Matrix -Related Stability and Viability of Microencapsulated Probiotics

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Abstract. We aimed to obtain and characterize (morphology, spectroscopy, viability) different types of microcapsules built with alginate or chitosan matrices which entrap *Streptococcus thermophilus* and *Lactobacillus delbrueckii subsp. bulgaricus* as probiotics. We used as matrices chitosan (CH) (1%, 1.5%, 2% w/v) and alginate (AG) (1%, 1.5%, 2% w/v) and the same percentage of probiotics (1mg/10ml matrix solution) to obtain microcapsules by coacervation. The concentration of AG and CH influenced the diameter, aspect, compactness of beads. Capsules of AG 1.5%, had the biggest area (4,1mm^2) and diameter (2mm) while CH 2% had the smallest area (1.5 mm^2) and diameter (2,1mm). As a complementary characterization the FTIR–HATR spectroscopy was able to fingerprint the free matrix, and encapsulated probiotics, identifying the specific markers located at 900-1100 cm^{-1}(carbohydrates), 1560-1620 cm^{-1} (for sugar ring stretching) and a large band (for water) at 3100-3700 cm^{-1}.The fingerprint of encapsulated probiotic is recognised by 2 signals identified at 2880 and 2920 cm^{-1}. The most suitable matrices for probiotic encapsulation were established to be AG 2%, AG 1.5%.The viability of probiotic powder in culture was 10^9 ml^{-1} while in capsules the viability was 10^6 ml^{-1} . We can notice that capsules with AG 1.5% and CH 1.5% released more bacteria and the viability was higher then in AG 2% and CH 2%, respectively.

Keywords: Alginate, Chitosan**, FTIR-HATR, Microencapsulation, Probiotic bacteria.

INTRODUCTION

Probiotics are living microorganisms which transit the gastrointestinal tract and bring benefits to the health of the consumer (Tannock et al., 2000). Their therapeutic benefits induced the incorporation of probiotic bacteria (such as lactobacilli and bifidobacteria) in dairy products, especially yoghurts (Lourens-Hattingh et al., 2001). The efficiency of added probiotics depends on their level and their viability must be maintained throughout storage, increasing shelf-life and they must survive the gut environment (Kailasapathy et al., 2000). Hence viability of probiotics bacteria is of paramount importance in the marketability of probiotic-based food products. Several reports have shown that survival and viability of probiotic bacteria is often low in yoghurt (Dave et al., 1997; Gilliland et al., 1977; Hull et al., 1984; Kailasapathy et al., 1997; Lourens- Hattingh et al., 2001; Schioppa, et al., 1981; Shah, 2000; Shah et al., 1995) and results in less than 10^8–10^9 cells daily recommended intake (Lourens-Hattingh et al., 2001). A number of different brands of commercial yoghurts have been analysed in Australia (Anon, 1992; Shah, 2000; Shah et al., 1995) and in Europe (Iwana et al., 1993) for the adequate presence of *L. acidophilus* and *Bifidobacteria*. Most of the yoghurts contained very low numbers of these organisms, especially *Bifidobacteria*.

Microencapsulation of bacterial cells is currently gaining attention to increase viability of probiotics bacteria in acidic products such as yoghurt (Godward et al., 2003; Kailasapathy,

2002; Krasaekoopt et al., 2003). We aimed to obtain and characterize (morphology, spectroscopy, viability) different types of microcapsules built with alginate or chitosan matrices which entrap *Streptococcus thermophilus* and *Lactobacillus delbrueckii subsp. bulgaricus* as probiotics.The viability of probiotics culture (like powder and from capsules) was also studied.

MATERIALS AND METHODS

Materials. Sodium alginate (AG) was purchased from Promova Biopolymer Norway, calcium Chloride (CaCl$_2$), chitosan (CH) (medium molecular weight), natrium tripolyphosphate (NaTPP), acetic acid from Sigma Aldrich. Probiotic bacteria (*Streptococcus thermophilus* and *Lactobacillus delbrueckii subsp. bulgaricus*) were purchased from MTC, Romania.

Preparation of beeds. Concentrations of alginate (2%,1.5%,1% w/v) (AG) and 0.1 g probiotic bacteria were dissolved in 10 ml of deionised water. Chitosan in different concentration (2%,1.5%,1% w/v) (CH) and 0.1g of probiotic bacteria was dissolved in 10ml solution (9.93 ml water and 0.07 ml acetic acid 0.7%). The emulsion obtained, was homogenized and dropped, using a syringe with needle (0.4 x 20mm) into a hardening bath 2% (w/v) solution of CaCl$_2$ (for AG) and bath 5% (w/v) solution of NATPP (for CH) to obtain the probiotic capsules made of alginate and chitosan. After 30 minutes, the capsules were separated from the hardening baths by filtration.

Beads Characterization: sizes and morphology. The obtained bead sizes, areas, perimeters, elongation and compactness were measured using the UTHSCSA ImageTool software.

FTIR-HATR analysis. The FTIR spectra were obtained with a Schimatzu IR Prestige-21 with HATR and an internal reflection accessory made of Composite Zinc Selenide (ZnSe) and Diamond crystals. Each spectrum was registered from 4000-650 cm^{-1}.The FTIR spectra were recorded for all samples in parallel with controls.

Viability of probiotics bacteria. Aliquots of 1mg of probiotic culture were inoculated in 180 ml MRS bullion. The MRS medium was skaked at 37°C, for 30hours. Aliquots were taken at 1h, 2h, 4h, 6h, 8h, 11h, 14h, 22h, 28h, 30h, diluted and inoculated on MRS agar (50 µl*3), incubated for 48 hours at 30°C.The probiotic capsules were added to MRS bulion.

RESULTS AND DISCUSSION

Beads Characterization. By dropping into the hardening baths the emulsions of AG with probiotic bacteria we obtained beads with diameter of 1-3 mm and almost spherical shape. From the emulsion of CH with probiotic bacteria we obtained beads with diameters under 1mm, oval and irregular. The concentration of AG and CH influenced the diameter of beads; increasing concentration of matrices (from 1% to 2%) determined harder beads with higher diameter. Fig.1 illustrates the differences between all probiotic capsules. Capsules diameter of CH 1% and AG 1% were less then 1mm and oval, while capsules of CH 1.5% and AG 1.5% were approximately spherical and had the diameter nearly of 2 mm.
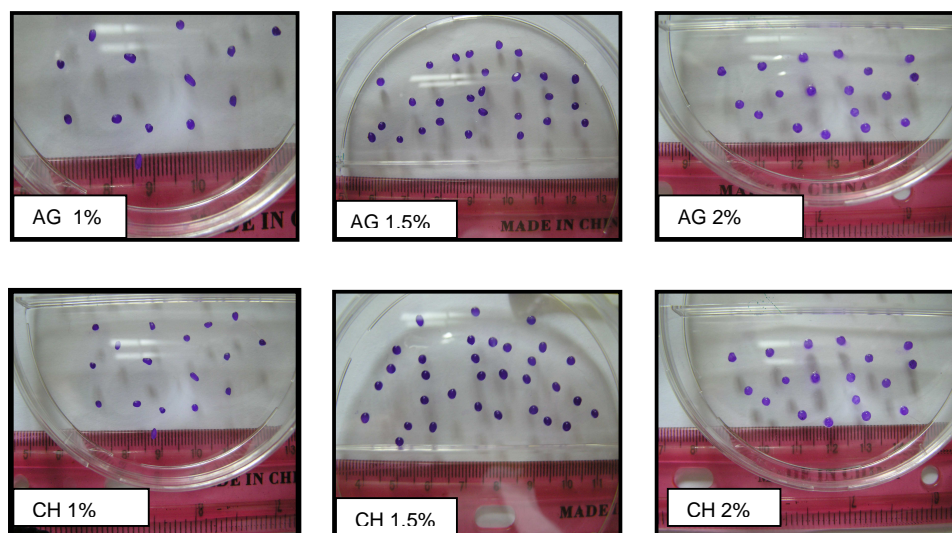
Fig 1. Represents beads containing probiotic bacteria: AG 2%, b. AG 1.5%, AG 1%, CH 2%, CH 1.5%, CH 1%.The scale of capsules measurement is exposed in cm.

In order to compare all the matrices and their concentrations used to obtain probiotic beads, (Fig.2), were measured their area, perimeter, elongation, roundness, diameter, compactness coefficient. Significant differences were observed especially in capsule area, perimeter and diameter. The most suitable for probiotic encapsulation were AG 2% and AG 1.5%. Capsules of AG 1.5%, had the biggest area (4,1mm^2) and diameter (2mm) while CH 2% had the smallest area (1.5 mm^2) and diameter (2,1mm). Roundness and compactness were nearly (1 mm) for all capsules. AG 1.5% had the largest perimeter (8 mm) while CH 2% had the lowest one (5 mm). Matrix with concentration of 1.5%, gives maximum water adsorption and area, diameter and perimeter was maximum. Capsules of AG are translucent, adsorbed water more then mat CH at the same concentration.
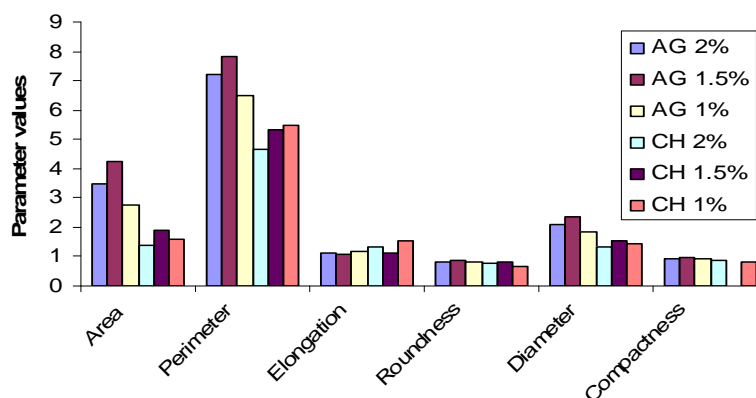

Fig 2. Comparative graphic representation of characteristics of AG 2%, AG 1.5%, AG 1%, CH 2%, CH 1.5%, CH 1%

FTIR –HATR analysis. The Comparative FTIR spectra of AG 2%, AG 1.5%, AG 1%, CH 2%, CH 1.5%, CH 1% matrices and probiotic capsules in AG 2%, AG 2%, AG 1.5%, AG 1%, CH 2%, CH 1.5%, CH 1% are plotted in Fig 3. The wavenumbers useful for matrices

discriminations were identified at 3244-3302 cm^{-1} (O-H stretch), 1400-1474 cm^{-1} (CH$_2$ bending), 1000-1200 cm^{-1} (C-O and C-C stretch), 924-1000 cm^{-1} (poly OH and CH$_2$ twist), 776-892 cm^{-1}(glycoside links) (Socaciu, 2009;Trif et al., 2007).

The alginate FTIR spectrum (Fig.3a) contains the characteristic peaks at 3242 cm-1 (OH$^-$ stretching) as mentioned before Lawrie, 2007, 1596 and 1407 cm^{-1} (COO– asymmetric and symmetric stretching), 1081-1024 cm^{-1} (C-O-C antisymmetric stretching), and carboxyl and carboxylate at about 1000 to 1400 cm^{-1} (Mayur, 2005).

In Fig 3 and Fig 4, are shown the wavenumbers characteristic considered for both matrices, located at 900-1100 cm^{-1}(carbohydrates) (1), 1560-1620 cm^{-1}(sugar ring stretching) (2) and a large band due to water 3100-3700 cm^{-1}(4). The encapsulated probiotic is recognised only by the group of 2 signals (3) identified at 2880 and 2920 cm^{-1} which correspond to same signals identified in pure probiotic culture (Fig 4).No differences were observed between the fingerprints of AG(Fig.3a) and CH(Fig.3b).
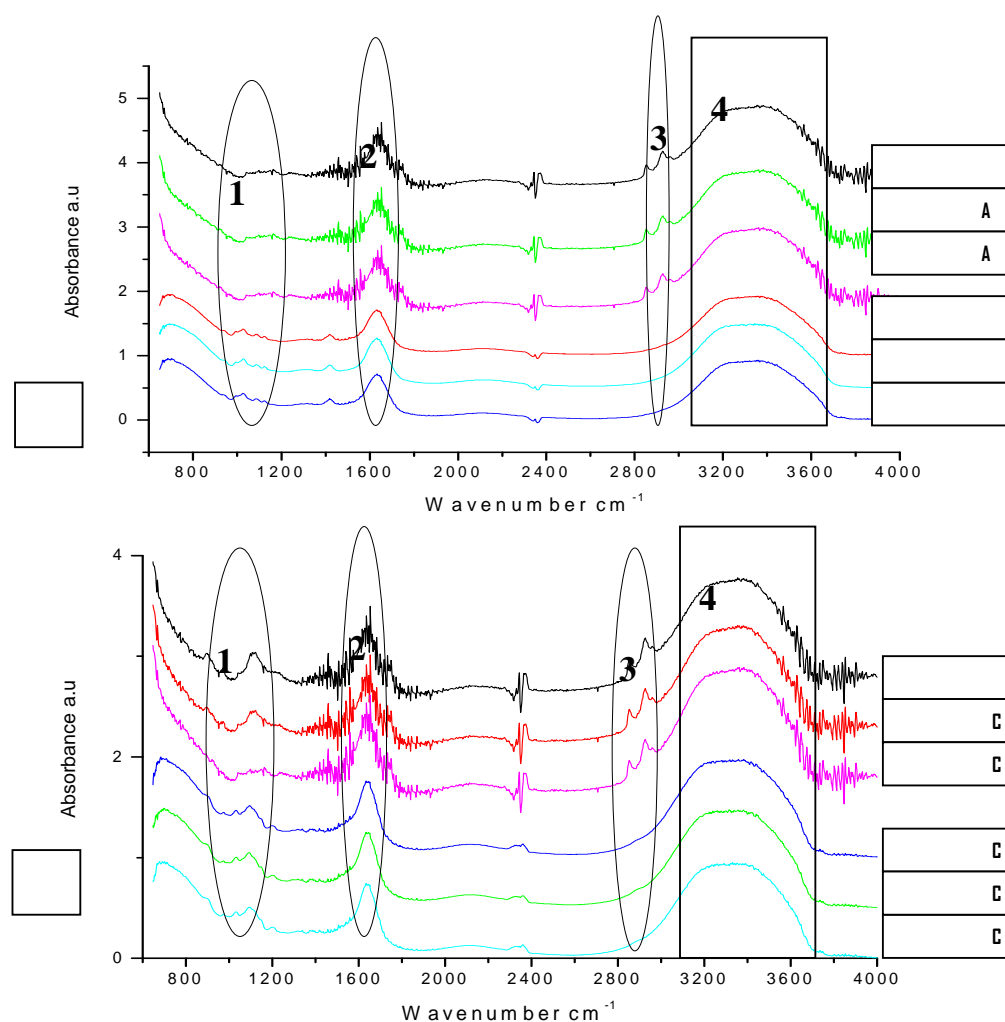

Fig. 3 Comparative FTIR fingerprint of matrices and capsules with probiotic bacteria of a: AG 2%, AG 1.5%, AG 1%,and b: CH 2%, CH 1.5%, CH 1%. (1)– carbohydrates; (2) - sugar ring stretching; (3) - bacterial fingerprint; (4) - water.
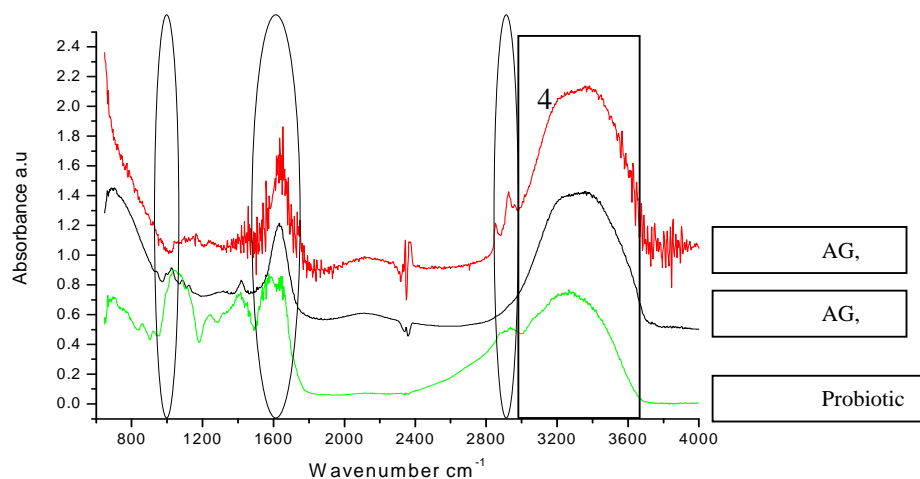
Fig. 4. Comparative FTIR fingerprint of: probiotic capsules AG 1.5%, matrices of AG 1.5%, and probiotic culture (1mg/10 ml water)

Viability of probiotics bacteria. Fig 5. represents the viability curve of probiotic bacteria (*Streptococcus thermophilus* and *Lactobacillus delbrueckii subsp. Bulgaricus)* on powder (━■━)(max. 14 C.F.U$*10^9$ ml^{-1}) used for encapsulation and the viability of probiotic capsules of CH 1.5% (━✳━)(max.12 C.F.U$*10^6$ ml^{-1}), CH 2% (━▲━) (max. 8 C.F.U$*10^6$ ml^{-1}), AG 1.5% (━■━)(max.15 C.F.U$*10^6$ ml^{-1}), AG 2% (━●━)(max. 14 C.F.U$*10^6$ ml^{-1}). Thus, it can be noted that capsules with AG 1.5% and CH 1.5% released more bacteria with a higher viability for the AG 2% and CH 2%.
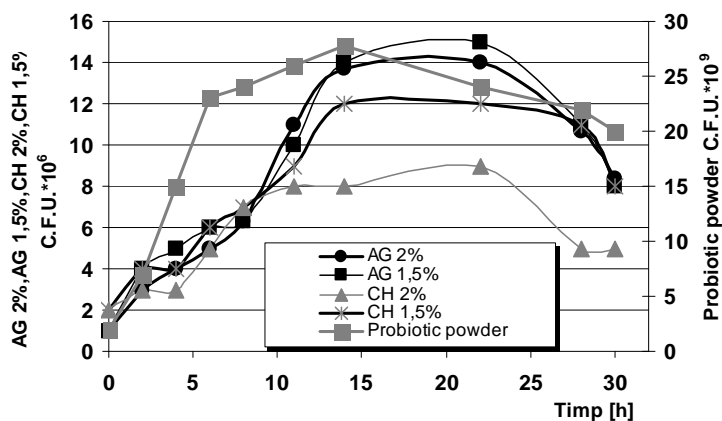

Fig.5. Comparative probiotic viability after release in media MRS bullion and cultivation on MRS agar: ━■━ probiotic powder, and capsules with probiotic bacteria of: ━✳━ CH 1.5%, ━▲━ CH 2%, ━■━ AG 1.5%, ━●━ AG 2%, for 30 hours at 37°C.

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CONCLUSIONS

The most suitable matrices for probiotic encapsulation with *Streptococcus thermophilus* and *Lactobacillus delbrueckii subsp. Bulgaricus* were alginate (AG 2% and AG 1.5%), considering their morphology and viability. FTIR fingerprint of encapsulated probiotic is recognised by the specific group of 2 signals identified at 2880 and 2920 cm^{-1} and represents an useful way to investigate non-destructively, such capsules. The probiotic viability of probiotic powder was superior (10^9 ml^{-1}) then for probiotic capsules (10^6 ml^{-1}). Thus, it can be noted that capsules with AG 1.5% and CH 1.5% released more bacteria with a higher viability for the AG 2% and CH 2%.

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