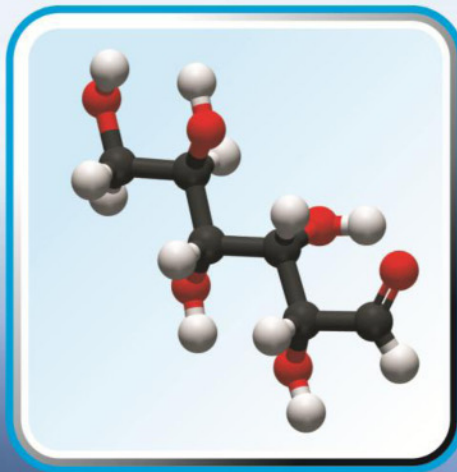



Македонски Фармацевтски Macedonian БИЛТЕН Pharmaceutical Bulletin



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5-ТИ
КОНГРЕС
НА ФАРМАЦИЈАТА
НА МАКЕДОНИЈА
СО МЕЃУНАРОДНО УЧЕСТВО

ПРЕДГОВОР

Овој број на Македонски фармацевтски билтен е специјален број на списанието посветен на Петтиот Конгрес на фармацијата на Македонија со меѓународно учество.

Во списанието се опфатени апстрактите кои од Научниот одбор се прифатени за презентација на Конгресот.

Апстрактите се печатени во оригиналната форма во која се доставени од авторите, без лекторирање и дополнително техничко обликување или пречукување па поради тоа овој број технички го нема вообичаениот стандард на Македонскиот фармацевтски билтен.

Авторите се потполно одговорни за содржините на нивните соопштенија.

PREFACE

The current issue of the Macedonian pharmaceutical bulletin is a special edition of the Fifth Congress of Pharmacy of Macedonia with International Participation.

The following issue of Macedonian pharmaceutical bulletin contains abstracts accepted by the Scientific Committee for the presentation at the Congress.

Abstracts were printed in their original form as submitted by the authors, without language corrections, technical editing or retyping.

Therefore, the layout doesn't meet the generally agreed upon standards required by the Journal.

Authors are fully responsible for the content of the submitted articles.



DEAR HONORED GUESTS, INVITED SPEAKERS, DELEGATES AND STUDENTS,

On behalf of the Scientific and Organizing Committee it is a pleasure to welcome you to Ohrid for the 5th Congress of Pharmacy in Macedonia with International Participation. The congress is organized as a joint activity of the Macedonian Pharmaceutical Association and Faculty of Pharmacy - Skopje as a quadrennial event since 1995.

This year's conference promises to be our most exciting and rewarding one yet, since more than 800 Pharmacists, among them scientists, educators, policy makers, professionals and the student community will be presenting and discussing some of the major opportunities and challenges affecting the pharmaceutical profession, today.

A great number of oral sessions and poster presentations will give both young and established scientists the opportunity to present their work and share ideas with colleagues on different topics. The opening lecture and the interdisciplinary plenary lectures presented by opinion leaders in particular fields are designed to create starting point for a fruitful congress for researchers, pharmacists and other health professionals. The scientific sessions that follow will start with opening lectures from a leading scientists in a particular field and will cover: I. Pharmaceutical Analysis / Quality Assurance / Regulatory Affairs, II. Pharmaceutical technology and biopharmacy, III. Pharmacoeconomy / Social Pharmacy, IV. Clinical biochemistry / Toxicology / Food and nutrition, V. Medicinal aromatic plants, VI. Clinical pharmacy, VII. Pharmaceutical chemistry / Biomolecular sciences. New trends in academia and education will be discussed during the half day Academic session. Two Workshops organized within the Congress, one on modern analytical techniques and one that will promote Regional ISPOR Chapter Macedonia will further enrich the activities planned for health professionals and scientists interested in those fields. The student session has a goal to introduce our students and other students from the region to experienced pharmaceutical and medical professionals, educators and scientists.

Best oral and poster presentations, selected by a Special Congress Award Committee will be awarded with prizes donated by our general sponsor.

You are invited to submit extended papers for publishing in Macedonian Pharmaceutical Bulletin immediately after the Congress.

We are especially grateful to the dedication of the entire faculty and all other colleagues and collaborators and our sponsors that have accepted to be a part of the Congress activities to ensure that this event is a success

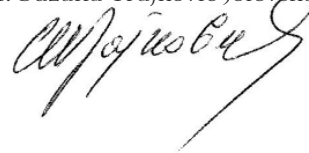
We wish you a successful Congress in Ohrid!

Regards,

Chair of the Scientific Committee
Prof. Katerina Goracinova



Chair of the Organizing Committee
Prof. Suzana Trajkovic Jolevska



ПОЧИТУВАНИ ГОСТИ, ПОКАНЕТИ ПРЕДАВАЧИ, ДЕЛЕГАТИ И СТУДЕНТИ,

Во име на Научниот и Организационен одбор задоволство е да Ви посакам добредојде во Охрид на 5-тиот Конгрес на фармацевти во Македонија со меѓународно учество. Конгресот е организиран од Македонското Фармацевтско Друштво и Фармацевтскиот факултет – Скопје, како настан кој се одржува секои четири години почнувајќи од 1995 година.

Програмата на овогодинашениот Конгрес е доказ за постојаниот напредок и промоција на овој настан. Повеќе од 800 делегати, меѓу нив и научници, професори, експерти и студенти, ќе презентираат и дискутираат за главните можности и предизвици кои ја засегаат фармацевтската професија.

Големиот број на орални и постер презентации, ќе им дадат можност како на младите така и на искусните научници да ја презентираат својата работа и меѓусебно да разменат идеи на различни теми. Воведното предавање и интередисциплинарните пленарни предавања презентирани од експерти во соодветните области ветуваат почеток на успешна работа на научниот дел од Конгресот. Научните секции кои следат, ќе започнат со воведни предавања од врвни научници во: 1. Фармацевтски анализи/ Обезбедување на квалитет/ Регулатива, 2. Фармацевтска технологија и биофармација, 3. Фармакоекономија/Социјална фармација 4. Клиничка биохемија/Токсикологија/Храна и исхрана 5. Медицински ароматични растенија 6. Клиничка фармација 7. Фармацевтска хемија/Биомолекуларни науки.

Во текот на Академската секција, ќе се дискутираат новите трендови во науката и образованието. Во рамките на Конгресот ќе се организираат две работилници. Првата ќе ги обработува современите аналитички техники, а во втората ќе се промовира регионалниот огранок на ISPOR, што дополнително ќе ги збогати активностите планирани за здравствените работници и научниците инволвирани во тие области. Студентската секција има за цел да ги промовира нашите и студентите од регионот, во професионалните, академските и научните кругови.

Најдобрите орални и постер презентации, одбрани од Комисијата за доделување награди и признанија, ќе бидат наградени од нашите генерални спонзори.

По завршување на Конгресот, ги покануваме авторите да поднесат проширена верзија на апстрактите во форма на оригинални научни трудови во Македонскиот Фармацевтски Билтен.

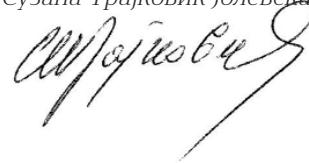
Особено сме благодарни на посветеноста на колегите од Фармацевтскиот Факултет - Скопје, сите останати соработници како и на нашите спонзори, кои прифатија да бидат дел од активностите на Конгресот.

Ви посакуваме успешен Конгрес во Охрид!
Со почит,

Претседател
на Научниот одбор
Проф. Д-р. Катерина Горачинова



Претседател
на Организационен одбор
Проф. Д-р. Сузана Трајковиќ Јолевска



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In food samples analyses mainly determined compounds are colorants (Tartrazine, Sunset Yellow, Ponceau 4R in various combinations). DS was used to evaluate the efficiency of phenylalanine removal for dietary supplements. Assays for malondialdehyde contents in infant milk formulas, protein and casein in milk as well as RPHPLC-second and fourth DS for milk proteins were evaluated. Applications of DS for the control of lipid oxidation in meats along with effectiveness of carnosine and dietary supplementation with α -tocopherol as well as assay of aromatic acids in cooked meat were outlined. Content of Vitamin C, plant pigments chlorophyll a and b, soy protein isolate, energizers in energy drinks, sorbic and benzoic acids in soft drinks and antibiotics in feeds were investigated using DS.

EVALUATION OF EP7 MONOGRAPH

Within our inter-laboratory evaluation of DS monograph (Ph.Eur.7) the comparison of analogue and digital (Savitzky-Golay algorithm) of second-order derivative spectra was carried out recording spectra on GBC-Cintra 20 (digital – smoothing points 5-21) and Thermo-Scientific - Evolution - 300 (analogue – smoothing – Low, Medium, High and Very-high or digital – smoothing points 3-121) using 0.02% V/V toluene in methanol solution. Requirement for resolution power defined as ratio A/B (A-amplitude ${}^2D_{265,263}$; B-amplitude ${}^2D_{263,261}$) is not less than 0.2 corresponds to resolution of shoulder and peak within the band. The results showed that the only possible comparison of instruments is for smoothing 7, and for both analogue and digital spectra with smoothing 7 and Medium.

IN CONCLUSION, along with substantial advantages of DS the limitations are caused by: a) dependence on instrumental parameters; b) non-robust character of the selected parameters of elaborated methods-parameters can be used only for system for which they are chosen and c) lack of harmonized protocol of parameter's optimization and results presentation.

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VIABILITY OF *L. CASEI* IN SYMBIOTIC CARROT JUICE DURING FERMENTATION AND STORAGE

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INTRODUCTION

Although dairy products are generally good matrices for the delivery of probiotics to humans and traditionally the most used, fruit juices are of growing interest due to their pleasant taste profile and refreshing characteristics (1). The increased demand of fruit juices for probiotic carriers can be explained by the lack of milk allergens and cholesterol (2). In addition, fruits and vegetables inherently contain essential nutrients such as vitamins, minerals and antioxidants (3). However, the low survival rate of probiotics in fruit juices resulting from acid environment is of concern. The viability of probiotic cells in fruit juices can be improved by adding of microencapsulated cells to the juice. In this study, carrot juice was inoculated with free probiotic cells of *L. casei* and synbiotic microparticles loaded with *L. casei* to compare the survival rate of the probiotic during fermentation and storage of the synbiotic beverages at 4 °C for 6 weeks.

MATERIALS AND METHODS

Carrot juice was prepared by extraction of washed and peeled carrots with no added water or any other nutrient. Then, the juice was pasteurized at 80 °C for 20 min. The cell suspension of probiotic *Lactobacillus casei* (Chr. Hansen, Denmark) was divided into two parts: one part was used for microencapsulation and another was used as free cells for direct adding in carrot juice. Prebiotic fructooligosaccharide (FOS) (Sigma-Aldrich, USA) was added simultaneously with free probiotic cells. The initial cell concentration of $7.4 \pm 0.1 \log_{10}$ cfu/ml was applied to ferment carrot juice using *L. casei* and FOS or synbiotic microparticles. This concentration was chosen according to the recommendations for minimum counts of $7.0 \log_{10}$ cfu per g or ml of probiotic food to exert beneficial effects (4). The microparticles were prepared by modified spray-drying method (5) when aqueous dispersion of alginate (LF 10/60, Protanal, FMC Biopolymers, USA), FOS and *L. casei* was submitted to spray-drying (nozzle diameter 0.7 mm, aspirator pressure 90%, flow rate 6 ml/min, inlet and outlet temperature, 120 °C and 60 °C, Büchi Mini Spray Dryer B-290, SW) followed by subsequent cross-linking and coating in solution of CaCl₂ (Merck, Germany) and chitosan (Chitine, France) in 1% w/w acetic acid. The microparticles formed were cured at least 3 h, separated and freeze-dried (-50 °C, 0.070 mbar, 24 h, Freeze-Dryer, Labconco, USA). An optimal formulation of micro-particles was prepared, with 4% w/w alginate, 0.5% w/w chitosan and 5% w/w CaCl₂. Positively charged microparticles (21.5 ± 1.5 mV) with d_{VS} $9.1 \pm 0.8 \mu\text{m}$, Ca-content of 9.4 ± 0.1 % and high cell viability of *L. casei* ($11.3 \pm 0.15 \log_{10}$ cfu/g) were obtained. Synbiotic microparticles were added to the carrot juice on the same day of preparation. All samples of synbiotic carrot juices were packed into sterile Erlenmeyers flasks closed with cotton plugs. The samples inoculated with free cells and added particles were firstly sub-

mit to fermentation and then stored at 4 °C. The fermentation process was carried out in an incubator for 24 h at 37 °C. The viability of *L. casei* in all experiments was determined periodically during 24 h and then on a weekly basis for 6 weeks of storage. When enumeration of bacteria was performed, 1 ml of the sample was mixed with 9.0 ml of peptone water, vortexed for 15 s and serially diluted with peptone water. The viable count was determined using plate-count method on MRS agar (Merck, Germany) after 72 h of incubation at 37 °C. Regarding the samples with added microparticles, the particles were removed from juice by filtration, washed with sterile saline solution and liquefied with phosphate buffer (pH 6.9). The suspension of particles was vortexed 30 s followed by standing at room temperature and then enumerate as described above. The pH of synbiotic carrot juices were also examined (pH meter PB 11 Sartorius, Germany).

RESULTS AND DISCUSSION

During the fermentation of carrot juice *L. casei* grew rapidly with increased population of free cells compared to encapsulated ones. At the end of the fermentation period the pH value of the juice inoculated with free cells was found to be lower than that of the microparticles added juice (Fig. 1).

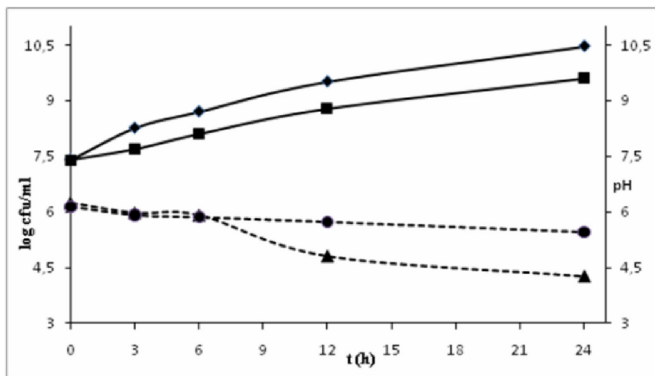


Fig. 1. Changes in viability and pH values during fermentation of carrot juice with free and microencapsulated *L. casei*

The viable cell counts of microencapsulated *L. casei* in fermented carrot juice was $8.1 \pm 0.13 \log_{10}$ cfu/ml after 6 weeks of cold storage at 4 °C, while that of free cells was only $4.89 \pm 0.1 \log_{10}$ cfu/ml. In addition, the pH values of the synbiotic carrot juices with free cells were lower than pH values of carrot juice with microencapsulated cells during all investigation period (Fig. 2).

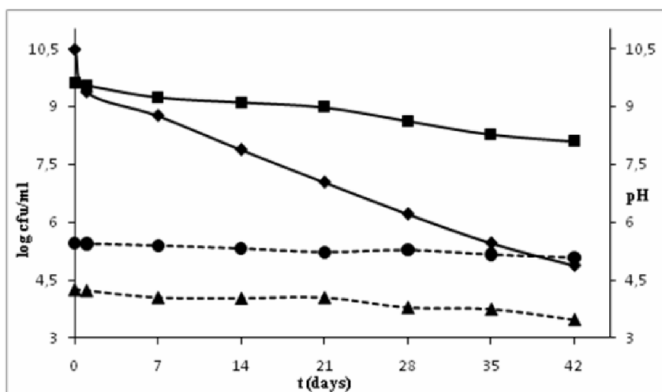


Fig. 2. Changes in viability and pH values during cold storage of fermented carrot juice with free and microencapsulated *L. casei*

The results showed that the survival rate of free probiotic cells in carrot juice was below the therapeutic level at the end of the test due to their sensitivity to the acidic conditions in the medium and need protection to maintain the viability during storage. Results also showed that adding of encapsulated *L. casei* in carrot juice as synbiotic chitosan-Ca-alginate microparticles might solve the problem. Regarding the sensory characteristics of the carrot juice with microparticles non-significant changes of the textural quality due to the low particle size was observed. Therefore, carrot juice containing synbiotic microparticles may be a new functional product and the effect of particles on the consumer acceptance should be further studied.

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ВИТАЛНОСТ НА *L. CASEI* ВО СИНБИОТСКИ СОК ОД МОРКОВ ВО УСЛОВИ НА ФЕРМЕНТАЦИЈА И ЧУВАЊЕ

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ВОВЕД

Покрај традиционалната примена на млечните производи за испорака на пробиотици во хуманиот ГИТ, пријатниот и освежувачки вкус на овошните сокови го зголемува интересот за апликација на пробиотиците во различни сокови (1). Интересот се должи на отсуството на млечни алергени и холестерол во овошните сокови (2), како и на присутните минерали, витамини и антиоксиданси во овошјето и зеленчукот (3). Киселата средина на сокот може значајно да ја намали виталноста на пробиотиците, а зголемување на виталноста може да се постигне со инкапсулирање на клетките. Во овој труд, следена е виталноста на *L. casei* во синбиотски сок од морков со слободни и инкапсулирани клетки за време на ферментација и чување во тек на 6 недели.

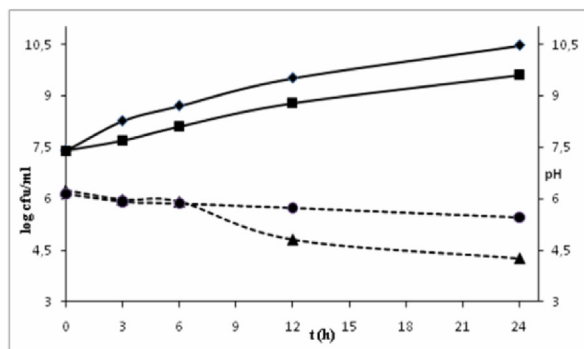
МАТЕРИЈАЛИ И МЕТОДИ

Сокот од морков беше подготвен со екстракција на измиени и излупени моркови без додадена вода и нутриенти и со примена на пастеризација (80 °C, 20 min). Клеточната суспензија на *L. casei* (Chr. Hansen, Denmark) беше поделена на два дела. Едниот беше наменет за микроинкапсулирање, додека другиот беше аплициран во сокот

заедно со пребиотикот фруктоолигосахарид (ФОС) (Sigma-Aldrich, USA). Почетната концентрација на *L. casei* ($7.4 \pm 0.1 \log_{10}$ cfu/ml) беше избрана врз основа на препораките за минимален број на витални клетки на g или ml производ за предизвикување на позитивни ефекти врз здравјето (4). Микрочестичките беа подготвени со модифициран метод на спреј сушење (5), така што водна дисперзија на алгинат (LF 10/60, Protanal, FMC Biopolymers, USA), ФОС и *L. casei* беше распрната со сушење (d диза 0.7 mm, проток 6 ml/min, аспирација 90%, влезна и излезна температура, 120 °C and 60 °C, Büchi Mini Spray Dryer B-290, SW) проследено со полиелектролитно комплексирање и вкрстено поврзување во раствор на цитозан (Chitine, France) и CaCl₂ (Merck, Germany) во 1% w/w оцетна киселина. По 3 h мешање, честичките беа одделени со центрифугирање и лиофилизирани (-50 °C, 0.070 mbar, 24 h, Freeze-Dryer, Labconco, USA). Со примена на 4% (w/w) алгинат, 0.5% (w/w) цитозан и 5% (w/w) CaCl₂, беше подготвена оптимална формулација на честички со позитивен површински полнеж (21.5 ± 1.5 mV), големина од $9.1 \pm 0.8 \mu\text{m}$, содржина на Ca од $9.4 \pm 0.1\%$ и виталност на *L. casei* од $11.3 \pm 0.15 \log_{10}$ cfu/g. Микрочестичките беа веднаш аплицирани во сокот. Потоа, примероците беа сместени во стерилни Erlenmeyer садови со памучни затворувачи и ферментирани во тек на 24 h на 37 °C во инкубатор. Виталноста на *L. casei* беше одредувана периодично во тек на 24 h и потоа еднаш неделно во услови на чување на 4 °C. Виталноста на *L. casei* беше определена со додавање на 9 ml пептонска вода на 1 ml примерок, вортексирање на содржината 15 s и сериско разредување со пептонска вода. Броенето на колониите беше вршено со примена на методот на броене на плоча на MRS agar (Merck, Germany) по инкубација на засадените плочи за време од 72 h на 37 °C. Виталноста на инкапсулираните клетки беше одредена со одделување на честичките од сокот со филтрирање. Суспензијата на честички во фосфатен пуфер (pH 6.9) беше вортексирана 30 s и оставена на собна температура. Потоа постапката за одредување на виталноста е идентична како што е претходно опишано. pH вредностите на синбиотските сокови од морков со слободни и инкапсулирани клетки од *L. casei* беа исто така испитувани (pH meter PB 11 Sartorius, Germany).

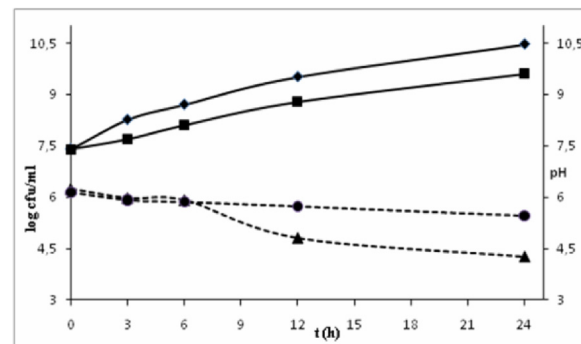
РЕЗУЛТАТИ И ДИСКУСИЈА

При ферментирање на сокот од морков беше забележан брз раст на *L. casei*, и тоа повисок раст покажаа слободните клетки. На крајот од ферментациониот период, pH вредноста на сокот со слободни пробиотски клетки беше пониска од сокот кој содржи инкапсулирани клетки (Сл. 1).



Сл. 1. Промени на виталноста на *L. casei* и pH вредноста на синбиотскиот сок од морков за време на ферментација

По 6 недели чување на 4 °C, виталноста на микроинкапсулираниот *L. casei* во ферментирани сок од морков беше $8.1 \pm 0.13 \log_{10}$ cfu/ml, додека виталноста на слободните клетки беше $4.89 \pm 0.1 \log_{10}$ cfu/ml. pH вредноста на сокот со слободни клетки беше пониска од pH вредноста на сокот со инкапсулирани клетки за целокупниот период на чување на 4 °C (Сл. 2).



Сл. 2. Промени на виталноста на *L. casei* и pH вредноста на ферментираниот сок од морков за време на чување на 4 °C

Резултатите покажаа дека виталноста на слободните пробиотски клетки во сок од морков не го задоволува терапевтското ниво во услови на чување што се должи на нивната осетливост кон киселата pH на сокот. Одржувањето на виталноста на *L. casei* при чување на сокот може да се подобри со инкапсулирање на клетките во облик на цитозан-Са-алгинатни честички. Со аплицирање на синбиотските микро-честички не беа забележани значајни промени на органолептичките својства и текстурата на сокот од морков како резултат на малите честички. Оттука произлегува можната употреба на сокот од морков со синбиотски микрочестички како нов функционален производ, при што следи проценка на ефектот на честичките врз прифатливоста од потрошувачите.

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