CO-ENCAPSULATION OF PROBIOTICS WITH PREBIOTICS INTO POLYSACCHARIDE PARTICLES AND ITS EFFECT ON VIABILITY IN SIMULATED GASTROINTESTINAL FLUID

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Abstract

Probiotic bacteria are live microorganisms that can provide beneficial effects on the human body. Microencapsulation helps to improve the survival of these bacteria because it protects them from harsh conditions, such as high temperature, pH, or salinity, during the preparation of a final food product and its gastrointestinal passage. The concept of co-encapsulation offers the potential for involved in symbioses and increased efficacy of functional foods by exploiting the synergy between prebiotic and probiotic ingredients.

This work was focuses on study co-encapsulation techniques used to enhance bacterial viability during utilization in functional foods and also for the targeted delivery in gastrointestinal tract. Probiotic strains *Bifidobacterium breve* and *Lactobacillus acidophilus* were used for encapsulation. Probiotics bacteria were separately co-encapsulated with prebiotics (green barley) and tested for their efficacy in improving the viability of bacteria and in improving stability of particles. Prepared particles long-term stability in model physiological conditions as well as in four different model foods was evaluated. Additionally, stability of selected particles in several real milk-based products was followed too. To analysis of probiotics optical and fluorescence microscopy were used. Viability of microorganisms using flow cytometry was determined too. The study revealed that co-encapsulation of probiotics with prebiotics exhibited longer survival than its free cells. Prepared particles are suitable for use to food product with beneficial effects on the human body.

Keywords:

Co-encapsulation, alginate, chitosan, probiotics, cell viability, release behavior

1. INTRODUCTION

Probiotics are defined as “live microorganisms which when administered in adequate amounts confer health benefits on the host”. Bacteria belonging to genera Bifidobacterium and Lactobacillus are often used as probiotic. One of the requirements for probiotic bacteria to be used as dietary adjuncts is the need to retain viability and activity during food processing and transit through gastrointestinal tract. A number of techniques have been used to improve the survival of probiotic bacteria. Microencapsulation has shown to protect probiotic bacteria not only during food fermentation and storage conditions but also from adverse gastro-intestinal environment. A variety of bio-polymers have been used as a capsular wall material, including alginate, chitosan, gelatin, carrageenan, cellulose, maize starch [1-3].

The live microbial additions (probiotics) may be used in conjunction with specific substrates (prebiotics) for growth. This combination could improve the survival of the probiotic organism, because its specific substrate is readily available for its fermentation, and result in advantages to the host that the live microorganism and prebiotic offer [1,2,4].

A prebiotic is a non-digestible food ingredient that beneficially affects the host by selectively stimulating the growth, activity, or both of one or a limited number of bacterial species already resident in the colon. For a food ingredient to be classified as a prebiotic, it must 1) neither be hydrolyzed nor absorbed in the upper part of the gastrointestinal tract; 2) be a selective substrate for one or a limited number of potentially beneficial

bacteria in the colon, thus stimulating the bacteria to grow, become metabolically activated, or both; and 3) be able as a consequence to alter the colonic microflora toward a more healthier composition [1,5]. Young green barley leaf is one of sources of a natural prebiotic. Green barley contains significant quantities of calcium, copper, iron, magnesium, potassium, zinc, β-carotene, folate, pantothenic acid, vitamins B1, B2, B6, C, and E, superoxide dismutase, catalase, and chlorophyll. Young barley leaf is consumed as a green-colored drink. Recently, several studies revealed that the young barley leaves helps to suppress a number of health disorders including obesity, diabetes, circulatory disorders, arthritis, anaemia, excessive cholesterol levels, renal difficulties, and cancer. Young barley leaves is rich in insoluble and water-soluble dietary fibers. The effects of dietary fiber on gastrointestinal functions have been described in many reports. Young barley leaves also possess polyphenols, including flavonoids, which are well-known antioxidants that prevent various diseases. Barley leaves extract is packed with essential vitamins, minerals, enzymes, chlorophyll, and antioxidants in a naturally-balanced form. In addition, Barley Leaves Extract has a purifying effect which enables the body to eliminate many toxins that would otherwise accumulate [6,7].

A combination of probiotics and prebiotics are defined as “synbiotics”. Results from various studies indicate that synergetic combination of probiotics and prebiotics showed enhanced beneficial effects compared to oral administration of probiotics or prebiotics only [1,4,5].

Effect of co-encapsulation with probiotics and prebiotics in improving the stability of microcapsules under simulated gastro-intestinal conditions and food storage conditions was investigated in this study. As well as, viability of co-encapsulated cells was tested.

2. EXPERIMENTAL

2.1. Methods

Bacterial strains *Lactobacillus acidophilus CCM 4833* and *Bifidobacterium breve CCM 7825T* used in this study were obtained from Czech Collection of Microorganisms in Brno. The strains were inoculated into MRS broth and incubated at 37 °C for 48 h. The cells were harvested by centrifugation at 3500 rpm for 10 min at low temperature (4 °C) and the cell pellet was washed with sterile distilled water. Cell suspension was mixed with sterile polysaccharide in order to obtain the final concentration of cell cultures 10^6 CFU/ml in capsules. Afterwards, suspensions were used for encapsulation.

As a material for particles preparation followed polymers were used: alginate, chitosan, chitosan-agar and alginate-starch. The particles were prepared by the method based on the principle of gelation and cross-linking polymers using the Büchi encapsulator B-395 Pro. The size/diameter of resulting particles was in range of 200 μm to 1000 μm.

The physicochemical evaluation of microparticles and the viability of *L. acidophilus* and *B. breve* during simulated gastrointestinal conditions were performed using microscopy and flow cytometry. Viability of encapsulation probiotic strains was determined also by fluorescence microscopy.

Simulated gastric fluids consisted of 2.5 g/L of pepsin with pH adjusted to 1.5 with hydrochloric acid. Simulated pancreatic intestinal fluids was prepared by dissolving pancreatin in intestinal solution (pH 7.5) to final concentrations of 2.5 g/L. Simulated intestinal bile fluids consisted of 4 g/L of bile salts.

In this work selected natural extracts of green barley were characterized. There substances were co-encapsulated into capsules with probiotic cells. In prepared particles long-term stability in model physiological conditions as well as in four different model foods (distilled water, acetic acid (3% solution), ethanol (10% solution), vegetable oil (25% emulsion)) was evaluated. Additionally, stability of selected particles in several real milk-based products was followed too. The stability of particles was determined spectrophotometrically. In natural extract a content of polyphenols, proteins, chlorophylls, as well as total antioxidant activity were analysed.

2.2. Results and discussion

In this work some natural polymers were used for preparation capsules with co-encapsulated probiotics strains and probiotic component. Such as prebiotics component in this work green barley was used. Particles were first prepared from alginate and chitosan. Further, particles were prepared also from mixture of two polymers, alginate-starch and chitosan-agar.

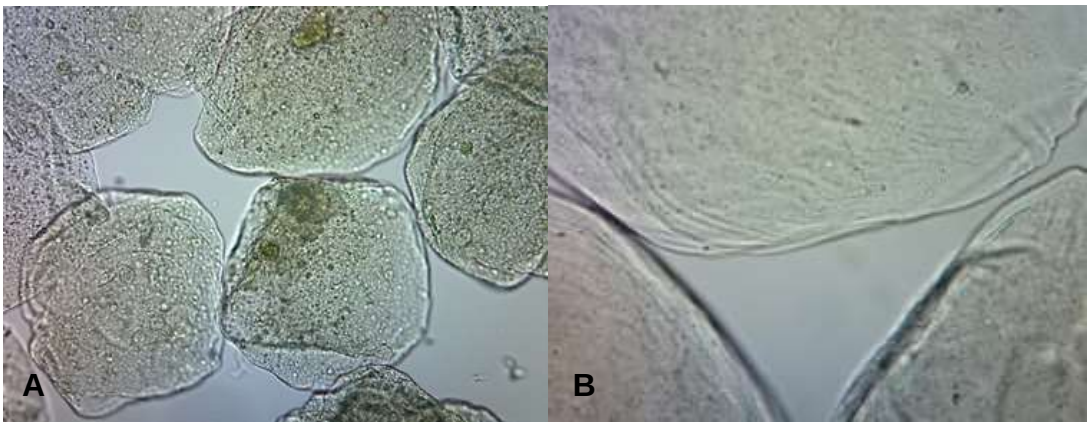

Fig.1: A) Alginate particles with co-encapsulation probiotics and green barley - 160x, B) Alginate particles with co-encapsulation probiotics and green barley- 640x

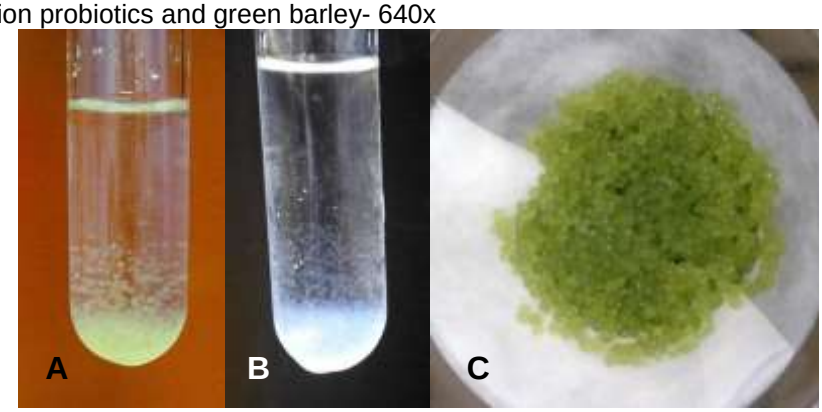

Fig.2: A) Particles with co-encapsulation probiotics and green barley - 400 μm, B) Particles with encapsulation probiotics - 300 μm, C) Particles with co-encapsulation probiotics and green barley - 900 μm

The physicochemical evaluation and mechanical stability of microparticles with co-encapsulation probiotics cells and prebiotics were performed using a light microscopy. In all of tested types of particles with co-encapsulated prebiotic and probiotic good mechanical stability were observed. The highest mechanical stability was found in alginate particle and chitosan-agar particle.

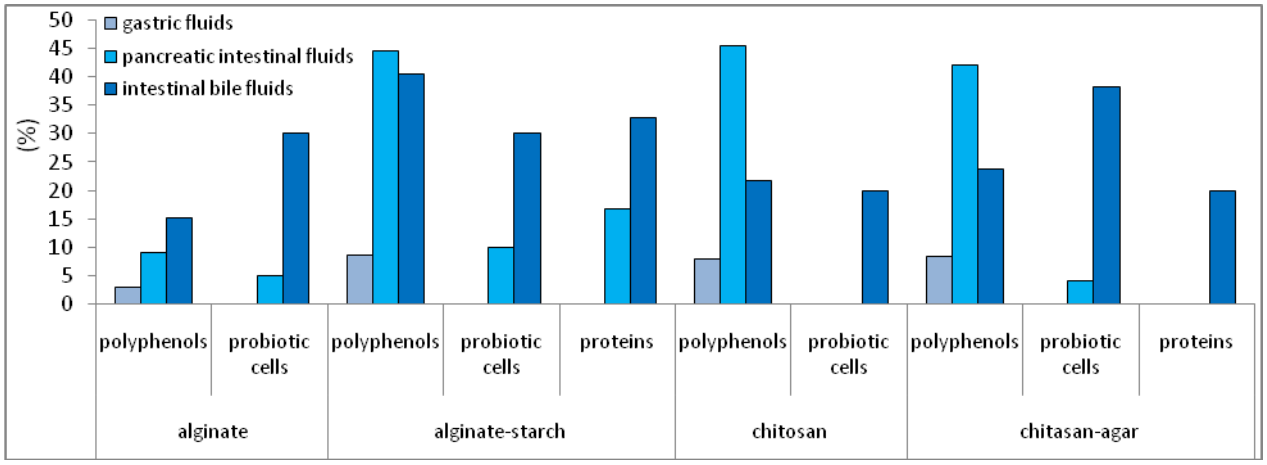

Fig.3: Amount of released components of particles with co-encapsulation probiotics and green barley during passage simulated gastrointestinal conditions

In prepared particles stability in model gastrointestinal conditions was evaluated. Particles are able to maintain their integrity during passage through the gastrointestinal tract until they reach their target destination, where they break down and release prebiotic and probiotic bacteria (Fig. 3). The best particle for control released in the gastrointestinal tract alginate-starch particle was found.

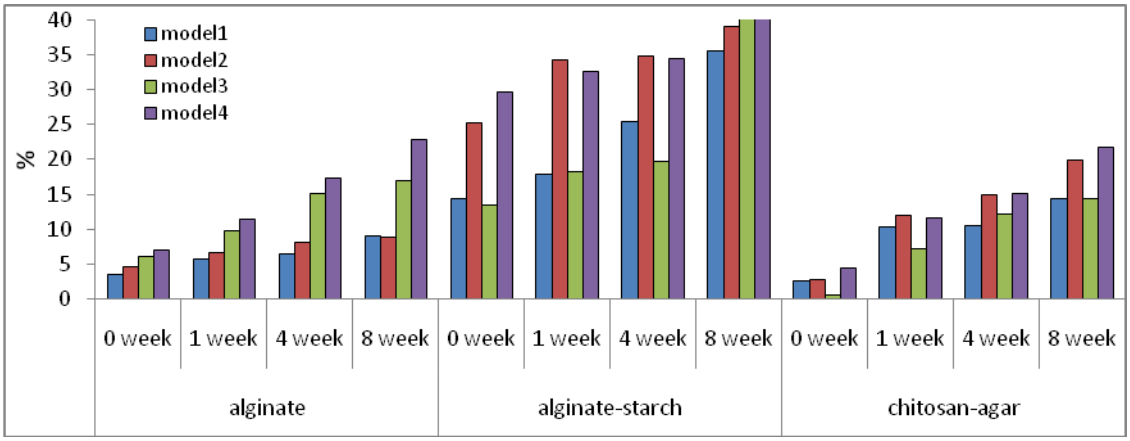

Fig.4: Amount of released polyphenols from alginate-starch particles with co-encapsulation probiotics and green barley during storage in model food

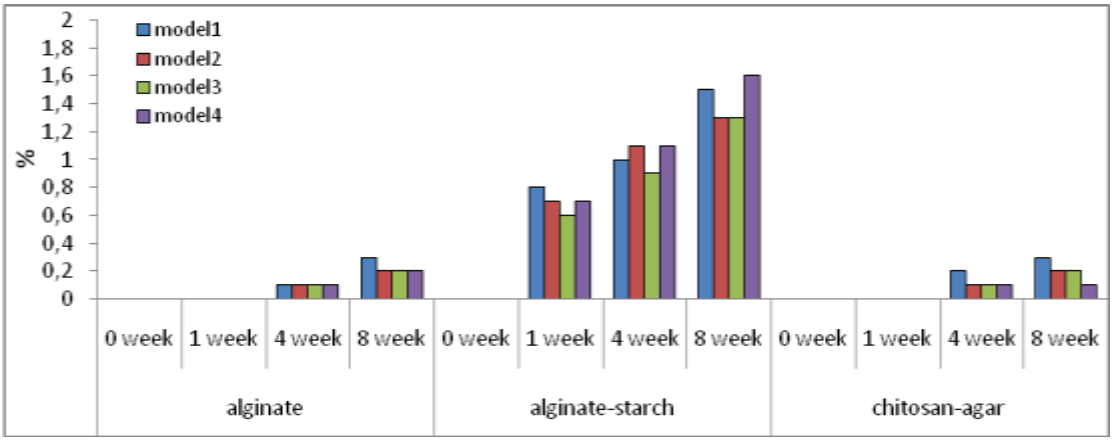

Fig.5: Amount of released probiotic from alginate-starch particles with co-encapsulation probiotics and green barley during storage in model food

Table 1: Amount of dead probiotic cell in alginate-starch capsules with co-encapsulated prebiotic and probiotic during storage in model food

type of capsules	time	% dead probiotic cells in capsules			
		model1	model2	model3	model4
alginate	0 week	0	0	0	0
	1 week	15,6	13,3	11,8	9,6
	4 week	35,6	31	29	16,9
	8 week	36,5	30,9	35,9	43,1
alginate-starch	0 week	0	0	0	0
	1 week	11	15,6	14,4	10,3
	4 week	23,3	20,6	19,2	34
	8 week	61,1	44,6	54,3	55,9
chitosan-agar	0 week	0	0	0	0
	1 week	10,6	16,3	15,5	9,2
	4 week	39,7	29,7	17,3	20,5
	8 week	59,6	41,7	47,3	35,5

These particles were exposed to model foods (model1: distilled water, model2: acetic acid (3% solution), model3: ethanol (10% solution), model4: vegetable oil (25% solution)). In these environments the particles were partially disintegrated and the release of probiotic cells can be evaluated (Fig.5). The release of prebiotics was determined too (Fig.4).

In all of tested types of particles with co-encapsulated microorganisms only about 1% released cells were detected after 8 week storage in each of model foods. In alginate-starch particle higher amounts of released prebiotic component was detected after storage in model foods.

Viability of released cells was determination by flow cytometry. The presence of living cells in encapsulated preparations was confirmed by fluorescence microscopy (Table 1). As a fluorescent probe propidium iodide was used. In all of tested types of particles with co-encapsulated probiotics about 10% to 15% dead cells were detected after 1 week and about 30% to 60% dead cells were found after 8 week storage in each of model foods.

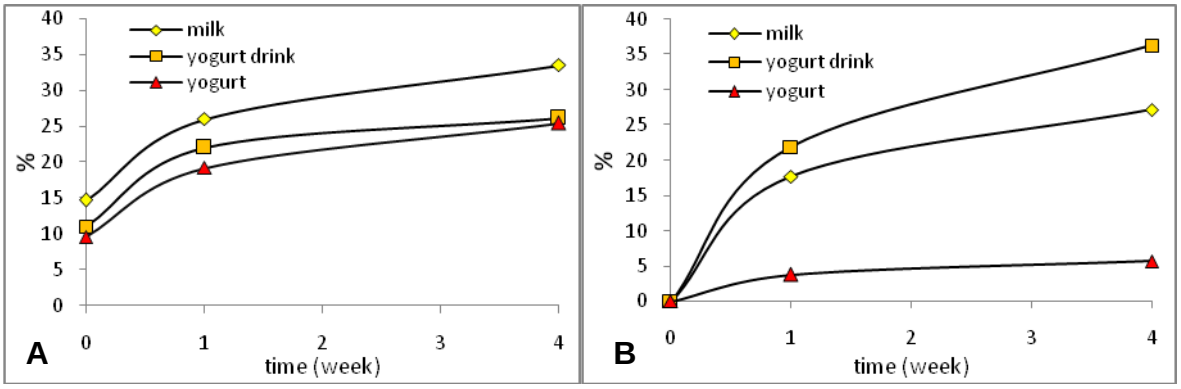

Fig.6: Amount of released A) polyphenols and B) probiotic from alginate-starch particles with co-encapsulated probiotics and green barley during storage in food

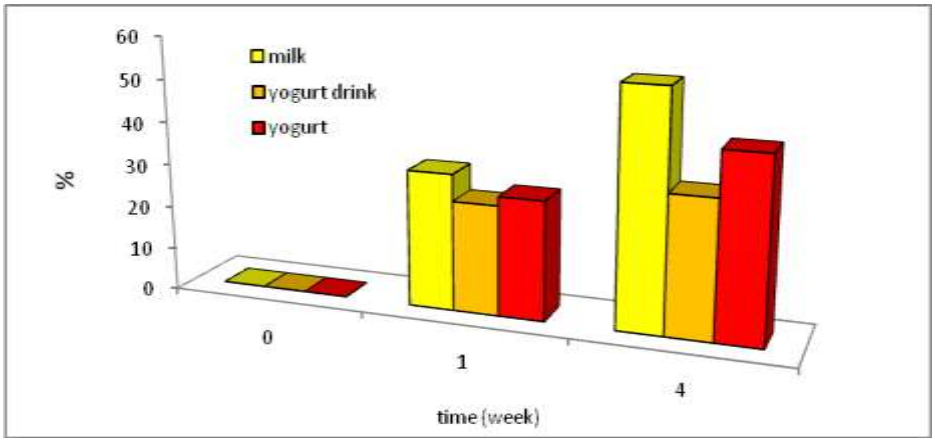

Fig.7: Amount of growth probiotic cell in alginate-starch capsules with co-encapsulated prebiotics and probiotics during storage in food

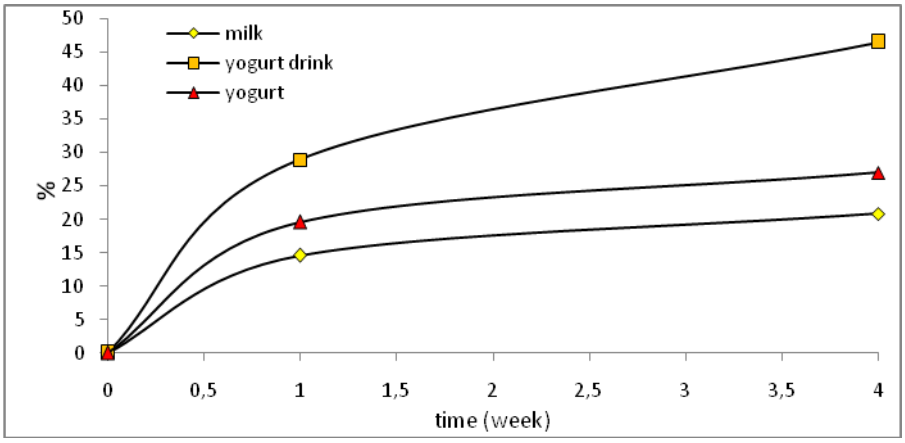

Fig.8: Amount of dead probiotic cell in alginate-starch capsules with co-encapsulated prebiotics and probiotics during storage in food

Stability of particles in several real milk-based products (milk, yogurt drink and yogurt) was evaluated. Particles were able to maintain their integrity during storage in food. In all of tested types of particles with co-encapsulated prebiotic and probiotic the most stable particle and the lowest amount of released components were found after storage in yogurt (Fig.6). In these particles about 5% released cells were detected after 4 week storage in food. Also, inside alginate-starch particles 45% growth cells were detected after 4-week storage in yogurt (Fig.7). In all of tested types of particles the lowest amount of dead cells were found after storage in milk. In alginate-starch particles 27% of dead cells were detected after 4-week storage in yogurt (Fig.8).

3. CONCLUSION

It can be concluded that co-encapsulation of probiotic bacteria with green barley leaves extract could be a suitable alternative for enhanced beneficial effects on gastrointestinal functions. Capsules are able to maintain their integrity during passage through the gastrointestinal tract until they reach their target destination, where they break down and release prebiotic and probiotic bacteria. In capsules high stability was found. Measurements confirmed that after co-encapsulation and during storage microorganisms survived inside the capsules and were still vital.

REFERENCES

[1] SATHYABAMA, S., M. RANJITH KUMAR, P. BRUNTHA DEVI, R. VIJAYABHARATHI a V. BRINDHA PRIYADHARISINI. Co-encapsulation of probiotics with prebiotics on alginate matrix and its effect on viability in simulated gastric environment. *LWT - Food Science and Technology* [online]. 2014, 57 (1), p. 419-425. DOI: 10.1016/j.lwt.2013.12.024.

[2] KRASAEKOOPT, W., S. WATCHARAPOKA, P. BRUNTHA DEVI, R. VIJAYABHARATHI a V. BRINDHA PRIYADHARISINI. Effect of addition of inulin and galactooligosaccharide on the survival of microencapsulated probiotics in alginate beads coated with chitosan in simulated digestive system, yogurt and fruit juice. *LWT - Food Science and Technology* [online]. 2014, 57 (2), p. 761-766. DOI: 10.1016/j.lwt.2014.01.037

[3] TRIPATHI, M.K. a S.K. GIRI. Probiotic functional foods: Survival of probiotics during processing and storage. *Journal of Functional Foods* [online]. 2014, 9, p. 225-241. DOI: 10.1016/j.jff.2014.04.030.

[4] OKURO, Paula K., Marcelo THOMAZINI, Júlio C.C. BALIEIRO, Roberta D.C.O. LIBERAL a Carmen S. FÁVARO-TRINDADE. Co- encapsulation of Lactobacillus acidophilus with inulin or polydextrose in solid lipid microparticles provides protection and improves stability: Survival of probiotics during processing and storage. *Food Research International* [online]. 2013, 53 (1), p. 96-103. DOI: 10.1016/j.foodres.2013.03.042

[5] GOURBEYRE, P., S. DENERY, M. BODINIER, Roberta D.C.O. LIBERAL a Carmen S. FÁVARO-TRINDADE. Probiotics, prebiotics, and synbiotics: impact on the gut immune system and allergic reactions. *Journal of Leukocyte Biology* [online]. 2011, 89 (5), p. 685-695. DOI: 10.1189/jlb.1109753.

[6] KAMIYAMA, Masumi a Takayuki SHIBAMOTO. Flavonoids with Potent Antioxidant Activity Found in Young Green Barley Leaves. *Journal of Agricultural and Food Chemistry* [online]. 2012, 60 (25), p. 6260-6267. DOI: 10.1021/jf301700j.

[7] IKEGUCHI, Motoya, Masahito TSUBATA, Akira TAKANO, Tomoyasu KAMIYA, Kinya TAKAGAKI, Hideyuki ITO, Yohko SUGAWA-KATAYAMA a Hideaki TSUJI. Effects of Young Barley Leaf Powder on Gastrointestinal Functions in Rats and Its Efficacy-Related Physicochemical Properties. *Evidence-Based Complementary and Alternative Medicine* [online]. 2014, p. 1-7. DOI: 10.1155/2014/974840.