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Enhancement in Vitamin B12 Production by Encapsulated *Propionibacterium Shermanii*

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ABSTRACT

In this study, evaluation of the ability of microencapsulated *Propionibacterium shermanii* by different methods (sodium alginate, K-carrageenan, skim milk and whey protein) to produce vitamin B12 using suitable growth media was studied. The study indicated that the microencapsulation 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. Encapsulated *Pr. shermanii* by sodium alginate was used to manufacture Tallaga cheese. The finished product was examined chemically, bacteriologically and organoleptically during storage period for 22 days. Count of encapsulated *Pr. shermanii* increased during storage period and reached maximum after 15 days of storage. The maximum production of Vitamin B12 was observed after 15 days of storage to reached 3.51µg/g in Tallaga cheese. The use of encapsulated strains improved the acceptable organoleptic properties and quality of Tallaga cheese.

Key words: microencapsulation, *Pr. shermanii*, Tallaga cheese, Vitamin B12.

Introduction

Vitamin B12 is an important cofactor for the metabolism of carbohydrates, lipids, amino acids and nucleic acids. It has numerous applications in medicine and nutrition (Spalla *et al.,* 1989). It is widely used to cure disorders of nervous system, arthritis and pernicious anemia (Shakirzyanova *et al.,* 2002). Vitamin B12 is produced by fermentation in an industrial scale using Propionibacteria (Crespo *et al.,* 1991; Nakano *et al.,* 1996). Dairy propionibacteria are important in food industry, with long tradition of use in manufacture of cheese, this organism also used as a production host for nutraceuticals, enzymes and antimicrobials such as propionicin (Jan *et al.* 2002).

In the production of vitamin B12, many fermentative processes routinely focus on bacterial growth to high cell densities and the nutrient composition of the medium. *Propionibacteria* produce vitamin B12 intracellularly and excrete mainly propionic acid and acetic acid extracellularly. The primary problem for vitamin B12 fermentation using *Propionibacteria* is that the end products such as propionic acid, etc. inhibit the cell growth (Hsu and Yang, 1991). It is, therefore, critical to remove the end-product and thus, to improve the cell growth, which results in the improved productivity of vitamin B12. A number of fermentation processes have been developed to remove propionic acid and acetic acid from fermentation broth. For example, fermentation with cross-flow filtration (Hatanaka *et al.,* 1998), electrodialysis culture (Zhang, *et al,.* 1993), and immobilized cell culture (Yang and Huang. 1995).

Microencapsulation or entrapment of cells in a gel matrix of alginates is the most popular system of immobilization (Champagne, *et al.,* 1994). Microencapsulation is defined as a technology of packaging solid, liquid or gaseous materials in miniature, sealed capsule that can release their content s at controlled rates under the influences of specific conditions (Anal and Stevens, 2005; Kailasapathay and Masondol, 2005). A microcapsule consists of a semi-permeable, spherical, thin and strong membrane surrounding a solid/liquid core with a diameter varying from microns to 1mm. Encapsulation can be used for many applications in the food industry, including stabilizing the core material, helps to separate a core material from its environment until it is released. It protects the unstable core from its environment, thereby improving its stability, extends the core's shelf life and provides a sustained and controlled release. Among the various developed methods for cell encapsulation using alginate, K-carrageenan, whey protein and casein which were used in many researches (Sheu *et al.,* 1993; El-Shafei *et al.,* 2003; Gilas *et al.,* 2009 and Heidebach *et al.,* 2009a&b).

Tallaga (refrigerated) cheese is an Egyptian white soft cheese variety characterized with a clean pleasant creamy low salty taste with a spreadable mellow soft body. It is a product closely related to Domiati cheese and mainly ready for consumption within one month of storage at refrigerator temperature (Hofi *et al.,* 1979; Mehanna and Rashed, 1990; Shehata *et al.,* 1995 and El-Kholy, 2005). Therefore, the main objective of this research was to produce Vitamin B12 using encapsulated *Pr. shermanii* specifying the best growth media for

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the vitamin B12 production, and the best method of encapsulation to be used in the manufacturing of Tallaga cheese as a function food.

Materials and Methods

1. Strains:

Propionibacterium sherminii was provided by Department of Food Technology, propionibacteria culture collection, Iowa State University and *Streptococcus thermophilus* were obtained from Chr. Hansen's Lab., Denmark.

2. Cultivation and harvesting of Pr. shermanii cells:

Sodium lactate broth (Champagne *et al.,*1989) was used to prepare the cell suspensions of *Pr. shermanii*. The medium was inoculated with 2% active cells and incubated at 30 °C for 36h under anaerobic incubation. Cells were harvested by centrifugation at 5000 rpm for 15 min at 4 °C., and were washed twice with saline and used to prepare capsules.

3. Preparation of microencapsulated cells culture:

3.1. 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.

3.2. 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.

3.3. Microencapsulation using sodium caseinat.

3.3.1. 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. Two grams of strain concentrate was thawed and mixed with 28 g of the casein dispersion, to create the protein–cell mixture.

3.3.2. 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.

3.4. Microencapsulation using skim milk:

3.4.1. 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. Two grams of strain concentrate was thawed and mixed with 28.0 g of the skim-milk-concentrate to create a milk-concentrate cell-mixture.

3.4.2. 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 grams 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.

4. Production of vitamin B12 using microencapsulated and free strain of Propionibacterium shermanii:

4.1. Production of vitamin B12 using different growth media:

Sterilized sodium lactate broth and whey permeate with sodium lactate broth media were inoculated by 2% of microencapsulated of *Pr. shermenii* with different methods of encapsulation and free cells at 30˚C for 3 days anaerobic incubation followed by incubated for other 3 days aerobically at the same temperature to study the intracellular production of vitamin B12 from *Pr. shermanii*.

Vitamin B12 was determined intracellulary with HPLC after extraction and conversion to cyanocobalamin as described by Piao *et al.,* (2004). Culture samples (20 ml) were harvested by centrifugation (10,000 rpm for 15 min at 4˚C). The cells pellets from each microencapsulated methods and free culture were washed once with 20 ml 0.2 M potassium phosphate buffer (pH 5.5), centrifuged (10.000 rpm for 15min) and resuspended in 1 ml of the same buffer containing 0.1% KCN. The suspension was autoclaved for 15 min at 121 ˚C and the cell debris was removed by centrifugation. The supernatant was filtered through a membrane filter (0.45 µm nylon membrane filter) before injection into HPLC equipment.

4.2. Production of Tallaga cheese containing vitamin B12:

Fresh buffalo`s milk standardized to 5% fat, pasteurized at 63 ˚C for 30 min then cooled, adjusted to 37 ˚C, calcium chloride and sodium chloride were added at the ratios of 0.02% and 2% (w/v), respectively and inoculated with *St. thermophilus*. The milk was divided into three portions: the first portion with *St. thermophilus* only and was regarded as control, the second portion was inoculated 1% free cells of *Pr. shermnii*, and the third portion was inoculated 1% encapsulated cells of *Pr. shermnii* with sodium alginate. Then, commercial rennet was added and the coagulation was performed after 3h. All milk portions were made into Tallaga cheese followed the conventional method of Domiati cheese (Fahmi and Sharara 1950). Cheese was packed in plastic cups filled with formerly boiled whey and storage under refrigeration 7 ˚C for 30 days.

4.2.1. Chemical analysis:

Moisture content, pH values of cheese and total nitrogen content were determined according to the method described by Ling (1963).

4.2.2. Determination of vitamin B12:

The sample extraction procedure was carried out according to literature (Albal´a-Hurtado *et al.,* 1997) in the following conditions: One g of the cheese samples which contain encapsulated and free cells of *Pr. shermnii* were accurately weighed, and 10 ml of double-distilled water were added into a 50 ml centrifuge tube (30 mm diameter). Then, 1 gram of solid trichloroacetic acid (TCA) and a magnetic stirring bar were added. The mixture was thoroughly shaken for 10 min over a magnetic stirring plate and centrifuged for 10 min at 1250 g to separate the two phases. After, 3 ml 4% TCA were added to the solid residue obtained, mixed comprehensively for 10 min, and centrifuged. Solid-phase was discarded. The two acid extracts were combined in a 10 ml volumetric flask and the volume was filled with 4% TCA. Samples should be always protected from light by covering tubes and flasks with aluminum foil and working under subdued lighting conditions. Then, acid extracts were filtered through a 0.45 µm filter prior to HPLC analysis.

Preparation of vitamin standards:

A stock standard solution (100 µg mL^{-1}) of cyanocobalamin prepared with water and stored at -20 °C. The standard solutions required for constructing a calibration graph were prepared from stock solution by serial dilution with water and were stored at 4 °C before use.

HPLC analysis:

HPLC analysis was performed with an Agilent 1260 HPLC system (Agilent Technologies, USA), equipped with a quaternary pump, auto sampler injector with 20 µl fixed loop injector, thermostat compartment for the column and photodiode array detector. The chromatographic column was C18 Zorbax XDB (250 mm x 4.6 mm, 5 µm film thicknesses). The column was kept at room temperature at a flow rate of 0.8 ml/min with a total run time of 12 min. Separation of vitamins was carried out by gradient elution with methanol (A) and 1% TFA containing water (B). the elute composition was initially 8 % A + 92 % B, held for 2 min, and changed linearly to 92 % A + 8 % B in the next 4 min and held for 6 min. Detection wave length for detection of cyanocobalamin was set at 254 nm. The retention time of cyanocobalamin was about 7.059 min.

4.2.3. Microbiological examination:

Propionibacteria counts were determined using sodium lactate agar medium according to (Champagne *et al.*, 1989). The plates incubated anaerobically at 30 ˚C for 72h. The plates were incubated at 37 °C for 48h. Also, streptococci counts were determined using M17 agar medium according to Terzaghi and Sandine, (1975).The plates were incubated at 37 °C for 48h.

4.2.4. Organoleptic assessment:

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

5. 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 replicated was carried out by using Duncan multiple test.

Results and Discussion

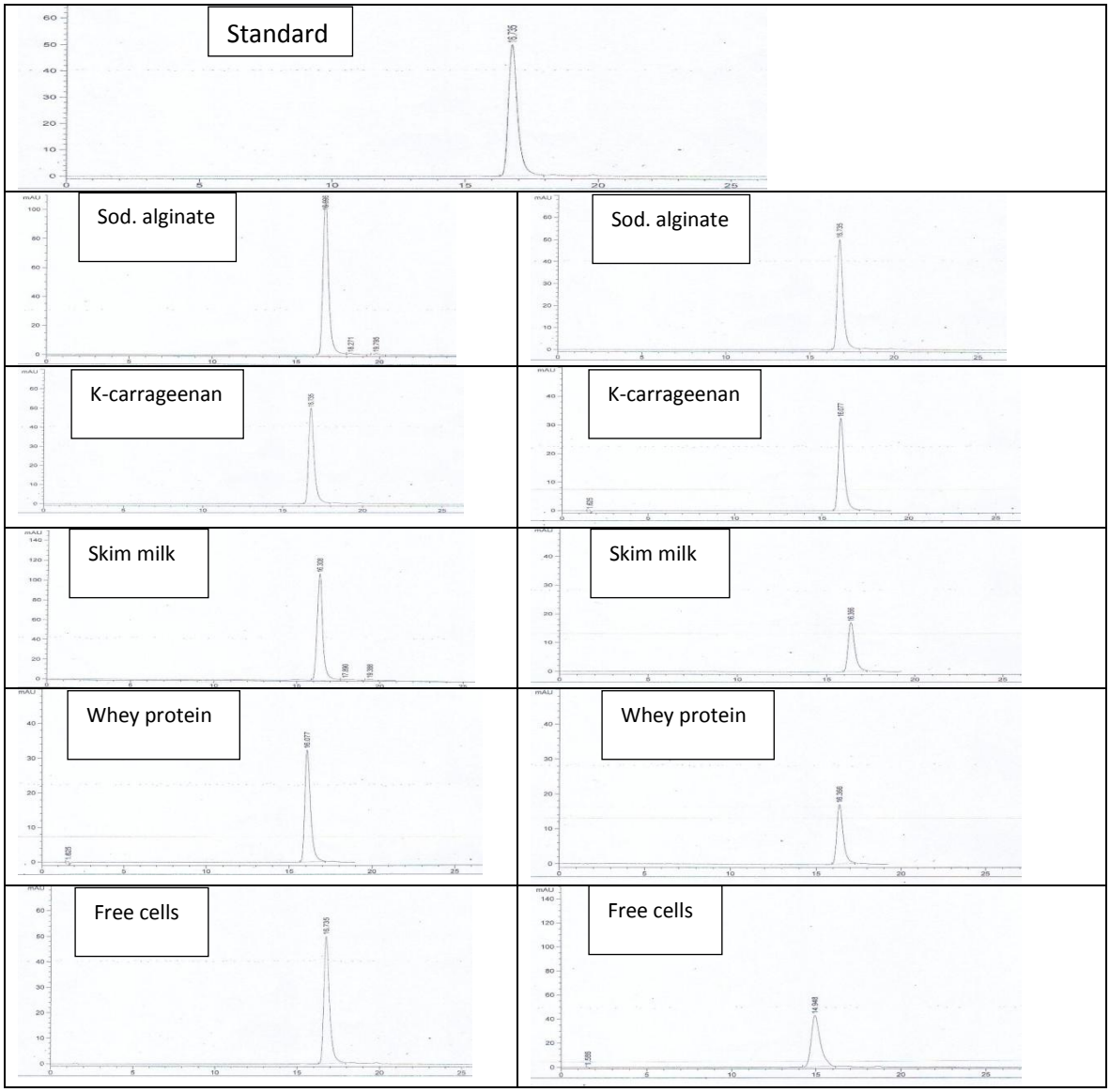
1. Production of vitamin B12 by different microencapsulation methods:

In the present study, remarkable differences in vitamin B12 production among the different microencapsulated *Pr. shermanii* and media are presented in Table (1) and Fig. (1). Production of vitamin B12 from microencapsulated using sodium lactate medium were significantly higher when compared to that produced using whey permeate medium, especially when *Pr. shermanii* encapsulated using sodium alginate and K-carrageenan. Production of vitamin B12 from encapsulated *Pr. shermanii* by sodium alginate reached 7.40 µg/ml on sodium lactate medium and followed by encapsulated with K-carrageenan where reached to 4.60 µg/ml on the same media compared with the other encapsulated methods and free cells. Hugenschmidt *et al.* ,(2010) found that the highest vitamin B12 production was measured for *Pr. freudenreichii* DF15 with 2.5 µg/ml using sodium lactate medium. Also, Yang *et al.* ,(2004) stated that changing in the nutrient composition of the growth medium affects the production of vitamin B12. Moreover, Marwah and Sethi (1984) found that *Pr. shermanii* 566 synthesized 5.6 mg vitamin B12 per liter of whey containing 4% lactose supplemented with 0.5% $(NH_4)_2HPO_4$, when fermentation was carried out at 30 ºC under anaerobic conditions for the first half (84h) followed by aerobic conditions for the second half of the fermentation (84h).

Table 1: Production of vitamin B12 (µg/ml) from *Pr. shermanii* encapsulated with different methods.

Encapsulated methods	Sodium lactate	Whey permeate	Overall means
Control	3.60	1.40	2.50^{b}
Sod. alginate	7.40	2.70	7.40^{a}
K- carrageenan	4.60	2.30	3.45^{b}
Skim milk	4.00	1.60	2.80^{b}
Whey protein	3.90	1.50	2.70^{b}
Overall means	4.70^{a}	2.84^{b}	

Data expressed as mean of 3 replicates. Means with the same letter are not significantly different (P≤ 0.05). Incubated at 30 ºC for 36h. Control: Free cells.



(1) Sodium lactate medium **(2) Whey permeate medium**

Fig. 1: Effect of growth media in the production of B12.

The same trend of results was observed with respect to the biomass production. (Table 2) showed that significantly, highest viable count was occurred when sodium lactate medium was used with encapsulated strain with sodium alginate (10.90 log cful/ml) followed by encapsulated K-carrageenan (9.70 log cfu/ml) compared with the other encapsulated methods and free cells using the same media. Champagne *et al.*,(1989) reported that immobilized systems can reach higher cell densities than classical free cell fermentation performed under the same conditions. Also, Arnauld *et al.,* (1992) reported that encapsulated culture provides high stability of cells and high productivity for metabolite production with high agitation rates. Champagne *et al.*,(1993) reported that it was possible to use encapsulated microorganisms to produce bacterial densities (123.1 x 10^8 ml^{-1}) 6 times higher than with classical cell free suspensions (18.6 x 10^8 ml/1).

Table 2. Effect of growth media on the viability (Log cfu/ml) of encapsulated *Pr. shermanii* with different methods.

Encapsulated methods	Sodium lactate	Whey permeate	Overall means
Control	7.94	7.45	7.69^{c}
Sod. alginate	10.90	9.15	10.90^{a}
K-carrageenan	9.70	8.50	9.10^{b}
Skim milk	8.66	8.00	8.33^{bc}
Whey protein	8.23	7.85	8.04^{c}
Overall means	9.08^{a}	8.54^{b}	

Data expressed as mean of 3 replicates. Means with the same letter are not significantly different (P≤ 0.05).). Incubated at 30 ºC for 36h. Control: Free cells.

2. Production of Tallaga cheese containing vitamin B12:

2.1. Moisture content:

Data concerning moisture content in Tallaga cheese samples manufactured with microencapsulated and free strain during storage period up to 22 days at 7 ºC are presented in Table (3). The data show that there were significant differences between the moisture content of control and all cheese treatments along the storage period. It was significantly slight decrease with increasing the storage period in all treated samples.

Table 3: Moisture content in Tallaga cheese manufactured with encapsulated *Pr. shermanii* and free cells during storage periods.

Treatments	Storage period (days)				
	Fresh	7	15	22	Overall means
Control	69.75^{d}	69.41^{e}	68.42^{f}	68.25^{f}	68.95^{c}
Enc. *Pr. shermanii*	70.27^{c}	71.21^{b}	71.12^{b}	70.30^{c}	71.72^{a}
Free cells	71.15^{c}	71.74^{a}	71.61^{a}	70.50^{b}	71.25^{b}
Overall means	70.39^{c}	70.74^{a}	70.43^{b}	69.90^{d}	

Data expressed as mean of 3 replicates. Means with the same letter are not significantly different (P≤ 0.05). Control: without *Pr. shermanii*.

Overall means of moisture content indicated that Tallaga cheese made with microencapsulated *Pr. shermanii* had the highest moisture content along the storage period Whereas, lowest moisture content was recorded in the control. Generally, the overall means of moisture content recorded at fresh as 70.39 for Tallaga cheese and significantly decreased to reach 69.90 at the end of storage. This might be due to the shrinkage of the curd as a result of acid development which helps to expel the whey from the cheese mass (Gafour, 2005). Dinakar and Mistry (1994) found that moisture content of cheddar cheese samples with immobilized bifidobacteria was higher than in control cheese samples.

2.2. pH values:

The effect of encapsulated and non encapsulated *Pr. shermanii* on the pH values of Tallaga cheese during storage at 7 ºC are presented in Table (4). Generally, the pH values significantly slight decreased in all cheese samples as the storage period increased. Furthermore, control had the highest pH values along the storage period and the encapsulated of *Pr. shermanii* had the lowest pH values when compared with free cell, which may be due to the high population cells in capsules of *Pr. shermanii*. These results in harmony with Mehanna *et al.* (2002) found that the pH value of control and soft cheese made with *Bif. bifidum* and lactobacilli strains decreased till the end of the storage period. Moreover, Sadek *et al.*, (2003) reported that increasing the incubation period of milk with immobilized *Leu. mesenteriodes* and *P. thoenii* from 24 to 72h had a slight effect on pH.

Table 4: PH values of Tallaga cheese manufactured with encapsulated *Pr. shermanii* and free cells during storage periods.

Treatments	Storage period (days)				
	Fresh	7	15	22	Overall means
Control	5.64^{a}	5.51^{b}	5.39^{c}	5.33^{d}	5.47^{a}
Enc. *Pr. shermanii*	5.36^{cd}	5.21^{e}	5.07^{f}	4.89^{g}	5.36^{cd}
Free cells	5.61^{a}	5.40^{c}	5.36^{cd}	5.18^{e}	5.39^{b}
Overall means	5.54^{a}	5.37^{b}	5.27^{c}	5.13^{d}	

Data expressed as mean of 3 replicates. Means with the same letter are not significantly different (P≤ 0.05).
Control: without *Pr. shermanii*.

2.3. Total nitrogen:

Table (5) illustrates that, change in % total nitrogen of Tallaga cheeses during refrigeration storage at 7 ^{o}C for 22 days. Statistical analysis indicated that the total nitrogen content of all cheese treatments was significantly affecting ($p<0.05$) by the refrigeration period (7 ^{o}C/22 days). Besides, it could be seen that, during the refrigeration period (7 ^{o}C/22 days), the control cheese had lower total nitrogen content as compared with those Tallaga cheese made with the encapsulated and non encapsulated *Pr. shermanii*. Also, it could be observed that, the % total nitrogen reached the lowest values at the end of the refrigeration period. These results are in harmony with those obtained by El- Zayat and Osman (2001), they mentioned that the decrease in the TN content during refrigeration in all treatments could be attributed to the protein degradation into SN and subsequently partial loss into the pickling solution.

2.4. Yield of vitamin B12:

Differences in the production of vitamin B12 using encapsulated strain and free cells in Tallaga cheese during storage are presented in Table (6) and Fig. (2). Significantly the highest yield of vitamin B12 was

collected after 15 days of storage period where the overall means reached to 3.18 µg/g. Encapsulated strain of *Pr. shermanii* significantly revealed the highest production since the amount of vitamin B12 reached to 3.51 µg/g followed by free cells which reached to 2.85 µg/g after 15 days of storage period. On the contrary, the amount of vitamin B12 declined to reach after 22 days to overall means 3.03 µg/g, which considered the highest yield observed with encapsulated *Pr. shermanii* followed by free cells. It should be noted that vitamin B12 in control during storage was not detected. These results may due to the high cell densities in capsules compared with free cells. Hugenschmidt *et al.,* (2010) found that the highest vitamin B12 production was measured for *Pr. freudenreichii* DF15 with 2.5 µg/ml. Moreover, Arnauld *et al.,* (1992) reported that encapsulated culture provides high stability of cells and high productivity for metabolite production. Also, Giroux *et al.,* (2013) optimized encapsulation of vitamin B12 in water-in-oil-in-water double emulsions to produce functional cream for cheese milk standardization. Encapsulation of vitamin B12 in double emulsions exhibited greater than 96% efficiency and prevented vitamin losses during in vitro gastric digestion. Less than 5% of the encapsulated vitamin B12 was released from double emulsion stabilized with sodium caseinate. Compared with non encapsulated vitamin B12, encapsulation in double emulsions reduced vitamin B12 losses in whey and increased retention in cheese from 6.3 to more than 90%.

Table 5: Total nitrogen content in Tallaga cheese manufactured with encapsulated *Pr. shermanii* and free cells during storage periods.

	Storage period (days)				
Treatments	Fresh	7	15	22	Overall means
Control	3.36^{abc}	3.21^{cde}	3.17^{de}	3.08^{e}	3.21^{b}
Enc. *Pr. shermanii*	3.42^{a}	3.34^{abc}	3.22^{cde}	3.23^{bcde}	3.30^{a}
Free cells	3.40^{a}	3.28^{abcd}	3.12^{de}	3.08^{e}	3.22^{b}
Overall means	3.39^{a}	3.28^{b}	3.17^{c}	3.13^{c}	

Data expressed as mean of 3 replicates. Means with the same letter are not significantly different ($P \leq 0.05$).
Control: without *Pr. shermanii*.

Table 6: Production of vitamin B12 (µg/g) in Tallaga cheese manufactured with encapsulated *Pr.shermanii* and free cells during storage periods.

	Storage period (days)				
Treatments	Fresh	7	15	22	Overall means
Control	ND	ND	ND	ND	ND
Enc. *Pr. shermanii*	3.05	3.09	3.51	3.31	3.24^{a}
Free cells	2.66	2.73	2.85	2.76	2.75^{b}
Overall means	2.85^{c}	2.91^{bc}	3.18^{a}	3.03^{b}	

Data expressed as mean of 3 replicates. Means with the same letter are not significantly different ($P \leq 0.05$).
Control: without *Pr. shermanii*.
ND: not detected.

2.5. Bacterial viable counts:

Differences in viable counts of encapsulated and free cells of *Pr. shermanii* in Tallaga cheese during storage period at 7 ºC are presented in Fig. (3). Viable counts of all Tallaga cheese treatments were significantly increased till 15 days of storage followed by a decreased at the end of the storage period. Viable counts of encapsulated *Pr. shermanii* was highest compared with free cells and control where its overall means during storage reached to 10.27 log cfu/g, followed by free cells of *Pr. shermanii* where overall means reached to 9.77 log cfu/g. Overall means along the ripening period for all Tallaga cheese samples indicated that, viable counts reached the highest viable counts after 15 days of storage period specially cheese sample containing encapsulated *Pr. shermanii*. This reflects the protective effect of microencapsulation on the viability of strains.

These results are in harmony with those obtained by Champagene *et al.,* (1989) who reported that immobilized system can reach higher cell densities than classical free cell fermentations performed under the same conditions. Furthermore, Sadek *et al.,* (2003) reported that the viable cell count of *Pr. thoenii* in beads when inoculated in milk for 24h ($5.5x10^{10}$ cfu/g) and increased after 48h to reached ($9x10^{10}$ cfu/g).

2.6. Organoleptic assessment:

The results of the organoleptic assessment of Tallaga cheese storage at 7ºC and for 22 days are given in Table (8). The data show that, there were significant differences between the control and all treatments samples along the storage period. Furthermore, score of flavor, body and texture and appearance significantly increased with the increase of storage period, the highest organoleptic scores were recorded after 15 days of storage period and decreased at the end of storage (22 days). Organoleptic scores indicate that all Tallaga cheese samples contained encapsulated *Pr. shermanii* had higher scores followed by free cells when compared with control. This study has indicated that Tallaga cheese could potentially be modified to provide additional dairy foods vitamins B12.

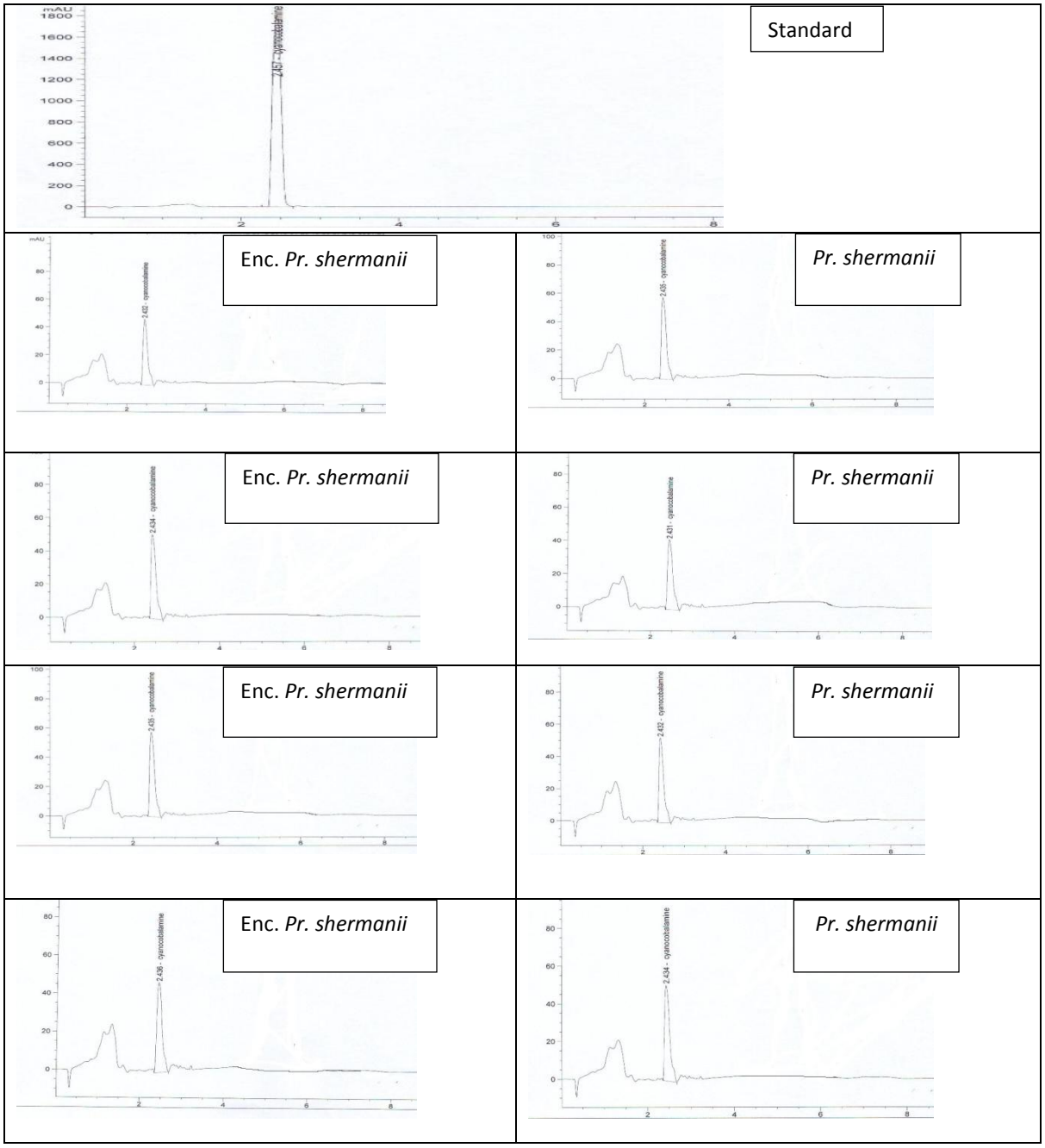
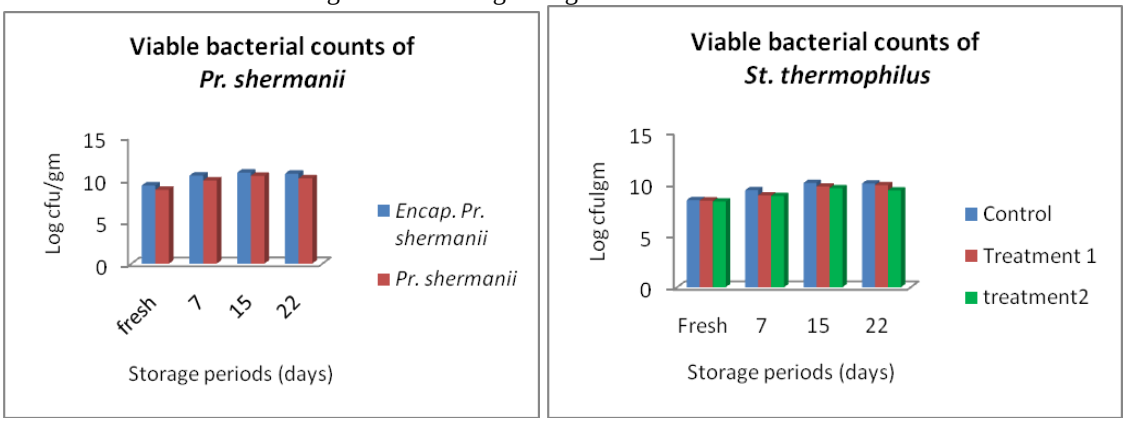


Fig. 2: Production of B12 in Tallaga cheese during storage.



Treatment 1: cheese containing Encap. *Pr. shermanii.* Treatment 2: cheese containing *Pr. shermanii.*

Fig. 3: Viable bacterial counts (Log cfu/gm) content in Tallaga cheese during storage periods.

Table 8: Organoleptic scores in Tallaga cheese manufactured with encapsulated and free cells during storage periods.

Treatments	Storage period (days)				
	Fresh	7	15	22	Overall means
Flavor scores					
Control	41^{de}	44^{bc}	40^{def}	39^{f}	41^{b}
Enc. *Pr. shermanii*	44^{bc}	45^{ab}	47^{a}	44^{bc}	45^{a}
Free cells	41^{de}	42^{cd}	44^{bc}	40^{def}	42^{b}
Overall means	42^{b}	44^{a}	45^{a}	40^{c}	
Body and texture scores					
Control	34^{b}	35^{ab}	36^{ab}	31^{c}	34^{b}
Enc. *Pr. shermanii*	35^{ab}	36^{ab}	37^{a}	35^{ab}	36^{a}
Free cells	34^{b}	34^{b}	35^{ab}	31^{c}	34^{b}
Overall means	34^{b}	35^{ab}	36^{a}	32^{c}	
Appearance scores					
Control	7.66^{bcd}	8.00^{bcd}	8.33^{abc}	7.33^{dc}	7.80^{b}
Enc. *Pr. shermanii*	8.00^{abc}	8.33^{abc}	9.33^{a}	8.66^{b}	8.66^{a}
Free cells	8.00^{bcd}	8.00^{bcd}	8.33^{abc}	7.00^{d}	7.83^{b}
Overall means	8.00^{b}	8.11^{b}	8.66^{a}	7.66^{b}	

Data expressed as mean of 3 replicates. Means with the same letter are not significantly different ($P \leq 0.05$).
Control: without *Pr. shermanii*.

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