Journal of Chemical, Biological and Physical Sciences

An International Peer Review E-3 Journal of Sciences

*Available online at*www.jcbsc.org

Section B: Biological Sciences

CODEN (USA): JCBPAT Research Article

Bioactive Properties and Stability of the Microcapsules with Lactobacillus Casei Probiotics and Arabinoxylans Fractions as Prebiotics

Gigi Coman[1], Elena Roxana Chiriac[2], Alexandru Chiriac[3]

[1]"Dunărea de Jos" University of Galati, Faculty of Food Science and Engineering, Galati, Romania;

[2]University of Medicine and Pharmacy "Carol Davila", Faculty of Pharmacy, Bucharest, Romania;

[3]"Dunărea de Jos" University of Galati, Faculty of Medicine and Pharmacy, Galati, Romania;

Received: 02 March 2017**; Revised:** 21 March 2017; **Accepted:** 24 March 2017

Abstract: Cereal bran arabinoxylans (AX) as prebiotics and *Lactobacillus casei* spp *paracasei* as probiotic have been encapsulated in sodium alginate, having as objective survival increasing during exposure to stress conditions while crossing the gastro-intestinal tract. The number of cells of *Lactobacillus casei* spp *paracasei (*LAB) increased in all samples after encapsulation, in a similar ratio for LAB + inulin, LAB + rye bran AX, LAB + oat bran AX. A high percentage was obtained for LAB+2% oat bran AX sample at a high value of encapsulation yield (E.Y.132, 72%). The significant resistance in simulated gastric juice has presented LAB+2% oat bran AX sample after 90 min and LAB+2% rye bran AX after 60 min exposure time. Viability was maintaining under simulated gastric juice (pH 2.0) and simulated intestinal juice conditions (pH 8.0) for all levels of exposure time in the case of LAB + rye bran AX and LAB + oat bran AX samples, demonstrating the protective role and nutritional enrichment of AX extracted from cereals bran. In conclusions, this study presents a microencapsulation method of probiotic (*Lactobacillus casei* spp *paracasei*) with

a prebiotic (cereal bran arabinoxylans), that makes to grow the resistance of the probiotic during exposure to adverse environmental conditions by gastro-intestinal tract.

Keywords: Arabinoxylan; Microencapsulation; Probiotics; *Lactobacillus casei* spp *paracasei*; simulated gastro-intestinal juices

INTRODUCTION

Lignocellulosic biomass is complex structure of polymeric components: cellulose, hemicellulose and lignin and represent an important resource for the production of industrial and pharmaceutical products[1]. Cellulose the main cell wall constituent, is a linear polymer of D-glucose residues β-(1→4)-linked which forms (1→4)-β-D-glucan with occasional (1→6)-β-linked branches[2]. Hemicelluloses are a heterogeneous class of polymers with complex structure containing: glucose, xylose, mannose, galactose, arabinose, rhamnose, glucuronic acid and galacturonic acid[3]. Hemicellulose serves as a binding between lignin and cellulose fibers and can be hydrolyzed by acid on base, as well as hemicellulases enzyme[4]. Lignin is a phenolic polymer composed by three primary monomers: monolignols p-coumaryl, coniferyl and sinapyl alcohols[5]. Its role is to function as a biological barrier and as adhesive to retain linked in to matrix the hemicelluloses and celluloses[6]. Presence of lignin tightly linked with cell wall polysaccharides (cellulose and hemicellulose) limited the accessibility of xylanase and cellulase enzymes during hydrolysis processes[7]. Due to this structural complexity of the lignocellulosic matrix, production of arabinoxylooligosaccharides (AXOS) from grain wastes requires pretreatment operation including alkaline extraction, precipitation and enzymatic hydrolysis. AXOS is obtained from xylan-rich hemicelluloses isolated by hydrolysis with potassium hydroxide from lignocellulosic materials and crude xylanase secreted by micro-organisms (fungus, actinomycetes) [8]. Hemicelluloses have important application for obtaining the pentose (xylose and arabinose) and hexose (glucose, galactose and mannose), their transformation into fuel ethanol by monosaccharides fermentation, chemical conversion into furfural, levulinic acid and xylitol. They are used in the medical field and for pharmaceutical product obtaining with the gastric mucosa protective action and also having antitussive, immunostimulatory and antitumor properties[1, 2].

Many works have shown recently that can function as prebiotics defined as non-digestible food ingredients that beneficially affect the host by selectively stimulating the growth and/or the activity of one or a limited number of bacteria in the colon, and thus improve host health[9-13]. Probiotics are live micro-organisms which, when ingested in adequate amounts, confer a health benefit on the host [14, 15]. Generally, are considered probiotics the *Lactobacillus* sp. and *Bifidobacterium* sp. strains which play an important role in the defense against enter pathogen infections and modulating the host immune response. To exert these protective actions the minimum number of bacteria per dose should be 10^8-10^9 viable cell per day/dose[15, 16]. Condition that these bacteria to colonize the intestinal tract (the distal ileum and colon) is to be brought into a form that can resist the harsh conditions in those environments. Microencapsulation on different biopolymers (alginate, pectin, chitosan) and on cellulose derivatives is a method that enhance the probiotic availability in acid conditions and their stability during time [17, 18]. One advantage of microencapsulation with hydrocolloids method is that cells are entrapped within the

matrix during the formation of the spheres, while in other techniques such as spray drying, freeze drying and fluidized bed drying, the micro-organisms are completely released into the product[18,19].

The aims of the present work were to study the viability of free and encapsulated *Lactobacillus casei* ssp. *paracasei* as probiotic with cereal bran extracted arabinoxylans as prebiotics under simulated conditions of gastrointestinal transit, to improve the availability of probiotics in foods.

MATERIAL AND METHODS

Materials: Probiotic culture *Lactobacillus casei* ssp. *paracasei* (*L. casei* 431®, *Lc*, Chr. Hansen, Hónsholm, Denmark) (LAB) as freeze-dried commercial starters was used as the active material for the microcapsules. The microencapsulating agents used were sodium alginate (acid alginic sodium salt obtained from brown algae, Sigma-Aldrich, Belgium), the prebiotic agent inulin by *Dahlia* spp. root with degree of polymerization DP ≥ 23 (Sigma-Aldrich, USA) and arabinoxylans with 3 ≤ DP ≤10 were extracted of cereals bran (wheat, rye, oats) in the Lab for Biomass Processing by Bioaliment Research Platform (www.bioaliment.ugal.ro). Microencapsulation was carried in a solution of calcium chloride (Sigma-Aldrich, Germany). For the cultivation of *Lactobacillus casei* ssp. *paracasei* has been used MRS-agar (Sigma-Aldrich Chemie GmbH, Germany) and MRS liquid medium (Sharlau Chemie S.A., Spain). For getting simulated gastric fluid (SGF) has been used sodium chloride (NaCl, Chimopar, Romania), acid hydrochloric (HCl, Sigma-Aldrich, Germany), pepsin from porcine stomach mucosa by activity of 600-1800U/mg protein (Sigma-Aldrich, USA).To achieving simulated intestinal fluid (SIF) has been used bile dried pure for microbiology (Merck, Germany) which corresponds to 10-12g natural bile, and a source of pancreatin was used pharmaceutical product "Poliferment" manufactured by Arena Group, S.A., Romania, which is a complex of the enzyme with 18 u.W. trypsin, 6 u.W. lipase, 6.5 g amylase. All reagents were analytical grade.

Prebiotics actions determination by arabinoxylans (AX) extracted from the bran of grain on the probiotic strain *Lactobacillus casei* spp. *paracasei*: A freeze-dried probiotic cell of *Lactobacillus casei* ssp *paracasei* destined for microencapsulation were propagated from 1% (v/v) inocula in MRS liquid medium for 18 h at 37°C under anaerobic conditions (GasPakPlus®Jar System, Becton Dickinson Microbiology Systems, Cockeysville, USA). To highlight the influence of bioactive compounds they have been introduced in the MRS medium with the probiotic strain. Control sample was MRS liquid medium without the addition of arabinoxylan. The samples from this work was represented by MRS medium to which was added 20 mg·mL^{-1} arabinoxylan (AX) extracted from the cereals bran (wheat, rye, oats). Control was represented by 2% inulin (Sigma-Aldrich). All samples and control were incubated at 37ºC for 4 h under anaerobic conditions, representing samples at 4 h. The samples were serially diluted in 9 g·L^{-1} sodium chloride and plated on MRS-agar as proposed Vinderola, C. G. and Reinheimer, J. A.[20]. The plates with samples on MRS-agar were incubated in anaerobic jars (GasPakPlus®Jar System -Becton Dickinson Microbiology Systems, Cockeysville, USA) at 37±1ºC, 72 h. The count of viable probiotic cells was carried out and expressed as log colony-forming units per gram (log CFU g^{-1}). Colony counting was performed on samples at 0 h and after 4 h. Data were expressed as the average of three independent experiments with two replicates.

Samples preparation and microencapsulation: Commercial culture dried by lyophilisation (*L.casei* 431®, LC, Chr.Hansen, Denmark) was rehydrated and incubated in MRS medium at 37°C for 4 h under anaerobic conditions, in order to determine the $\log_{10}$ CFU·cm $^{-3}$ corresponding to 1g microbial powder. Counting the bacteria was carried out in Petri dishes on agar MRS medium in anaerobic conditions at 37°C incubation for 3 days. These data were needed for the determination of encapsulation efficiency and were expressed as the average of three experiments with two replicates.

Solution of 10% arabinoxylan extracted from the cereals bran (wheat, rye, oats) was mixed with 1.5% solution of sodium alginate (1:3 ratio) after which was then subjected to ultra-sonication operation for 3 min, 4 pulsation,70% amplitude to Bandelin Sonopuls System (Bandelin Electronic GmbH & Co. KG, Germany). Then over ultra-son solution was added 0.5 g *L.casei* 431® commercial freeze-dried cells and the mixture was homogenized using a magnetic stirrer. The obtained mass was subjected to extrusion using a 5 mL sterile syringes (Braun®, Germany) fitted with a needle 21G x 11/2 "(Braun®, Germany). Thus the suspension was extruded under aseptic conditions in 0.1M calcium chloride (100 mL) at a distance of 20 cm between the needle tip and the air-solution interface at a flow rate of 0.33 mL·min^{-1} which corresponds to a volume 7.14 µL per drop. In contact with a 0.1M calcium chloride the droplets of alginate and prebiotic co-immobilized with biomass were order in the sphere shapes. After 30min, beads were separated by decantation and washed with 0.1M calcium chloride by vacuum pump filter (Nalgene Corporation, USA).

Assay moisture loss and the degree of swelling of the microcapsules: To determine the *moisture loss* were weighed daily analytical balance (Metller Toledo, Spain) samples of microcapsules obtained. It has also been determined the *degree of swelling* of the microcapsules by immersing them in miliQ water for 3 hours. Weigh out analytical balance before and after 3 hours by immersion at temperature of 37°C, after they have absorbed enough water. The *moisture loss* (ML) was determined by the following formula (Eq. (1)):

$$\text{ML (\%)} = \frac{m_2 - m_1}{m_1} \cdot 100 \qquad (1)$$

Where MS is the moisture loss (%), m_1 is the weight of products before water immersing (g) and m_2 is the weight after that operation (g) [21].

The *degree of swelling* was tested by solutions with different pH values, corresponding to the acid range (as in the gastric juice, pH 5.0), neutral (pH 6.5, buffer Clark-Lubs) and alkaline (similar intestinal juice, pH 8.0). Clark-Lubs buffer solution was achieved by mixing 50 mL 0.1M KH_2PO_4 (13.6 g/L) and 13.9 mL 0.1 M NaOH in 1000 mL distilled water.

Encapsulation yield determination: Encapsulation yield (EY) (g·100g^{-1}) was represented survival rate during the microencapsulation process (Eq. (2)) and was calculated as following formula (Eq. (2)), proposed by Picot and Lacroix [22]:

$$E.Y. = \frac{N}{N_0} \cdot 100 \qquad (2)$$

Where N is the number of viable cells (log CFU g^{-1}) after encapsulation and N_0 is the number of viable cells (log CFU g^{-1}) before encapsulation[23].

The viability of encapsulated bacteria to simulated gastric fluid (SGF) and simulated intestinal fluid (SIF) conditions: Evaluation of the protective effect by low pH values corresponding to simulated gastric fluid (SGJ) with pepsin was performed using the method proposed by Sun and Griffiths[24] where SGJ has presented the following composition ($g \cdot L^{-1}$): 9 NaCl, 3 pepsin by swine gastric mucosa, 3 mL HCl. Hydrochloric acid was added to achieve the pH value at 1.8-2.0 range values suitable for the gastric juice. Also, the choice of the value at 1.8 to SGJ was achieved taking into account that pepsin activity is maximum in the pH ranges[25, 26] between 1.7-3.0 . The probiotic bacteria was cultivated, in MRS medium for 18 h at 37°C and 0.2 mL *Lactobacillus casei* ssp *paracasei* cell suspension was mixed in 10 mL SGJ to which was added 1 mL of a solution of arabinoxylan (wheat, rye, oats) so that to achieve a final concentration in the reaction medium by 20 $mg \cdot mL^{-1}$ and the bacterial inactivation was evaluated at 30, 60, 90 min under constant agitation rate at 50 rpm (orbital shaking incubator Ivynem System, Lab Companion Comecta S.A., Spain). For the control was used inulin disposed in the medium at the same concentration as the other samples. Counting bacteria was performed in the Petri dishes on MRS-agar medium in anaerobic conditions at 37°C for 3 days [23, 27].

Simulated intestinal fluid (SIF) has presented the following composition ($g \cdot L^{-1}$): 4.5 biliary salts, 1 pancreatin. These substances were dissolved in the Clark Lubs buffer for which the pH value was adjusted to 8.0 with 0.1N NaOH. Probiotic bacteria was cultivated in MRS medium for 18 h at 37°C and 0.2 mL of *Lactobacillus casei* ssp *paracasei* cell suspension was mixed in 10 mL SIF then was added 1 mL arabinoxylan (wheat, rye, oats) solution as to achieve final concentration by 20 $mg \cdot mL^{-1}$ and bacterial inactivation was determined by 30, 60, 90 min under constant agitation rate (50 rpm). For the control was used inulin disposed in the medium at the same concentration as the other samples (2%). Counting bacteria was performed in the Petri dishes on MRS agar medium under anaerobic conditions at 37°C for 3 days (Binder, Germany). The data represent the mean of three experiments with two replicates each [23, 28].

Viability determination of *Lactobacillus* after lysis of alginate microcapsules: Alginate microcapsules were solubilized in the Clark Lube buffer, pH 6.5, 60 min under slowly agitation rate (IKA RetBasic, India), and 1 mL sample were collected and inoculated in the liquid MRS for 18 h at 37°C under anaerobic conditions after which they were achieved serial dilutions to determine[23, 29] the number of $CFU \cdot cm^{-3}$.

***Lactobacillus* viability determination by microscopically analysis of alginate microcapsules:**

Where taken 1 g alginate microcapsules containing *Lactobacillus* co-immobilized with cereal bran arabinoxylans and inoculated in MRS liquid medium for 72 h at 37°C under anaerobic conditions. At the end of cultivation were harvested microcapsules, were compressed between two glass slides to give a fingerprint which was then stained with 1% methylene blue solution or 1% fuxin base to visualize the microbial cells. Microscopically analysis was performed under the optical microscope Optika Microscopes (Italy) at a magnification of 1.500 x. At the same time, have been achieved serial dilutions by culture medium on the MRS-agar in the Petri dishes which were cultured at 37°C under anaerobic conditions for 72 h to count the $CFU \cdot cm^{-3}$ for bacteria developed in the external environment of microcapsules.

Statistical analysis: ANOVA and Tukey's mean comparison tests ($p \leq 0.05$) were used to evaluate the data obtained from the test using the Statistica 7.0 (Statsoft, Tulsa, USA). All experiments and analyses were run in triplicates.

RESULTS AND DISCUSSION

The viability of the *Lactobacillus casei* spp. *paracasei* in the presence of arabinoxylan extracted from the cereals bran with prebiotic action: According to the FAO/World Health Organization[14], probiotics are living micro-organisms which confers benefits to their host when they are consumed in appropriate amount as part of food composition. Among the known probiotic micro-organisms, *Lactobacillus* strains are usually associated with fermented dairy products and their consumption has been associated with healthy living habits[30].Food fibers consist of the non-starchy polysaccharides including the cellulose, hemicellulose, pectin, β-glucans, gums and lignin. Fruits, vegetables, grains and legumes are the foods that are rich in these components [31, 32]. Some fibers have prebiotic effects which influence the state of the individual's health by selectively stimulating the growth of one or more bacteria strains in the colon [31]. The symbiotic term is used when a product contains probiotic and prebiotic ingredients [33]. It suggested that these products can increase the probiotic bacteria survival during transition through the gastrointestinal tract.

The study of the influence of prebiotics represented by arabinoxylans extracted from bran cereals (wheat, rye, oats) on the probiotic bacteria strain showed no significant changes compared with the control (LB + 2% inulin (A1)). Compared to this, the number of CFU·cm^{-1} was greater at 4 h of incubation for LB + wheat bran AX (A2), and for LB + rye bran AX (A3) and LB + oat bran AX (A4) was observed a slight increase for this value to 72 h of incubation (**Figure 1**).

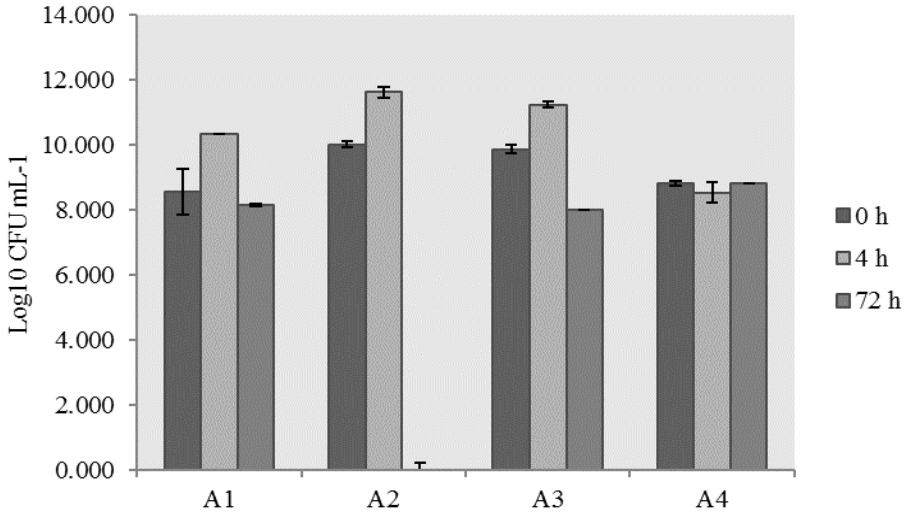


Figure 1: *Lactobacillus casei* spp *paracasei* (LB) viability in the presence of various encapsulating agents at a temperature of 37ºC in anaerobic conditions and at different time intervals: LB+2% inulin (Control) (A1); LB + wheat bran AX (A2); LB + rye bran AX (A3); LB + oat bran AX (A4). Error bars represent the mean ± SD of the results of the experiment.

Guergoletto *et al.*[34] in a study on the prebiotic fiber effects on the *Lactobacillus casei* viability showed that apple fibers have expressed 64% viability, and inulin had a lower percentage of viability by 55%. Several authors have reported that inulin show a better effect on the bifid-bacteria protection during exposure to simulated gastric fluid. Barclay *et al.*[35] considers that inulin would be ideal for transporting substances to the colon, since it is stable in a range of pH and ionic strength of the human gastrointestinal tract. Moreover, Mantzouridou *et al.* [36] believe that inulin molecules are not hydrolyzed by enzymes of the gastrointestinal tract and, therefore, it provides a beneficial effect on health as dietary fiber. The present study demonstrates that arabinoxylans have an improved ability to prebiotic function to *Lactobacillus casei*, inulin having a good physical and chemical stability and adequate bioavailability.

Moisture of loss, degree of swelling and encapsulation yield of the alginate microcapsules with *Lactobacillus casei* spp *paracasei* co-immobilized with arabinoxylans (AX): Many efforts were carried to improve the stability of bacteria probiotics bacteria during storage and to minimize transport and storage costs. Freeze-drying or spray-drying is a promising method to increase the survival of the bacteria during storage at room temperature.

Evolution of the moisture loss of symbiotic microcapsules revealed a stable character for LB + 2% inulin who presented a hydration state by 92% compared to the other samples which showed a loss of moisture ranges between 17-20% after 144 h of storage at room temperature (25ºC). The highest value of moisture loss presented LB + oats AX sample with 20% percentage (**Figure 2**).

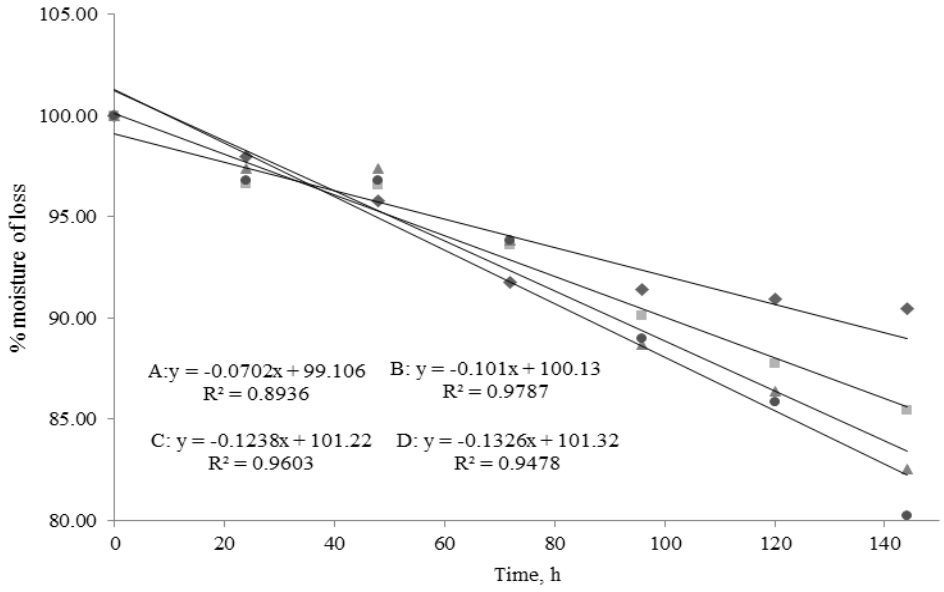


Figure 2: Evolution of the hydration of co-immobilized microcapsules of *Lactobacillus paracasei* spp *casei* with 2% AX obtained from cereal bran during the 144h at room temperature: ♦ A: LB + inulin; ■B: LB + wheat bran AX; ▲C: LB + rye bran AX; ●D:LB + oat bran AX. The lines represent first order regression.

The degree of swelling of the microcapsules in the SGJ and SIF is shown in **Figure 3**. The degree of swelling increases in case of incubation with SIF microcapsules LB + AX rye and for other samples, but with lower levels. A stable degree of swelling was registered for all the samples incubated at 6.5 pH value. A increased degree of swelling to incubated microcapsules with SGJ has presented the samples LB + inulin (control) and LB + wheat AX, while a decrease degree has presented the samples LB + rye AX and LB + oats AX. These results confirm in this study, on the one hand that microcapsules of alginate are much more stable in SIF than SGJ and on the other hand the arabinoxylan content influences the resistance to environmental conditions with pH very low value.

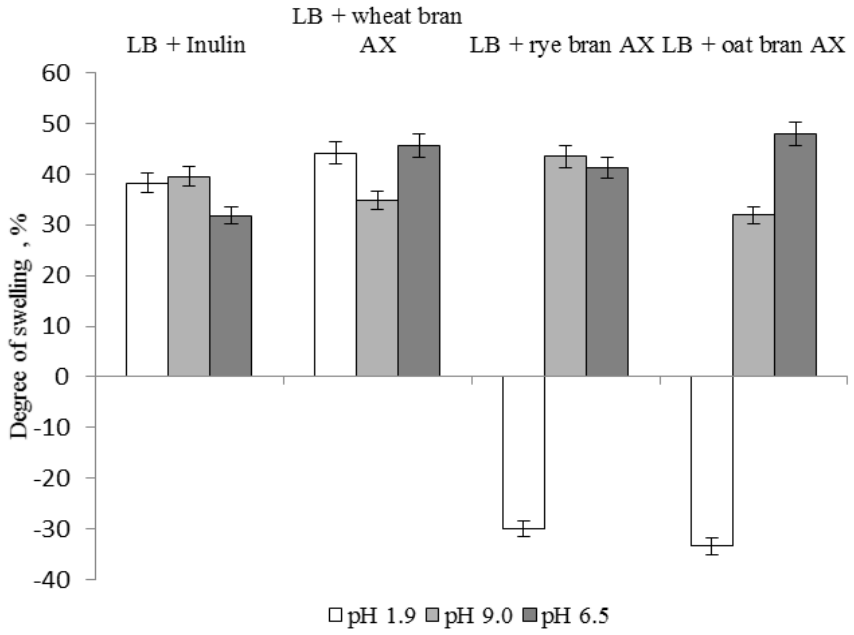


Figure 3: The degree of swelling of microcapsules with *Lactobacillus paracasei* spp *casei* co-immobilized with 2% AX obtained from bran cereal after 3h at 37°C. LB + inulin were the control sample. Error bars represent the mean ± SD of the results of the experiment.

Kanmani *et al*.[37] they have shown in a study of cryopreservation for alginate-chitosan microcapsules with the *Enterococcus faecium* MC13 probiotic that the degree of swelling increased when they were incubated in SIF. For SGJ was not observed the phenomenon of swelling. A 30% degree of swelling was observed after 144 h in SIF. The dimensions of the microcapsules have remained stable in SGF case and only 10% in size decreased. Wenrong and Griffiths[38] have shown that the microcapsules of alginate does not swell when incubated at low pH, carboxyl group of alginate remains unbound by Na^+ at high pH.

Viability in lysates symbiotic microcapsules: As shown in **Figure 4**, the increased viability in lysate microcapsules has presented a sample LB + rye AX, followed in equal measure by the other two samples LB + wheat AX and LB + oats AX. This was confirmed by viability determination in the external environment microcapsules where the lowest number of

microorganisms has presented the LB + rye AX samples and increased number for LB + wheat AX and LB + oat AX. Sample with inulin presented an equal number of free and encapsulated microorganisms. Arabinoxylans extracted from rye bran by their composition interact better with alginate matrix. Also, this arabinoxylan gives good gastrointestinal passage resistance and he had a good effect prebiotic for *Lactobacillus casei* spp *paracasei* strain. Cells number of *Lactobacillus casei* spp *paracasei* used as probiotics increases in all samples after encapsulation in a similar ratio to LB + inulin, LB + rye bran AX, LB + oat AX as shown in **Figure 4**. A higher ratio is for LB + oats AX sample which confirms as well the highest value for encapsulation yield.

El-Diebe et al.[39] in a study on the growth behavior for *Lactobacillus casei* and *Bifidobacterium bifidum* Bb-12 by bio-yogurts, have shown that microencapsulation offers a potential increase of viability for two strains during the storage of refrigerated conditions. Muthukumarasamy and Holley[40] shown that microencapsulation can improve the viability of the bacterial cells by keeping them in a protective matrix or the polymer membrane.

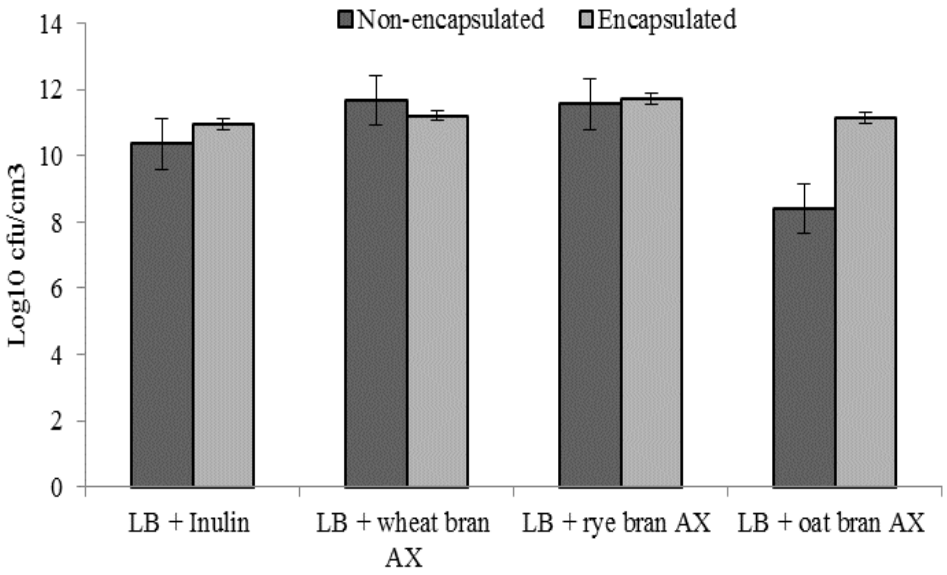


Figure 4: Comparative analysis of the number of cells of *Lactobacillus casei* spp *paracasei* co-immobilized with 2% AX from bran cereals before and after immobilization.

The yield of probiotic encapsulation with prebiotic compounds represented by arabinoxylans obtained from cereal sources: The encapsulation yield was calculated as the ratio between the microbial population after and before encapsulation. In order to achieve equivalence between the two measurements was reported number of bacterial cells by 1g biomass. As shown in **Figure 5** the largest yield encapsulating it has presented LB + oats AX sample and the smallest yield has presented LB + wheat AX sample.

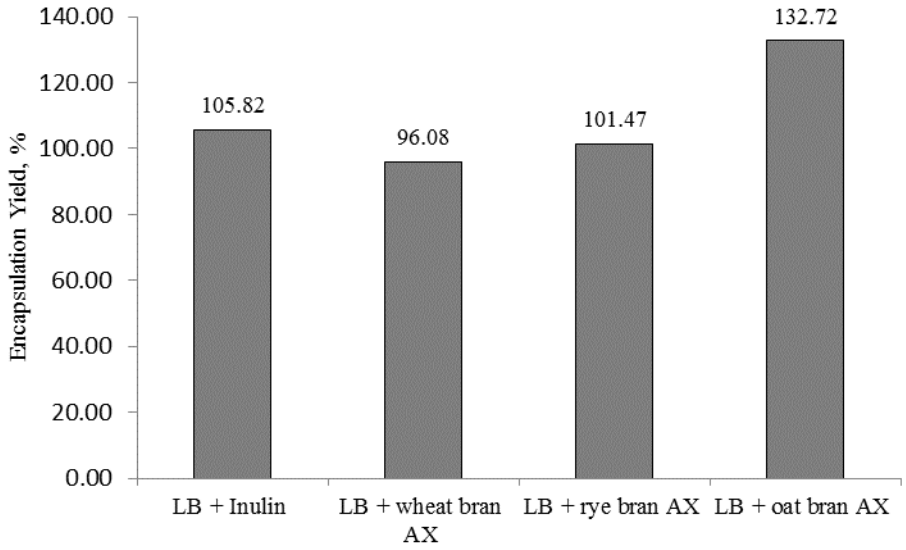
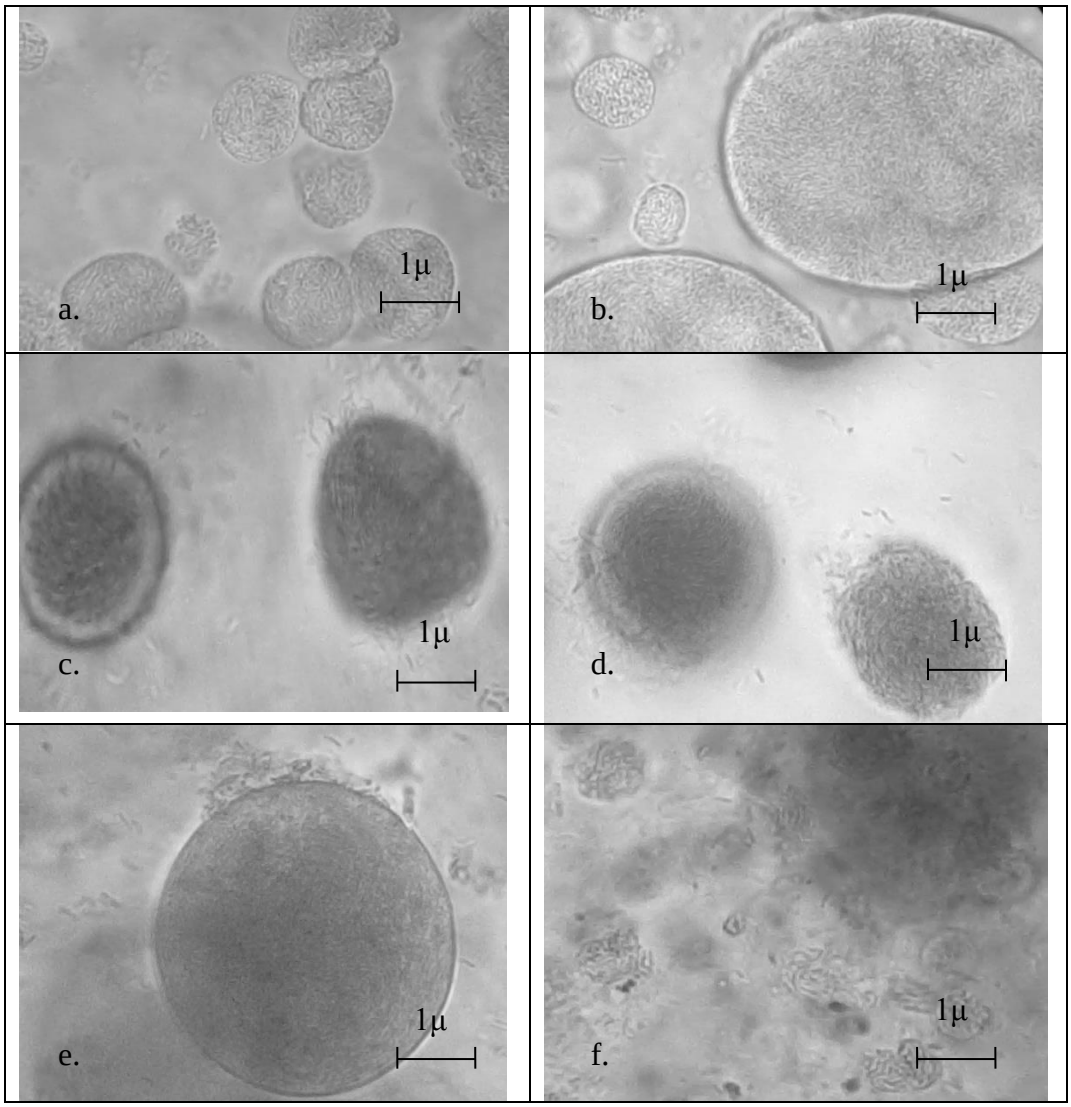


Figure 5: Encapsulation yields for co-immobilized *Lactobacillus casei* spp *paracasei* with 2% AX from bran cereals.

The appearance and structure determined by optical microscopy of *Lactobacillus casei* ssp *paracasei* microcapsules co-immobilized with arabinoxylans: The main reason for bacteria encapsulating is to increase the rate of their survival during production and storage of functional foods and protect them from physical and chemical stress during progression in the digestive tract. A key factor in obtaining the probiotics microcapsules is choosing the encapsulating material, depending on the desired physical and chemical properties and the method of micro-encapsulation. Microcapsules should be stable and maintain integrity during passage through the digestive tract until it reaches the destination where the capsule should break and release their contents. The encapsulation material should retain bacteria, and should limit the diffusion of hydrogen and digestive enzymes by microcapsules. For alginate capsules, a number of factors determine the internal structure, including intramolecular distribution and guluronic acid and mannuronic acid ratio, concentration and distribution of mono-and bivalent cations and pH values [41].

Inside the capsule alginate appears as "mesh-like" network among them bacteria are distributed individually or grouped. Some studies with crio-fracturing SEM microscopy[41] showed the presence of bacteria inside the gaps disposed inside the alginate microcapsule. Micro-granular appearance of inside the microcapsules seen by immersion optical microscopy (1500X) confirms the presence of alginate network. Outwards the microcapsules present a delimitation layer like cell membrane structure. The cells of *Lactobacillus casei* spp *paracasei* are disposed on the outside and the inside microcapsules. Their presence at the inside is confirmed by the cells agglomeration that occurs near a microcapsule in dissolution (**Figure 6f**). Much of the cells are placed on the microcapsules surface thus demonstrating their ability to bind to the alginate matrix (**Figure 6e**). There is a difference in terms of apparence size of microcapsules after prebiotic co-immobilization agent and by type of arabinoxylan used. Thus, the LB + inulin microcapsules

(**Figure 6 a-b**) have variable dimensions and tend to be firmer with a lower microbial load, while samples LB + wheat AX and LB + rye AX shows a higher adhesion to bacterial cells surface (**Figure 6c-d**). The microcapsules dissolution degree is greater compared with the control sample (LB + inulin). The LB + oats AX microcapsules are smaller compared to the other samples (**Figure 6 g-h**).



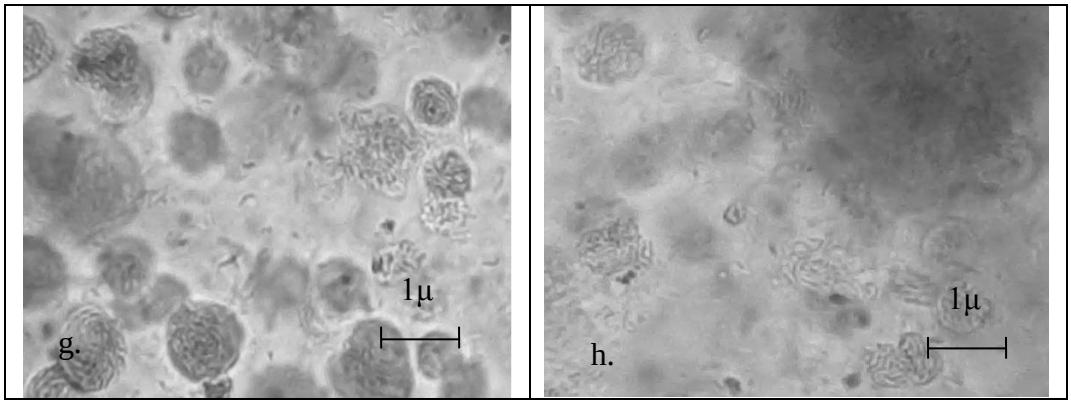


Figure 6: Microscopic image of microcapsules of *Lactobacillus casei* spp *paracasei* co-immobilized with 2% bran cereal AX, 1.500 X. (a), (b) LB+ 2% inulin (c), (d) LB + 2% wheat AX (e), (f) LB + 2% rye AX (g), (h) LB + 2% oat AX.

Testing the arabinoxylan oligosaccharides (AXOS) fractions on the bacteria viability with prebiotics under in vivo simulated conditions: Before *Lactobacillus casei* spp *paracasei* can be used in the functional product, it must survive the until they reach in the gastrointestinal tract, passing through and get to colonize the large intestine[42,43]. One of the major problems of efficiency of probiotic foods is low survival rate of microorganisms in the gastric pH and in the high bile salts concentration by intestinal juice [43,44] .

Figure 7 show data about free probiotic cell viability in the presence of 2% prebiotic represented by arabinoxylans obtained from grain bran exposed to simulated gastric fluid with 0.3% pepsin. In the study a increased decline of the free cells number is observed in the SGJ presence after 60 min exposure for probiotic samples in the presence of 2% inulin and 2% extracted arabinoxylans from wheat bran and good resistance for samples with 2% rye bran and oat extracted arabinoxylan. The best resistance has presented LB + 2% oats AX sample after 90 min exposure and LB + 2% rye AX after 60 min. For these samples is observed an increase in the number of micro-organisms at 60 min for SGJ exposure and a slight reduction to 90 min exposure. Because low resistance of probiotic with inulin or wheat arabinoxylan it's necessary microencapsulation thereof.

The probiotics free cell viability in the presence of 2% arabinoxylans extracted from cereal bran exposure to the intestinal juice at pH 8.0 and a content of 0.45% bile salts are presented in **Figure 8**. A $\log_{10}$ CFU · cm^{-1} reduction is observed in the control sample (probiotic with 2% inulin) and probiotic sample (2% arabinoxylan from wheat bran) at 60 min exposure and complete growth inhibition at 90 min. For samples with arabinoxylans obtained by rye and oat bran was observed a constancy of $\log_{10}$ CFU cm^{-1} number both at 30 min and at 60 and 90 min.

Dainty *et al*.[45] have reported that breaking of the calcium alginate matrix without chitosan coating takes place by the chelating action of phosphate ions in the phosphate buffer at pH values greater than 5.5. For the encapsulation yield determining was necessary to count the encapsulated cells of *Lactobacillus* and the free bacterial cells. The lysis of microcapsules was carried out in the Clark-Lubs buffer, pH 6.5. The phosphate ions by monopotassium phosphate in this buffer bind and

sequester the calcium ions by alginate matrix destabilizing the system and causing the dissolution of the particle, releasing the *Lactobacillus* cells.

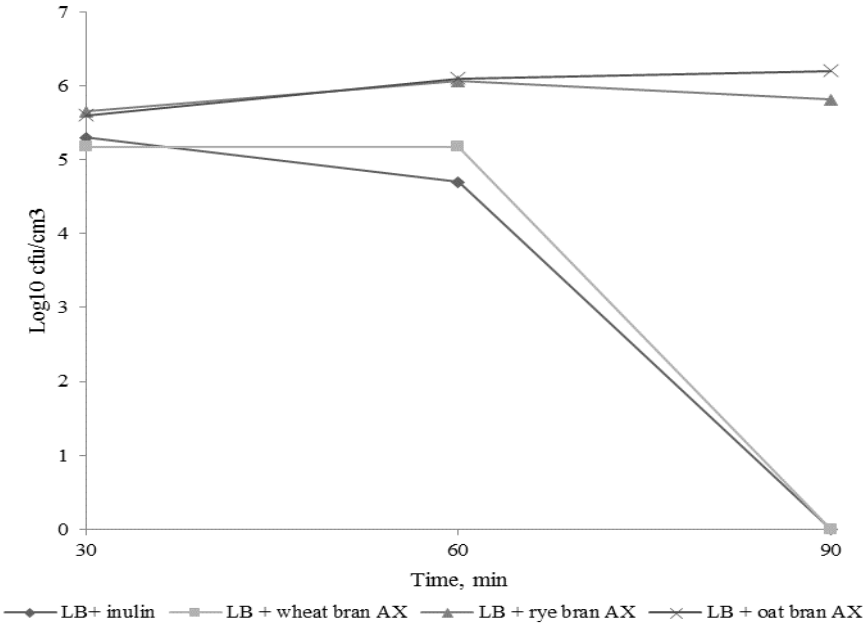


Figure 7: The survival rate from *Lactobacillus casei* spp *paracasei* in the presence of 2% cereal extracted arabinoxylans from different exposure times to simulated gastric juice, pH 2.0

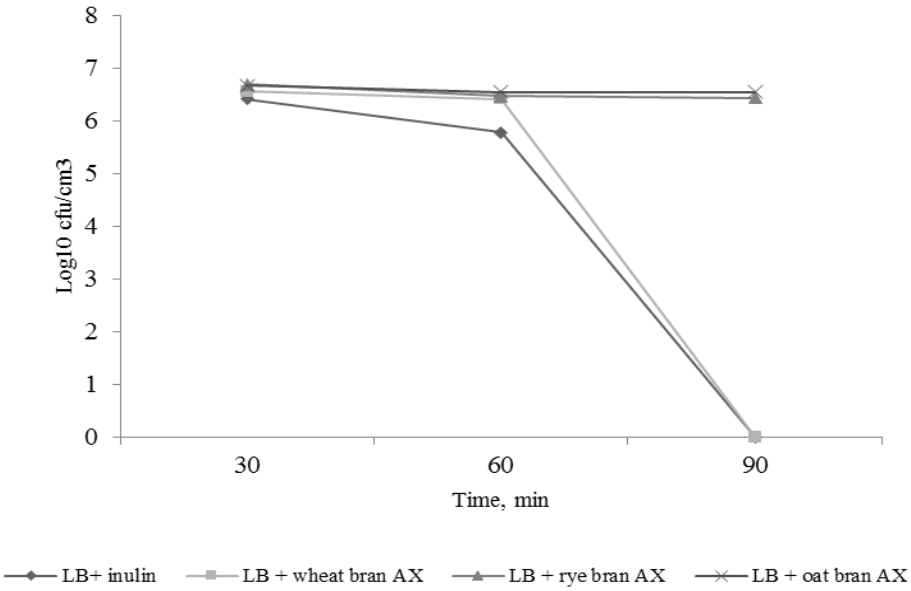


Figure 8: The survival rate from *Lactobacillus casei* spp *paracasei* in the presence of 2% cereal extracted arabinoxylans from different exposure times to simulated intestinal juice, pH 8.0.

CONCLUSION

Microencapsulation with alginate and prebiotics represented by cereal bran extracted arabinoxylans (oat bran AX, rye bran AX) provided the protection for *Lactobacillus casei* spp *paracasei* from the harsh acidic conditions of simulated gastric juice. The microcapsules are disintegrated into the simulated intestinal fluid and held released of entrapped cells. The degree of hydration of symbiotic microcapsules showed a stable character for LB + 2% inulin sample. The degree of swelling of the microcapsules is influenced by the pH of the medium. The microcapsules of alginate are more stable at the pH of simulated intestinal fluid (SIF) compared with the pH of the simulated gastric juice (SGJ). Inulin and wheat bran AX affects the micro-particles resistance in the acidic environment and rye bran AX and oat bran AX in the alkaline environment. A growing and viability maintaining under simulated gastric juice (pH 2.0) at all levels of exposure time they presented LB + rye bran AX and LB + oats bran AX samples showing the protective role of AX. A similar situation has shown the exposure in simulated intestinal juice conditions (pH 8.0). The largest viability for the *Lactobacillus casei* spp *paracasei* from lysates microcapsules was recorded for 2% of rye bran AX co-immobilization followed equally by 2% bran wheat AX or oat bran AX samples, respectively. For confirmation was performed the viability determination by *Lactobacillus* cells in the outside by microcapsules (MRS medium). The results showed a low viability test for the AX extracted from rye bran samples and slightly increased for AX samples extracted from wheat or oat bran, respectively. Benchmarking of cell viability by *Lactobacillus casei* spp *paracasei* co-immobilized with 2% of cereal bran AX before and after immobilization by sodium alginate matrix of the samples showed off an increase for the immobilized samples to the free samples. Sample LB + oat bran AX shown the greatest ratio between viability after and viability before immobilization. The highest encapsulation yield it has presented LB + oat bran AX sample. Future research activities will target the development of specific food and pharmaceutical application together with methods of microcapsules stabilizing and conditioning.

ACKNOWLEDGEMENT

This work has benefited from financial support through the 2010 POSDRU/89/1.5/S/52432 project, „ORGANIZING THE NATIONAL INTEREST POSTDOCTORAL SCHOOL OF „APPLIED BIOTECHNOLOGIES" WITH IMPACT ON ROMANIAN BIOECONOMY", project co-financed by the European Social Fund through the Sectoral Operational Programme Human Resources Development 2007-2013. The material support was assured by Integrated Center for Research and Education, Biotechology Applied in Food Industry – BIOALIMENT (www.bioaliment.ugal.ro).

REFERENCES

1. F.Mandelli, L.B.Brenelli, R.F.Almeida, R.Goldbeck, L.D.Wolf, Z.B.Hoffmam, R. Ruller, G.J.M.Rocha, A.Z.Mercadante, F.M.Squina, Simultaneous production of xylooligosaccharides and antioxidant compounds from sugarcane bagasse via enzymatic hydrolysis. *Ind. Crops and Prod.* 2014,52,770-775.

2. F.Peng, P.Peng, F.Xu, R.C.Sun, Fractional purification and bioconversion of hemicelluloses. *Biotech. Adv.*2012,30,4,879-890.
3. F.M.Girio, C.Fonseca, F.Carvalheiro, L.C. Duarte, S.Marques, Rouge-Lukasik, Hemicelluloses for fuel ethanol: A review. *Biores. Techn.*, 2010, 101,4775-4800.
4. F.Talebnia, D.Karakashev, I.Angelidaki, Production of bioethanol from wheat straw: An overview on pretreatment, hydrolysis and fermentation. *Biores. Technol.* 2010, 101, 4744-4753.
5. S.H.Ghaffar, M.Fan, Lignin in straw and its applications as an adhesive. *Int. J. Adhes and Adhesi.*, 2014, 48, 92-101.
6. A.Toledanoa, L.Serranoa, A.Pinedab, A.A.Romerob, R.Luqueb, J.Labidia, Microwave-assisted depolymerisation of organosolv lignin via mildhydrogen-free hydrogenolysis: Catalyst screening, *App. Catalysis B: Environ.* 2014,145,43-55.
7. J.Zeng, Z.Tong, L.Wanga, J.Y. Zhu, L.Ingram, Isolation and structural characterization of sugarcane bagasse lignin after dilute phosphoric acid plus steam explosion pretreatment and its effect on cellulose hydrolysis. *Biores. Technol.* 2014,154,274-281.
8. J.Bian, F. Peng, X.P.Peng, P.Peng, F.Xu, R.C.Sun, Structural features and antioxidant activity of xylooligosaccharides enzymatically produced from sugarcane bagasse, *Biores.Techn.* 2013,127,236-241.
9. G.R.Gibson, M.B.Roberfroid, Dietary modulation of the human colonic microbiota: introducing the concept of prebiotics. *J. Nutr.* 1995, 125, 1401-1412.
10. G.R.Gibson, Dietary modulation of the human gut microflora using prebiotics. *Br. J. Nutr.* 1998,80,209-212.
11. J.E.Teitelbaum, W.A. Walker, Nutritional impact of pre- and probiotics as protective gastrointestinal organisms, *Annu Rev Nutr.*, 2002, 22,107-138.
12. M.Ventura, S.O'Flaherty, M.J.Claesson, F.Turroni, T.R.Klaenhammer, D.van Sinderen, Genome-scale analyses of health-promoting bacteria: probiogenomics, *Nat. Rev. Microbiol.*, 2009,7,61-71.
13. J.Grimoud, H.Durand, C.Courtin, P.Monsan, F.Ouarné, V.Theodorou, C.Roques, In vitro screening of probiotic lactic acid bacteria and prebiotic glucooligosaccharides to select effective synbiotics, *Anaerobe*, 2010, 16,493-500.
14. FAO/WHO Guidelines for the evaluation of probiotics in food. Food andAgriculture Organization of United Nations and World Health Organization Working Group report, London, Ontario, 2002.
15. B.Albertini, B.Vitali, N.Passerini, F.Cruciani, M.Di Sabatinoa, L.Rodriguez, P.P. Brigidi, Development of microparticulate systems for intestinal delivery of *Lactobacillus acidophilus* and *Bifidobacterium lactis*, *Euro. J. Pharma.Sci.*, 2010, 40,359-366.

16. A.L.Servin, Antagonistic activities of lactobacilli and bifidobacteria against microbial pathogens, *FEMS Microbiol. Rev.* 2004, 28,405-440.
17. C.Calinescu, M.A.Mateescu, Carboxymethyl high amylose starch: chitosan self-stabilized matrix for probiotic colon delivery, *Eur. J. Pharm. Biopharm.*, 2008, 70,582-589.
18. G.B.Brinque, M.A.Z. Ayyub, Effect of microencapsulation on survival of *Lactobacillus plantarum* in simulated gastrointestinal conditions, refrigeration, and yogurt, *J. Food Engin.*, 2011, 103,123-128.
19. W.Krasaekoopt, B.Bhandari, H.Deeth, The influence of coating materials on some properties of alginate beads and survivability of microencapsulated probiotic bacteria, *Int. Dairy J.*, 2004, 14, 8,737-743.
20. C.G.Vinderola, J.A.Reinheimer, Culture media for the enumeration of *Bifidobacterium bifidum* and *Lactobacillus acidophilus* in the presence of yoghurt bacteria, *Int. Dairy J.*, 1999, 9,497-505.
21. A.I.Raafat, E.Arabyb, S.Lotfya, Enhancement of fibrinolytic enzyme production from *Bacillus subtilis* via immobilization process onto radiation synthesized starch/dimethylaminoethyl methacrylate hydrogel, *Carbohyd. Polym.* 2012, 87, 1369-1374.
22. A.Picot, C.Lacroix, Encapsulation of bifidobacteria in whey proteinbased microcapsules and survival in simulated gastrointestinal conditions and in yogurt, *Int. Dairy J.* 2004, 14,505-515.
23. C.B.F. Freire, E.S. Prudencio, S.S. Pinto, I.B.Munoz, D.M.C.R. Amboni, Effect of microencapsulation on survival of *Bifidobacterium* BB-12 exposed to stimulated gastrointestinal conditions and heat tratments, *LWT* 2013, 50,39-44.
24. W.R. Sun, M.W. Griffiths, Survival of bifidobacteria in yogurt and simulated gastric juice following immobilization in gellan-xanthan beads, *Int. J. Food Microb.* 2000,61,1,17-25.
25. N.A.Tobey, S.S.Hosseini, C.Caymaz-Bor, H.R.Wyatt, G.S.Orlando, R.C.Orlando, The role of pepsin in acid injury to esophageal epithelium, *Am. J. Gastroenterol.* 2001,96,11,3062-3070.
26. G.K.Gbassi, G.K.T.Vandamme, S.Ennahar, E.Marchioni, Microencapsulation of *Lactobacillus plantarum* spp in an alginate matrix coated with whey proteins, *Int. J.Food Microb*., 2009, 129,103-105.
27. C.Iyer, K.Kailasapathy, Effect of co-encapsulation of probiotics with prebiotics on increasing the viability of encapsulated bacteria in simulated gastrointestinal conditions and in yoghurt, J. Food Sci., 2005, 70, 18-23.
28. H.L.Truelstrup, P.M.Allan-Wojtas, Y.L.Jin, A.T.Paulson, Survival of free and calcium-alginate microencapsulated *Bifidobacterium* spp. in simulated gastro-intestinal conditions, *Food Microbiol.*, 2002, 19, 35-45.

29. T.Y.Sheu, R.T.Marshall, H.Heymann, Improving survival of culture bacteria in frozen desserts by microentrapment, *J. Dairy Sci.*, 1993,76,1902-1907.
30. W.G. Elmer, V.L. Mcfarland, M. Mcfarland, The power of probiotics: Improving your health with beneficial microbes. The Harworth Press, Inc., 2007, 236.
31. G.R.Gibson, Prebiotics, *Best Pract. Res. Clin. Gastroenterol*, 2004, 2, 18,287-298.
32. F.Figuerola, M.L. Hurtado, A.M. Estévez, I.Chiffelle, F.Asenjo, Fibre concentrates from apple pomace and citrus peel as potential fibre sources for food enrichment, *Food Chem.*, 2005, 91, 3,395-401.
33. M.B.Roberfroid, Concepts and strategy of functional food science: The European perspective, *Am. J. Crit. Nutr.*, 2000, 7, 1660-1664.
34. K.B.Guergoletto,M. Magnani, J.San Martin, C.G.T.J. Andrade, S.Garcia, Survival of *Lactobacillus casei* (LC-1) adhered to prebiotic vegetal fibers, *Innov. Food Sci. and Emerg. Technol.*, 2010, 11,415-421.
35. T.Barclay, M.Ginic-Markovica, P.Cooper, N.Petrovsky, Inulin a versatile polysaccharide with multiple pharmaceutical and food chemical uses, *J. Excip.Food Chem.*, 2010, 1, 27-50.
36. F.Mantzouridou, A.Spanou, V.Kiosseoglou, An inulin-based dressing emulsion as a potential probiotic food carrier, *Food Res. Int.* 2012, 46,260-269.
37. P.Kanmani, R.S.Kumar, N.Yuvaraj, K.A.Paari, V.Pattukumar, V.Arul, Effect of cryopreservation and microencapsulation of lactic acid bacterium *Enterococcus faecium* MC13 for long-term storage, *Biochem. Engin. J.*, 2011, 58,140-147.
38. S.Wenrong, M.W.Griffiths, Survival of bifidobacteria in yoghurt and simulated gastric juice following immobilization in gellan–xanthan beads, *Int. J.Food Microbiol.* 2000,61,17-25.
39. S.M. El-Dieb, F.H.R.Abd Rabo, S.M.Badran, A.M.Abd El-Fattah, F.M.F. Elshaghabee, The growth behaviour and enhancement of probiotic viability in bioyoghurt, *Intern. Dairy J.*, 2012, 22, 44-47.
40. P.Muthukumarasamy, R.A.Holley, Microbiological and sensory quality of dry fermented sausages containing alginate-microencapsulated *Lactobacillus reuteri*, *J. Food Microb.*, 2006,111,164-169.
41. P.Allan-Wojtas, H.L.Truelstrup, A.T.Paulson, Microstructural studies of probiotic bacteria-loaded alginate microcapsules using standard electron microscopy techniques and anhydrous fixation, *LWT*, 2008, 41,101-108.
42. H.S.Kim, B.J.Kamara, I.C.Good, G.I.Jr Enders, Method for the preparation of stabile microencapsulated lactic acid bacteria, *J. Ind. Microb.* 1988,3,253-257.
43. D.L.Pedroso, M.Thomazini, R.J.B.Heinemann, C.S.Favaro-Trindade, Protection of *Bifidobacterium lactis* and *Lactobacillus acidophilus* by microencapsulation using spray-chilling, *Int. Dairy J.*, 2012, 26,127-132.

44. L.Sabikhi, R.Babu, D.K.Thompkinson, S.Kapila, Resistance of microencapsulated *Lactobacillus acidophilus* LA1 to processing treatments and simulated gut conditions, *Food and Biopr. Technol.*, 2010, 3,586-593.
45. A.L.Dainty, K.H.Goulding, P.K.Robinson, I.Simpkins, M.D.Trevan, Stability of alginate-immobilized algal cells, *Biotechnol. Bioeng*, 1986, 28,210-216.

Corresponding author: Coman Gigi,

Faculty of Food Science and Engineering,
Bioengineering in Food Industry Department, "Dunărea de Jos" University,

111 Domnească Street, 800201, Galaţi, Romania, comangigi@yahoo.com

On line publication Date: 24.03.2017