{a}	9.0^{a}	9.0^{a}
6	9.0^{a}	6.6^{b}	6.6^{b}	6.6^{b}	8.6^{a}	8.6^{a}	9.3^{a}
Color							
0	9.6^{a}	9.6^{a}	9.6^{a}	9.6^{a}	9.6^{a}	9.6^{a}	9.6^{a}
2	9.3^{ab}	8.0^{bc}	7.6^{c}	8.3^{abc}	9.6^{a}	9.6^{a}	9.6^{a}
4	9.6^{a}	8.0^{b}	8.0^{b}	8.0^{b}	9.6^{a}	9.6^{a}	9.6^{a}
6	9.3^{a}	7.0^{b}	7.0^{b}	7.0^{b}	9.6^{a}	9.3^{a}	9.6^{a}
Mouthfell							
0	9.6^{a}	9.6^{a}	9.6^{a}	9.6^{a}	9.6^{a}	9.6^{a}	9.6^{a}
2	9.0^{a}	7.8^{b}	7.6^{b}	7.8^{b}	9.6^{a}	9.6^{a}	9.3^{a}
4	9.0^{a}	7.3^{c}	7.0^{c}	7.0^{c}	8.0^{b}	8.3^{ab}	8.6^{ab}
6	9.0^{a}	6.3^{c}	6.3^{c}	6.8^{c}	8.0^{b}	8.0^{b}	8.5^{ab}
Taste							
0	9.6^{a}	9.6^{a}	9.6^{a}	9.6^{a}	9.6^{a}	9.6^{a}	9.6^{a}
2	9.0^{a}	7.8^{b}	7.6^{b}	7.8^{b}	9.6^{a}	9.6^{a}	9.3^{a}
4	9.0^{a}	6.6^{b}	6.8^{b}	6.6^{b}	8.3^{a}	8.3^{a}	8.5^{a}
6	9.0^{a}	6.3^{b}	6.5^{b}	6.8^{b}	8.3^{a}	8.3^{a}	8.5^{a}
Overall acceptability							
0	9.6^{a}	9.6^{a}	9.6^{a}	9.6^{a}	9.6^{a}	9.6^{a}	9.6^{a}
2	9.0^{a}	7.0^{c}	7.6^{abc}	7.3^{bc}	8.3^{abc}	8.3^{abc}	8.5^{ab}
4	9.0^{a}	6.6^{bc}	6.3^{c}	6.3^{c}	8.0^{ab}	8.0^{ab}	8.6^{a}
6	9.0^{a}	6.0^{c}	6.0^{c}	6.0^{c}	8.0^{ab}	7.3^{bc}	8.6^{ab}

Different alphabets within the same raw are significantly ($P<0.05$).

CONCLUSION

It could be concluded that, a good quality mayonnaise could be prepared by incorporating encapsulated lactobacillus and bifidobacteria in calcium alginate beads. Calcium alginate provided an excellent protection of probiotic bacteria

from the bactericidal effects in mayonnaise medium. Incorporating encapsulated probiotic bacteria in mayonnaise improved the microbiological characteristics, oxidative stability, sensory characteristics and inhibited the growth of pathogenic bacteria up to five months. Mayonnaise containing encapsulated bifidobacteria and lactobacillus had high level of viable aforementioned probiotic bacteria during refrigerated storage; thereby it could be a good source for providing live probiotic bacteria to consumers.

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خصائص جودة المايونيز الغنى فى محتواة ببكتيريا البروبيوتك

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يهدف هذا البحث الى انتاج مايونيز غنى فى محتواة من بكتيريا *Lactobacillus casei* DSMZ 20011 and *Lactobacillus acidophilus* ATCC 4321 و*Bifidobacterium longum* ATCC 15707 ودراسة تأثير هذه البكتيريا على خصائص الجودة لهذا المنتج أثناء فترة التخزين لمدة ستة أشهر على درجة حرارة الثلاجة. وقد تم اضافة Lactobacillus و *Bifidobacteria* الى المايونيز أثناء الاعداد فى صورة خلايا حرة وأخرى مكبسلة باستخدام الجينات الكالسيوم. وأوضحت النتائج المتحصل عليها عدم وجود أى خلايا حية من Lactobacillus و *Bifidobacteria* فى عينات المايونيز المحضرة باستخدام الخلايا فى الصورة الحرة بعد ثلاثة شهور من التخزين المبرد. وقد حدث انخفاض فى عدد الخلايا المكبسلة أثناء فترة التخزين الا انها لم تصل الى عدد اقل من الحد الأدنى المطلوب وجودة فى المنتج (10^{5}-10^{6} خلية/جم) والذى يمثل العدد المطلوب للحصول على المنافع المرجوة من وجود هذة الخلايا البكتيرية فى المنتج. وقد لوحظ زيادة طفيفة فى العدد الكلى للبكتيريا أثناء فترة التخزين. كما ان كل عينات المايونيز المحضرة كانت خالية من وجود الـ *salmonella* . بينما *staphylococcus* إختفت تماما من كل العينات المحضرة بعد شهر من التخزين. وقد تم تقدير قيم كل من رقم الأس الهيدروجينى، البيروكسيد، الأنيسيدين، الأكسدة الكلية والخصائص الحسية أثناء فترة التخزين. وقد حدث انخفاض فى قيم الأس الهيدروجينى مع تقدم فترة التخزين حتى وصلت الى أقل القيم فى نهاية الفترة حيث تراوحت بين ٤,٨ فى العينة الكنترول و٤,٠٥ فى العينات المعاملة. كما وجد أن عينات المايونيز المحضرة باستخدام الخلايا المكبسلة كان لها ثبات عال ضد اكسدة الليبيدات بدرجة معنوية ($p<0.05$) أثناء فترة التخزين مقارنة بعينات المايونيز المحضرة باستخدام الخلايا الحرة. كما أنه لم يكن هناك فرق معنوى ($p>0.05$) فى خصاص الجودة الحسية لعينات المايونيز المحضرة باستخدام الخلايا المكبسلة والأخرى غير المعاملة.