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Comparative evaluation of the efficiency of the microencapsulation methods to improve the flavor production by *Leuconostoc mesenteroides* and *Streptococcus lactis diacetilactis.*

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ABSTRACT

In this study, evaluation of the ability of microencapsulated *Leuconostoc mesenteroides* and *Streptococcus lactis diacetilactis* by different methods (sodium alginate, K-carrageenan, skim milk and sodium casienate) for production of flavor compounds (acetaldehyde and diacetyl) using the suitable incubation period, temperature degrees, pH values and growth medium . The greatest production of flavor compounds was achieved at 25 ºC for 24 h, pH 6 using Elliker growth medium by encapsulated *Leu. mesenteroides* with sodium alginate, and for encapsulated *S. lactis diacetilactis* with sodium alginate was occurred at 37 °C for 24h, pH 6 using Elliker medium. Encapsulated strains was used to prepare stirred using sodium alginate. Treatment samples and control were analyzed chemically ,bateriologically and organoleptically during cold storage for 15 days.Yoghurt with *Leu. mesenteroides* recorded highest score which reflected the ability to produce more flavor compounds to improve the quality of yoghurt. The present results revealed that encapsulated strains improved the acceptable organoleptic properties and quality of stirred yoghurt. However, the use of encapsulated bacteria to produce economical quantities flavor needs to numerous experiments .

Keywords: Flavor compound, microencapsulation, *Leuconostoc mesenteroides* - *Streptococcus lactis diacetilactis*

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INTRODUCTION

Microencapsulation in which the cells are retained within an encapsulating matrix or membrane has emerged as an alternative for protection of probiotics, providing a particular and convenient micro-environment for the encapsulated microorganism, enhancing their viability, and enabling controlled release of cells in the intestinal tract . Encapsulation technology has been proved to be one of the most effective ways to protect probiotics during processing and subsequent storage. Among the various developed methods for encapsulation of bacteria using alginate, K-carrageenan, whey protein and casein which were used in many researches (1,2,3,4). The encapsulation of the bacterial cells in capsules offers space for the cell growth and good diffusion properties, which seems to offer many advantages over free cells such as maintenance of stable and active cells for extended periods of time (5) as well as the continuous production of starter culture (6).

The applications of encapsulation of LAB in dairy product fermentations have been studied intensively, including the production of lactic acid, diacetyl, and concentrated starters which increase in milk quality and production tests for cream yogurt and fresh cheese(7). Heidebach *et al.*, (2009a and 2009 b) (8&9)used casein and milk protein in probiotic cells microencapsulation in food. Microencapsulation of enzymes in inorganic matrices is very useful in practical applications due to the preserved stability and catalytic activity of the immobilized enzymes under extreme conditions (10). Magee *et al.*, (1981) (11)studied the use of encapsulated bacteria free cells extract of *Streptococcus lactis* subsp *diacetilactis* to produce diacetyl and acetoin in cheese. They found that the concentration of diacetyl and acetoin cheese containing encapsulated enzymes increased during ripening to eight fold compared control cheese. So, Gardner and Champagne, (2005) (12) studied the effect of immobilization of cultures in alginate beads on the production of secondary metabolites, and therefore, the use of microencapsulation can improve the intermediate compounds from probiotic bacteria.Sharaf et al., (2014)(13)found that the microencapsulated *Propionibacterim shermanii* by sodium alginate gave the greatest production of vitamin B12 in sodium lactate medium at 30 ºC for 36h under anaerobic condition and pH 6. Mawgoud et al.,(2016) (14)studied the influence of nitrogen source on lactic acid production from whey permeate by immobilized *Lactobacillus bulgaricus Lb-12*.

The present study was carried out with the aim to evaluate the microencapsulation methods using sodium alginate, K-carrageenan, skim milk and whey protein to produce acetaldehyde and diacetyl using encapsulated *Leu. mesenteriodes* and *Streptococcus lactis diacetilactis* and using it to produce stirred yoghurt.

MATERIALS AND METHODS

Strains

Streptococcus lactis diacetilactis CHI DRT-VAC, and *Leuconostocc mesenteroides* provided by the Northern Regional Research Laboratory. Illinois, USA.

Cultivation and harvesting of lactococci cells.

M17 broth (Oxoid) was used to prepare the cell suspensions. The M17 medium was inoculated with 5% active cells with initial count (10^7cfu/ml) and incubated at 37 °C for 24h. Cells were harvested by centrifugation at 5000 rpm for 15 min at 4 °C., and cells were washed twice with saline and used to prepare capsules.

Preparation of microencapsulated cells culture:

Microencapsulation using sodium alginate.

A suspension of cells was mixed with an equal volume of sodium alginate (4%). The mixture was added drop-wise into solution of sodium chloride (0.2mol/L) and calcium chloride (0.5mol/L) and magnetically stirred at 200 rpm/min till alginate beads were formed according to Klinkenberg *et al.,* 2001(15).

Microencapsulation using K- carrageenan

Prepared by mixing 20 g cells (wet weight) in 1000 ml of a sterile solution of K-carrageenan (2%), then the mixture was added drop-wise into potassium chloride (3%) under agitation. K-carrageenan beads were formed within 10 min according to Dinakar & Mistry ,(1994)(16).

Microencapsulation using sodium caseinate.

Preparation of the protein–cell mixture:

The protein suspensions were prepared by dispersing sodium caseinate in double distilled water to a concentration of 15% (w/w). After 2 h of stirring, the pH of the casein suspension was adjusted to 7.0 and the solution was stirred overnight at 4 ˚C before further use. Tow g of strain concentrate was thawed and mixed with 28 g of the casein dispersion, to create the protein–cell mixture.

Encapsulation process:

Transglutaminase enzyme (TGase) was added to the protein–cell mixture with an enzyme concentration of 10 U TGase per g substrate protein at 30 ˚C. Directly after TGase addition, 30 g of the protein–cell mixture containing strain was added to 150 g of tempered (40 ˚C) sunflower oil in a 200 ml Erlenmeyer flask and stirred at a constant speed of 900 rpm with a magnetic stirrer for 120 min. The temperature was maintained during the process in a water bath controlled by thermostat. During the process the emulsified droplets of protein–cell mixture were converted into gel particles according to Heidebach *et al.,* 2009a(8).

Microencapsulation using skim milk.

Preparation of the milk-concentrate-cell-mixture:

Skim-milk-powder was dispersed in double-distilled water to obtain a 35% (w/w) solution and stirred overnight at 10 ˚C. Tow g of strain concentrate was thawed and mixed with 28.0 g of the skim-milk-concentrate to create a milk-concentrate cell-mixture.

Encapsulation process:

The 30 g milk-concentrate-cell-mixture was cooled to 5 ˚C, incubated with 400 ml rennet stock-solution, and then kept at 5 ˚C to perform the cleavage of the k-casein. After 60 min incubation, 180 ml 10% (w/v) $CaCl_2$ solution was added to the mixture and the encapsulation process was subsequently initiated. Fifteen g of the cold-rennet mixture was added to 150 g of tempered (5 ˚C) vegetable oil in a 200 ml Erlenmeyer flask and magnetically stirred at 500 rpm for 5 min to emulsify the mixture into the oil. Subsequently, the gelatinized microcapsules were separated from the oil by gentle centrifugation (500 g , 1 min) according to Heidebach *et al.,* 2009b(9).

Production of acetaldehyde and diacetyl using encapsulated and free cells

All tests were run in 250 ml conical flasks containing 100 ml of the exanimate skimmed milk to study the production of acetaldehyde and diacetyl. Sterilized skimmed milk 11% (w/v) was inoculated by 2% of each microencapsulated strains resulted from different methods and free strains was carried out during studying the production of acetaldehyde and diacetyl.

Effect of incubation periods on the production of acetaldehyde and diacetyl

The inoculated milk with microencapsulated and free strains was incubated at 35 ˚C for 6, 12, 18, 24 and 48h.

Effect of temperature degrees on the production of acetaldehyde and diacetyl

The inoculated milk with microencapsulated and free strains was incubated at 25, 30, 37 and 40 ˚C for 24h.

Effect of pH values on the production of acetaldehyde and diacetyl

The pH of sterilized milk was adjusted to pH 4.0, 4.5, 5.0, 5.5, 6.0 and 6.5 by 10% sterilized lactic acid (w/v) and was inoculated by microencapsulated and free strains (2%) then incubated at 35 ˚C for 24h to study the effect of pH.

Effect of growth media on the production of acetaldehyde and diacetyl

Different sterilized three growth media (skim milk, Elliker broth and M17 broth) was inoculated by 2% of each microencapsulated strains resulted from different methods and free strains incubated at 35 ºC for 24h.

Determination of acetaldehyde and diacetyl

Acetaldehyde and diacetyl were estimated as given by Magee *et al.* (1981)(11) using conway microdiffusion-semicarbazide methods. One ml of micromole semicarbazide solution was pipetted in the inner wall of conway microdiffusion cell. Three ml of sample were rapidly pippeted in the outer and the sample was covered and placed in the incubator at 30 ºC for 15 min. The solution in the inner wall transferred to 10 ml volumetric flask and made up to volume. The absorption for acetaldehyde was measured at 224 nm and at 270 nm for diacetyl.

The efficacy of selected microencapsulated strains with sodium alginate to improve the quality of stirred yoghurt

The selected microencapsulated method using sodium alginate was applied for manufactured stirred yoghurt. Fresh buffalo`s milk standardized to 5% fat, heated at 90 ºC for 30 min then cooled and adjusted to 42 ºC according to Abd El-Khalek (2001)(17), The milk was divided into four portions: the first portion inoculated with (1:1%) *St. thermophilus* and *Lb. bulgaricus* and was regarded as control, the second portion was inoculated with (1:1%) *St. thermophilus* and *Lb. bulgaricus* and 1% encapsulated *Leu. mesenteroides*, the third portion was inoculated with (1:1%) *St. thermophilus* and *Lb. bulgaricus* and 1% encapsulated *Lac. lactis diacetilactis* while the fourth portion was inoculated with (1:1%) *S. thermophilus* and *Lb. bulgaricus* and 1% encapsulated *S. lactis diacetilactis* Then, samples were transferred into 40 ml plastic cups and incubated at 42 ºC for 2 to 4h until coagulation, after which the cups were stirred and stored at 7 ºC for 15 days. The produced stirred yoghurt treatments were analyzed when fresh, and after 3, 7, 10 and 15 days of storage at 7 ºC.

Chemical analysis

1. Total solid content and the pH of cheese were determined according to the method described by Ling (1963)(18).
2. Acetaldehyde and diacetyl were estimated as mentioned before by Magee *et al.* (1981)(11).

Microbiological examination

Leuconostoc and Streptococci counts were determined using M17 agar according to Terzaghi and Sandine (1975)(19)but , the plates were incubated at 35 °C and 37°C for 48h respectively .

Sensory evaluation

The organoleptic properties of stirred yoghurt samples were assessed by a regular taste panel of the staff- members of the dairy science department, National Research Center. Stirred yoghurt samples were evaluated for flavor (50 points), body and texture (40 points) and appearance (10 points) according to Bodyfelt *et al.,* (1988)(20).

Statistical analysis

The data were analyzed according to Statistical Analysis System Users Guide (SAS,1994) (SAS Institute,Inc,U.S.A.) . Separation among means in three replicates was carried out by using Duncan multiple test (21).

RESULTS AND DISCUSSION

Effect of incubation periods on the acetaldehyde and diacetyl production

There were variations in the acetaldehyde and diacetyl **produced by the encapsulated bacteria after 6,12,18,24 and 48 hrs. at 35** ºC **.**Tables (1& 2) shows the highest yield of acetaldehyde and diacetyl were collected at 24 h for all strains specially the strains which encapsulated by sodium alginate since the acetaldehyde values were reached to 37.79 and 37.88 m mol/100 ml and diacetyl values were reached to 36.97 and 29.90 m mol/100 ml for encapsulated strains of *Leu. mesenteriodes*, and *S. lactis diacetilactis* with sodium alginate respectively compared by other encapsulated methods and free strains. This was followed by the strains encapsulated with K-carrageenan since the acetaldehyde values were reached to 34.07 and 34.20 m mol/100 ml while diacetyl values were 34.87 and 25.22 m mol/100 ml after 24h for encapsulated *Leu. mesenteriodes* and *S. lactis diacetilactis* respectively. On the contrary, the lowest yield was recorded when strains encapsulated by sodium caseinate where acetaldehyde values reached to 27.68 and 29.10 m mol/100 ml and diacetyl values were 26.89 and 17.96 m mol/100 ml for encapsulated strains of *Leu. mesenteriodes* and *S. lactis diacetilactis* respectively. The amount of acetaldehyde and diacetyl produced are declined significantly ($p \leq 0.05$) after the first 24h for all strains. In this respect the studies carried out by Ibragimova *et al*.,(1980)(22)showed that milk cultures of *Streptococcus lactis*, *S. cremoris* and *S. diacetilactis* produced high amounts of 2,3-butanedione and acetaldehyde in 24 h at 30 °C. Cultures with the best aroma contained 2-5 parts acetaldehyde to 1 part 2, 3-butanedione. Also, Narvhus *et al.*, (1998) (23)reported that the production of diacetyl, acetoin, acetaldehyde and the malty aldehydes from *Lactococcus lactis* subsp. *lactis* biovar.*diacetylactis* INF-DM1 occurred during the period of active growth in fermented products.

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Table 1. Effect of incubation periods on the production of acetaldehyde (mmol/100ml) by encapsulated strains with different methods.

Encapsulation methods	Incubation periods (h)									
	Leu. mesenteriodes					*S. lactis diacetilactis*				
	6	12	18	24	48	6	12	18	24	48
Sod. alginate	19.17^{ghi}	26.92^{ef}	35.72^{ab}	37.79^{a}	28.23^{de}	19.17^{jk}	26.89^{ef}	32.70^{bc}	37.88^{a}	32.58^{bc}
K-carrageenan	17.05^{ijk}	19.96^{gh}	29.83^{cd}	34.07^{b}	25.66^{f}	16.00^{lm}	20.53^{ij}	25.73^{fg}	34.20^{b}	28.00^{de}
Skim milk	15.11^{k}	19.60^{gh}	27.04^{ef}	30.92^{c}	21.40^{g}	13.76^{n}	18.31^{k}	22.13^{hi}	31.24^{c}	23.93^{gh}
Sod. caseinate	12.29^{l}	17.79^{hij}	21.25^{g}	27.68^{def}	19.35^{ghi}	11.18^{o}	15.23^{mn}	19.30^{jk}	29.10^{d}	20.92^{ij}
Free cells	12.28^{l}	16.80^{jk}	19.57^{gh}	26.37^{ef}	17.71^{hij}	9.81^{o}	13.27^{n}	17.91^{kl}	26.77^{fg}	19.81^{jk}

Data expressed as mean of 3 replicates. Means with the same letter are not significantly different (P≤ 0.05). Incubated at 35 ºC.

Table 2. Effect of incubation periods on the production of diacetyl (m mol/100ml) by encapsulated strains with different methods.

Encapsulation methods	Incubation periods (h)									
	Leu. mesenteriodes					*S. lactis diacetilactis*				
	6	12	18	24	48	6	12	18	24	48
Sod. alginate	23.52^{fgh}	27.99^{de}	32.52^{c}	36.97^{a}	27.97^{de}	16.22^{fgh}	20.70^{d}	26.14^{b}	29.90^{a}	24.18^{c}
K-carrageenan	18.97^{m}	22.88^{ghi}	29.53^{d}	34.87^{b}	24.19^{fg}	13.80^{ij}	16.97^{ef}	21.43^{d}	25.22^{bc}	19.97^{d}
Skim milk	16.27^{n}	22.08^{hij}	24.13^{fg}	28.28^{de}	21.30^{ijk}	11.80^{lm}	14.87^{hi}	17.94^{e}	20.52^{d}	16.56^{efg}
Sod. caseinate	15.32^{n}	20.19^{ijkl}	21.19^{ijkl}	26.89^{e}	19.30^{lm}	12.29^{kl}	14.33^{i}	15.64^{ghi}	17.96^{e}	14.90^{hi}
Free cells	12.62^{o}	19.45^{klm}	19.58^{klm}	24.99^{f}	16.02^{n}	10.76^{m}	11.77^{lm}	13.64^{ijk}	17.07^{ef}	12.73^{kl}

Data expressed as mean of 3 replicates. Means with the same letter are not significantly different (P≤ 0.05). Incubated at 35 ºC

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Effect of temperature degrees on the acetaldehyde and diacetyl production

As shown in Tables (3&4),the optimum temperature for acetaldehyde and diacetyl production was 25 ºC for *Leu. mesenteriodes* specially which encapsulated with sodium alginate, yielding 37.61 and 32.96 m mol/100 ml for acetaldehyde and diacetyl respectively, followed by encapsulated with K-carrageenan, yielding 34.31 and 28.85 m mol/100 ml for acetaldehyde and diacetyl respectively compared with free cells. Moreover, calculating the quantity of acetaldehyde and diacetyl formed revealed a maximum of 38.55 m mol/100 ml and diacetyl reached to 32.65 m mol/100 respectively when encapsulated *S. lactis diacetilactis* with sodium alginate was grown at 37 ºC ,followed by the same strains encapsulated with K-carrageenan .

Table 3. Effect of temperature degrees on the production of acetaldehyde (m mol/100ml) by encapsulated strains with different methods.

Encapsulation methods	Temperature degrees (ºC)							
	Leu. mesenteriodes				*S. lactis diacetilactis*			
	25	30	37	40	25	30	37	40
Sod. alginate	32.96^{a}	28.79^{b}	24.68^{cd}	20.86^{e}	22.95^{cd}	27.16^{b}	32.65^{a}	21.81^{def}
K-carrageenan	28.85^{b}	23.46^{d}	19.53^{e}	16.18^{f}	21.74^{cde}	23.30^{cd}	28.48^{b}	19.09^{efg}
Skim milk	26.19^{c}	20.38^{e}	16.58^{f}	14.61^{fg}h	18.06^{gh}	18.57^{fg}	24.53^{c}	15.11^{ij}
Sod. caseinate	21.07^{e}	19.09^{e}	15.16^{fg}	12.98^{h}	16.21^{hi}	18.03^{gh}	21.17^{def}	14.07^{ij}
Free cells	19.96^{e}	16.53^{f}	13.67^{gh}	10.98^{i}	15.41^{hij}	16.47^{ghi}	20.96^{ef}	13.25^{j}

Data expressed as mean of 3 replicates. Means with the same letter are not significantly different (P≤ 0.05).

Table 4. Effect of temperature degrees on the production of diacetyl (m mol/100ml) by encapsulated strains with different methods.

Encapsulation methods	Temperature degrees (ºC)							
	Leu. mesenteriodes				*S. lactis diacetilactis*			
	25	30	37	40	25	30	37	40
Sod. alginate	37.61^{a}	31.17^{c}	28.81^{de}	23.98^{h}	32.27^{cd}	33.61^{bc}	38.55^{a}	28.66^{g}
K-carrageenan	34.31^{b}	29.47^{cd}	25.96^{fg}	21.26^{i}	29.81^{efg}	31.53^{de}	34.65^{b}	25.71^{h}
Skim milk	29.95^{cd}	24.56^{gh}	21.87^{i}	17.95^{j}	28.81^{h}	19.19^{fg}	31.20^{def}	23.20^{i}
Sod. caseinate	27.53^{fe}	21.32^{i}	20.35^{i}	14.22^{k}	20.75^{j}	23.00^{i}	28.95^{g}	19.87^{j}
Free cells	24.31^{gh}	21.31^{i}	16.61^{j}	11.71^{l}	18.92^{jk}	20.88^{j}	26.02^{h}	17.52^{k}

Data expressed as mean of 3 replicates. Means with the same letter are not significantly different (P≤ 0.05).

Effect of pH values on the acetaldehyde and diacetyl production

Significantly the highest acetaldehyde levels of 38.84 and 38.93 m mol/100 ml and the highest diacetyl level of 37.18 and 36.44 m mol/100 ml were produced at pH 6.0 by encapsulated strains with sodium alginate *Leu. mesenteriodes* and *S. lactis diacetilactis* respectively . These results may due to the high population cells at the same pH value with encapsulated strains using sodium alginate and K-carrageenan compared with other encapsulated methods and free cells (Tables,5&6) .

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Table 5. Effect of pH values on the production of acetaldehyde (m mol/100ml) by encapsulated strains with different methods.

pH values												
Encapsulation methods	***Leu. mesenteriodes***						***S. lactis diacetilactis***					
	4.0	4.5	5.0	5.5	6.0	6.5	4.0	4.5	5.0	5.5	6.0	6.5
Sod. alginate	18.84^{hi}	27.18^{d}	34.91^{b}	35.58^{b}	38.84^{a}	30.53^{c}	15.55^{l}	19.64^{hij}	28.78^{cd}	35.49^{b}	38.93^{a}	33.42^{b}
K-carrageenan	14.85^{j}	22.18^{fg}	30.37^{c}	30.15^{c}	34.47^{b}	23.88^{ef}	11.09^{mn}	15.94^{kl}	25.70^{fg}	30.84^{c}	34.24^{b}	28.05^{de}
Skim milk	10.92^{k}	17.69^{i}	27.08^{d}	26.72^{d}	30.87^{c}	18.27^{hi}	10.00^{n}	12.85^{m}	19.11^{ij}	24.84^{fg}	30.64^{c}	25.09^{fg}
Sod. caseinate	10.05^{kl}	14.49^{j}	23.33^{fg}	22.35^{fg}	27.01^{d}	14.84^{j}	7.87^{o}	11.09^{mn}	17.69^{jk}	21.78^{h}	26.57^{ef}	21.48^{h}
Free cells	8.27^{l}	11.62^{k}	20.31^{gh}	20.18^{gh}	24.97^{de}	13.98^{j}	7.07^{o}	10.51^{n}	14.90^{l}	20.16^{hi}	24.06^{g}	18.94^{j}

Data expressed as mean of 3 replicates. Means with the same letter are not significantly different (P≤ 0.05).

Table 6. Effect of pH values on the production of diacetyl (m mol/100ml) by encapsulated strains with different methods.

pH values												
Encapsulation methods	***Leu. mesenteriodes***						***S. lactis diacetilactis***					
	4.0	4.5	5.0	5.5	6.0	6.5	4.0	4.5	5.0	5.5	6.0	6.5
Sod. alginate	20.49^{klm}	26.04^{de}	31.36^{c}	34.65^{b}	37.18^{a}	30.03^{c}	16.13^{i}	23.42^{d}	35.56^{ab}	31.01^{abc}	36.44^{a}	31.57^{ab}
K-carrageenan	17.21^{op}	21.27^{ijk}	31.01^{c}	29.98^{c}	33.93^{b}	25.13^{ef}	13.92^{k}	19.10^{f}	10.78^{m}	28.24^{abc}	33.12^{abc}	27.69^{ab}
Skim milk	13.88^{r}	19.28^{lmn}	22.67^{hij}	23.65^{fgh}	27.99^{d}	$19.02l^{mn}$	7.53^{o}	11.24^{lm}	16.28^{i}	23.90^{d}	28.22^{abc}	23.96^{d}
Sod. caseinate	11.23^{s}	15.89^{pq}	22.74^{hi}	23.01^{ghi}	26.10^{de}	18.90^{mno}	7.20^{o}	10.73^{m}	14.78^{j}	20.82^{e}	24.16^{cd}	20.19^{ef}
Free cells	11.41^{s}	14.60^{qr}	21.90^{ijkl}	20.65^{jklm}	24.92^{efg}	17.96^{no}	9.56^{n}	13.25^{kl}	26.82^{ab}	18.54^{fh}	21.36^{de}	17.68^{h}

Data expressed as mean of 3 replicates. Means with the same letter are not significantly different (P≤ 0.05).

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Acetaldehyde and diacetyl production in growth media

Acetaldehyde and diacetyl production by encapsulated strains with sodium alginate on Elliker and M17 were generally significantly higher when compared to that produced from other microencapsulated methods or free strains (Tables 7&8).The amount of acetaldehyde produced from encapsulated *Leu. mesenteriodes* and *S. lactis diacetilactis* by sodium alginate on Elliker broth medium were 38.03 and 38.90 m mol/100 ml respectively. Diacetyl productions from the same encapsulated strains with sodium alginate on Elliker medium were 37.82 and 35.95 m mol/100 ml respectively. Jyoti *et al.* (2003) (24) used *Lb.rhamnosus* to produce diacetyl and acetoin . The productivity of diacetyl on medium containing glucose and citrate was higher, than that on citrate alone. Also, Urbach (2005) (25) stated that diacetyl and acetaldehyde are their main contributions to the flavor of cultured milks and fresh cheeses .

Table 7. Effect of growth media on the production of acetaldehyde (mmol/100ml) by encapsulated strains with different methods.

Encapsulation methods	*Leu. mesenteriodes*			*S. lactis diaceti lactis*		
	Skim milk	Elliker	M17	Skim milk	Elliker	M17
Sod. Alginate	31.05^{c}	37.82^{a}	35.31^{b}	27.18^{c}	35.95^{a}	29.92^{b}
K-carrageenan	$25.86e^{f}$	35.41^{b}	29.03^{cd}	23.49^{ef}	31.40^{b}	25.05^{de}
Skim milk	21.02^{hi}	27.19^{de}	22.26^{gh}	20.70^{gh}	26.14^{cd}	19.33^{hi}
Sod. caseinate	18.25^{i}	24.15^{fg}	24.17^{fg}	18.17^{ij}	22.59^{fg}	16.37^{j}
Free cells	18.01^{i}	22.64^{gh}	18.61^{i}	18.50^{ij}	20.89^{gh}	17.36^{ij}

Data expressed as mean of 3 replicates. Means with the same letter are not significantly different (P≤ 0.05).

Table 8. Effect of growth media on the production of diacetyl (mmol/100ml) by encapsulated strains with different methods.

Encapsulation methods	*Leu. mesenteriodes*			*S. lactis diacetilactis*		
	Skim milk	Elliker	M17	Skim milk	Elliker	M17
Sod. Alginate	33.53^{b}	38.03^{a}	38.19^{a}	30.48^{cd}	38.90^{a}	40.00^{a}
K-carrageenan	28.12^{d}	35.30^{b}	32.89^{bc}	28.02^{ef}	34.42^{c}	35.85^{b}
Skim milk	22.73^{e}	30.74^{c}	26.99^{d}	23.11^{g}	31.26^{c}	31.42^{c}
Sod. caseinate	19.78^{fg}	26.30^{d}	22.90^{e}	19.84^{h}	28.62^{def}	30.19^{cde}
Free cells	18.21^{g}	25.51^{d}	21.69^{ef}	17.64^{i}	26.65^{f}	28.26^{ef}

Data expressed as mean of 3 replicates. Means with the same letter are not significantly different (P≤ 0.05).

Improvement of the stirred yoghurt quality by encapsulated *Leu. mesenteriodes* and *S. lactis diacetilactis*

Chemical analysis

Data given in Table (9) showed that using encapsulated strains in manufacturing of stirred yoghurt had no effect on total solid content of all fresh treatments compared to control, whereas they showed slight decrease in all treatments during cold storage period where the overall means of total solid in fresh stirred yoghurt reached to 13.59 which significantly decreased to reach to 13.29 after 15 days of cold storage. The highest total solid content was observed in both encapsulated *Leu. mesenteriodes* and control. Moreover, the pH of all stirred yoghurt samples significantly decreased during storage period. This might be attributed to the continuation of metabolic activity of starter culture (26 & 27).

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Table 9. Chemical analysis in stirred yoghurt manufactured with encapsulated strains during storage periods.

Treatments	Storage period (days)									
	Fresh	3	7	10	15	Fresh	3	7	10	15
	Total solid content					pH values				
Control	13.65^{b}	13.55^{b}	13.49^{cde}	13.46^{cde}	13.35^{fg}	4.75^{b}	4.61^{d}	4.50^{e}	4.48^{ef}	4.41^{g}
Leu. mesenteroides	13.73^{a}	13.63^{b}	13.50^{cd}	13.41^{ef}	13.32^{g}	4.92^{a}	4.70^{c}	4.51^{e}	4.36^{gh}	4.33^{hi}
S. lactis diacetilactis	13.49^{cde}	13.46^{cde}	13.43^{def}	13.31^{g}	13.20^{h}	4.76^{b}	4.46^{ef}	4.35^{ghi}	4.31^{hij}	4.27^{j}

Data expressed as mean of 3 replicates. Means with the same letter are not significantly different (P≤ 0.05).

Acetaldehyde and diacetyl contents

The acetaldehyde and diacetyl contents of stirred yoghurt treatments are presented in Table (10). At the fresh samples, highest content of either acetaldehyde or diacetyl in stirred yoghurt samples was achieved with encapsulated *Leu. mesenteriodes* and *Lc. lactis diacetilactis*. This might be due to the action of those organisms in producing more acetaldehyde and diacetyl than other strains. In general, the acetaldehyde and diacetyl contents in samples that contain encapsulated strains were obviously more than control and reached its maximum production after 10 days of storage but decreased by the end of storage. This might attributed to the high population of viable counts at the 10th day of storage especially at encapsulated strains compared with control. In addition to the citrate metabolism into diacetyl. Also, possibly to that the diacetyl and acetoin accumulated because citrate repressed the synthesis of diacetyl reductase (DR) (28). Gilliland (1985) (29) reported that, in mixed cultures diacetyl production is enhanced by the rapid drop in pH associated with the growth of Streptococci.

Table 10. Acetaldehyde content in stirred yoghurt manufactured with encapsulated strains during storage periods (m mol/100g).

Treatments	Storage period (days)									
	Fresh	3	7	10	15	Fresh	3	7	10	15
	Acetaldehyde content					Diacetyl content				
Control	13.50 h	15.48^{fgh}	15.90 fg	16.16 efg	14.70 gh	8.33^{j}	9.23^{ij}	9.81^{ghi} j	10.31^{f} ghij	9.36^{hij}
Leu. mesenteriodes	17.36 cdef	18.63^{cd}	23.33 b	28.33 a	22.00 b	11.33 efghi	15.05 abc	15.40 ab	16.43^{a}	14.60^{abc} d
S. lactis diacetilactis	14.83 gh	16.71^{efg}	18.90 c	21.66 b	19.42 c	9.70^{ghi} j	11.73 defghi	13.20 bcdef	14.51^{a} bcd	12.26^{def} gh

Data expressed as mean of 3 replicates. Means with the same letter are not significantly different (P≤ 0.05).

Microbiological analysis

All treatments showed appropriate growth of encapsulated strains compared with control Table (11). Viable counts of all treatments slightly increased during storage reaching a maximum after 10 days , thereafter, the counts gradually decreased along storage period . This increase reveals the protective effect of microencapsulation on the viability of strains. These results are in harmony with those obtained by Godward and Kailasapathy, (2003) (30). Moreover, Jayalalitha *et al.*, (2011)(31)found that all the methods of encapsulation improved the viability of probiotic strains .

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Table 11. Total viable bacterial counts in stirred yoghurt manufactured with encapsulated strains during storage periods (log cfu/gm).

Treatments	Storage period (days)				
	Fresh	3	7	10	15
Control	8.36^{k}	8.55^{k}	8.94^{j}	9.58^{fg}	9.08^{ij}
Leu. mesenteroides	9.07^{ij}	9.69^{e}	10.15^{de}	10.71^{a}	10.03^{e}
S. lactis diacetilactis	9.10^{ij}	9.55^{fg}	10.29^{cd}	10.55^{ab}	10.34^{bcd}

Data expressed as mean of 3 replicates. Means with the same letter are not significantly different (P≤ 0.05).

Sensory evaluation

Scores for sensory evaluation of yoghurt through storage for 15 days in refrigerator are presented in Table (12) . It seemed that prepared yoghurt treatments with *Leu. mesenteriodes* and *S. lactis diacetilactis* was obtained highest scores ,this may be attributed to the ability of encapsulated strains to produce more flavor compounds to improve the flavor of stirred yoghurt. On the other hand, the lowest significant total scores were observed in control. Also, data in Table (12) show that sensory evaluation reached the highest scores after 10 days of storage period .No change was observed in stirred yoghurt 's properties when Kailasapathy (2006) (32) incorporated encapsulated probiotic cells in the product. In another study, Adhikari *et al.*,(2003)(33) stated that consumers detected a grainy texture in yogurts prepared by incorporation of encapsulated bifidobacteria into stirred yogurt (size range particles about 22-50 µm) . However , this is a major problem for consumer acceptance , but it can be overcome by good microencapsulation .

Table 12. Sensory evaluation of stirred yogurt manufactured with encapsulated strains during storage periods.

Treatments	Storage period (days)				
	Fresh	3	7	10	15
	Flavor scores (50)				
Control	40.00^{fgh}	40.66^{efg}	41.66^{def}	44.00^{bc}	41.33^{defg}
Leu. mesenteroides	40.00^{fgh}	42.66^{cde}	44.66^{bc}	46.00^{a}	43.33^{bcd}
S. lactis diacetilactis	39.33^{gh}	45.00^{ab}	45.33^{ab}	46.66^{a}	44.33^{bc}
	Body and texture scores (40)				
Control	30.33^{f}	32.00^{def}	32.66^{de}	34.00^{bcd}	31.33^{ef}
Leu. mesenteroides	31.00^{ef}	34.00^{bcd}	34.00^{bcd}	36.00^{a}	33.00^{cde}
S. lactis diacetilactis	30.33^{f}	33.66^{bcd}	35.00^{abc}	36.33^{a}	33.33^{cde}
	Appearance scores (10)				
Control	7.33^{c}	9.00^{a}	9.00^{a}	9.00^{a}	7.66^{bc}
Leu. mesenteroides	8.00^{b}	9.00^{a}	9.00^{a}	9.00^{a}	9.00^{a}
S. lactis diacetilactis	8.00^{b}	9.00^{a}	9.00^{a}	9.00^{a}	7.33^{c}

Data expressed as mean of 3 replicates. Means with the same letter are not significantly different (P≤ 0.05).

CONCLUSION

The highest yield of acetaldehyde and diacetyl were reported by encapsulted *Leu.mesenteroides* using sodium alginate at 25˚C , pH 6 for 24 hr , in Elliker medium . Manufactured stirred yoghurt with this bacteria recorded highest scores which reflected the ability to produce more flavor compounds to improve the quality of yoghurt . The use of encapsulated strains improved the acceptable organoleptic properties and quality of stirred yoghurt. However, the use of encapsulated bacteria to produce economical quantities flavor needs to numerous experiments .

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