Nucl. Sci. and Tech, Vol.5, No. 4 (2015), pp. 09-15

Nuclear Science and Technology

Journal homepage: https://jnst.vn/index.php/nst

Study on the immuno stimulation of radiation degraded *β*-glucan in swiss mice

Nguyen Thanh Long[1], Le Quang Luan[2*]

1) Company for Production of Vaccines and Biological Substances of Nha Trang, 26 Han Thuyen, Nha Trang, Khanh Hoa

2) Center for Nuclear Techniques, 217 Nguyen Trai, District 1, Ho Chi Minh City
**Email: lequangluan@gmail.com*

Abstract: The mixtures *β*-glucan extracted from the yeast cell wall were irradiated under gamma rays from a Co-60 source at doses of 100, 200 and 300 kGy in order to prepare water-soluble *β*-glucan. Yields of the water soluble *β*-glucan produced are 25.9, 49.1, 66.71%, and their molecular weights (Mw) are 30.5, 24.9 and 10.8 kDa, respectively. There are no any new peak in the IR spectra of the irradiated *β*-glucan samples, but the intensity ratio between the peaks at wavenumber of 1156 cm^{-1} (assigned to C-O-C bond) and of 1040 cm^{-1} (assigned to C-C bond) in glycosidic linkages was reduced with irradiation dose. These results revealed that gamma irradiation did not cause any change in the *β*-glucan structure except the scissions of glycosidic linkages. In this study, immuno stimulation of the irradiated *β*-glucan was also investigated for the Swiss mice. After 28 days supplying with the irradiated *β*-glucan, not only cellular indexes (white blood cell, neutrophils and lymphocytes counts), but also humoral immunity indexes (IgA and IgM) of the mice significantly increased and the highest effects was obtained for the mice supplied with the oligo*β*-glucan prepared by gamma irradiation at 200 kGy. Thus, the water soluble oligo*β*-glucan with Mw ~ 24.9 kDa prepared by gamma radiation much stimulated the natural immune system (non-specific immunity) in mice including both the cellular and humoral immunities. Particularly, the irradiated *β*-glucan is a very promising product for preparation of functional foods aiming at cancer prevention.

Key words: *oligoβ-glucan, gamma irradiation, degradation, immune stimulation, Swiss mice*.

I. INTRODUCTION

Several reports informed that *β*-glucan extracted from yeast cell wall in particular, or from fungy, bacteria and grain in general has potential ability for using as functional food or medicine due to the stimatation of natural immuno system, antitumor activity, increase of the resistance of infectious challenge, anticarcinogenic activity, enhancement of wound healing and so on [1-6]. From both practical and academic points of view, *β*-glucan also draws great attention as potential additive feed for enhancing the growth and immunostimulant in animals, shrimps and fishes [7-10]. Sung et al. [7] and Suphantharika et al. [8] have proved that yeat *β*-glucan stimulated significantly the immune activity of *Penaeus monodon* hemocytes and protection of this shrimp from pathogenic *Vibrio spp*. According to Jorgensen et al. [9] and Robertsen et al. [10], the supplementation of 1 ppm *β*-glucan into the feed enhanced non-specific disease resistance that leads to increase of the suvial of Alantic salmo (*Salmo salar*).

Recently, several reseseachers have paid their attentions to degrade of this biopolymer, because *β*-glucan extracted from yeast cells is water-insoluble and has some limitations for application. In addition, Buyn et al. [11] informed that the immunostimulant of low molecular weight (Mw) *β*-glucan was much

https://jnst.vn/index.php/nst/article/view/201
Received 02 December 2015, accepted 15 December 2015

higher than that of high Mw *β*-glucan. Sor far, three main methods have been used for preparation oligo-*β*-glucan with low Mw and water-solube oligomer [12-15]. Along with the tradiational method using specific enzyme for degradation [12] and chemical method using strong oxidative agents for degradation [13], radiation degradation has been reported as a useful method with several advantages such as the ability to degrade Mw reproducibly and quantitatively without the introduction of chemical reagents and without the need for special equipment to control for temperature, environment, and additives [14,15]. Therefore, this technology is unique and more environmentally friendly than conventional methods. The aim of this study is to salvage the spent brewer's yeast, a large discard waste released from beer production industry for preparation of *β*-glucan and reduction of environmental pollution as well. The research also tagetted to prepare water-solube oligo*β*-glucan by gamma irradiation and to evaluate its effects on the innate immuno system of mice.

II. EXPERIMENTAL

Materials

β-glucan was extracted from the cell wall of brewer's yeast collected from Saigon-Binhduong Brewery, Saigon-Binhtay Joint stock company. Swiss mice with 4-weeks old used for study were supplied by Pasteur Institute in Hochiminh City. Gamma cell used for irradiation was a gamma Co-60 source from India (BRIT 5000) at Dalat Nuclear Research Institute with dose rate about 3 kGy/h.

Radiation degradation of β-glucan

β-glucan powder was suspended, incubated and swollen in deionized water overnight at room temperature, then stirred for 3 h to obtain 10% (w/v) mixtures. The *β*-glucan mixtures were degraded by gamma irradiating at room temperature and doses of 100, 200 and 300 kGy.

Determination of the water-solubility of radiation degraded β-glucan

For determining the difference in water-solubility of the radiation degraded *β*-glucan, the irradiated mixtures were first lyophilized as described by Byun et al. [11]. Two grams of sample powder were put into a 50-ml glass tube with a cap, vortexed with 10 ml deionized water for 20 min, and centrifuged at 3500 × g for other 20 min. The supernatant was separated and dried at 100^{o}C for 2 h, and the weight of the dried products was determined. The water-solubility was calculated as follows:

Water-solubility (%) = 100 × (weight of dried supernatant)/(weight of initial *β*-glucan powder).

Mw determination

Gel permeation chromatography (GPC) was applied to monitor the changes in the average molecular weight (Mw) of *β*-glucan by gamma irradiation. GPC was implemented using the Agilent 1100 GPC system (USA) equipped detector RID G1362A and a Bin pump G1312A. Ultrahydrogel columns model 250 and 500 from Waters (USA) (7.8 id × 300 mm) equipped with a Ultrahydrogel guard column from Waters (USA) (6 id × 40 mm) were operated at 40^{o}C and eluted with distilled water at a flow rate of 1.0 ml min^{-1}. The *β*-glucan sample concentration was 0.1% (w/v) and 20 µl of sample solution was loaded into the GPC system. The column was calibrated using six pullulan standard samples with Mw values of 7.78, 12.2, 23.7, 48, 100 and 380 kDa (Polymer Laboratories, USA) [16].

FTIR spectra

FTIR spectra of degraded β-glucans were obtained on a Shimadzu FTIR-8100A spectrophotometer, which was linked to a Shimadzu DR-8030 computer system, in the

wavelength region between 4000 and 400 cm^{-1}. Samples were prepared in KBr pellet formed by well-dried mixtures of 3 mg sample and 100 mg KBr. All obtained spectra were the results of 128 scans at ambient temperature and a spectrophotometer resolution of 4 cm^{-1}.

Testing in mice

The 4-week-old mice with average body weight about 20 g/head were daily oral supplied with 100 µl of 2% unirradiated *β*-glucan and irradiated *β*-glucan solutions (2 mg/head). The control were supplied with the same volume of distilled water. After 28 days, the blood of mice was collected and put into tubes contained heparin for analysis. The cellular immunity indexes included total white blood cell counts, lymphocytes, and neutrophils were analyzed by an automatic haematology analyzer 18 para-meters Celltac α (Nihon Kohden, Japan). For analysis of the humoral immunity indexs, the colleted blood was centrifuged at 5000 rpm for 10 min. to separate the serum. The obtained serum was then used for analyzing IgG (immunoglobulin G) and IgM (immunoglobulin M) by ELISA (enzyme-linked immunosorbent assay) method at Laboratory of Stem Cell Research and Application, National Unviersity in Hochiminh City. All experiments were repeated three times. Data were statistically analyzed using the ANOVA test. The means were compared using the least significant difference (LSD) at a 5% probability level, and the standard deviations were calculated.

III. RESULTS AND DISCUSSION

Change in water-solubility and Mw of β-glucan by irradiation

Degradation to reduce the molecular size or depolymerization is a popular way for preparation of water-soluble *β*-glucan. Several methods such as acid and alkaline hydrolyses, or enzymatic digestion have been applied for degradation of *β*-glucan [13, 17-29]. Although these methods are effective in decreasing the molecular weight, they do have certain disadvantages such as a high cost, low yield, long processing time, and disposal of wastes. Recently much attention has been paid to apply the radiation processing technology for obtaining degraded polysaccharides such as alginate and chitosan due to several superiority of this method over enzymatic and chemical degradation processes. Main advantages include: degradation reaction can be carried out at room temperature, after processing the degraded alginate can be used without purification, simplicity of controlling the whole process and above all the large scale application [20,21]. Even so, the study on radiation degradation of *β*-glucan is still limited.

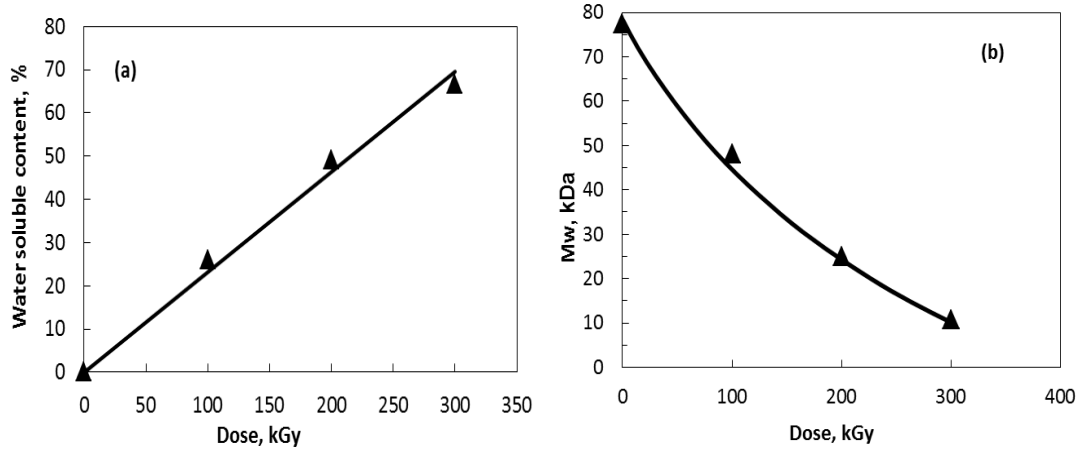

Fig. 1. Change in water soluble content (a) and Mw (b) of *β*-glucan by gamma irradiation

In this study, *β*-glucan extracted from brewer's yeast cell wall is water-insoluble, which were degraded by gamma irradiation, and the radiation effects on the water-solubility and molecular weight of *β*-glucan were investigated at different doses. The results in Fig. 1 indicate that the content of water soluble *β*-glucan linearly increased with radiation dose, from 25.89% at 100 kGy to 66.71% at 300 kGy. On the contrary, the Mw of water-soluble *β*-glucan was decreased from 77.4 kDa to 10.8 kDa by the increase of radiation dose up to 300 kGy. These results are in agreement with those reported by Byun et al. [11] and Methacanon et al. [4] that the gamma irradiation could degrade *β*-glucan in solution, then reduce its Mw and increase the content of water-soluble β-glucan.

FTIR characterization

The FTIR spectra of irradiated β-glucan were invertgated and the results are showed in Fig. 2a and the obtained results were recognized by the increase of peak intensity at 1731 cm^{-1} assigned to C=O linkages in reducing end-group. In addition, it can be seen clearly from Fig. 2b that intensity ratios between C-O-C glycosidic linkages (the peak which appears at 1156 cm^{-1}) and C-C linkages (the peak which appears at 1653 cm^{-1} was proposed to be almost stable by irradiation [22,23]) were decreased by the increasing of irradiation dose. These results indicated that scission in molecular chain of β-glucan by radiation occurred at glycoside linkages. The results are also in good agreement with our previous study on degaration of alginate by gamma irradiation [17].

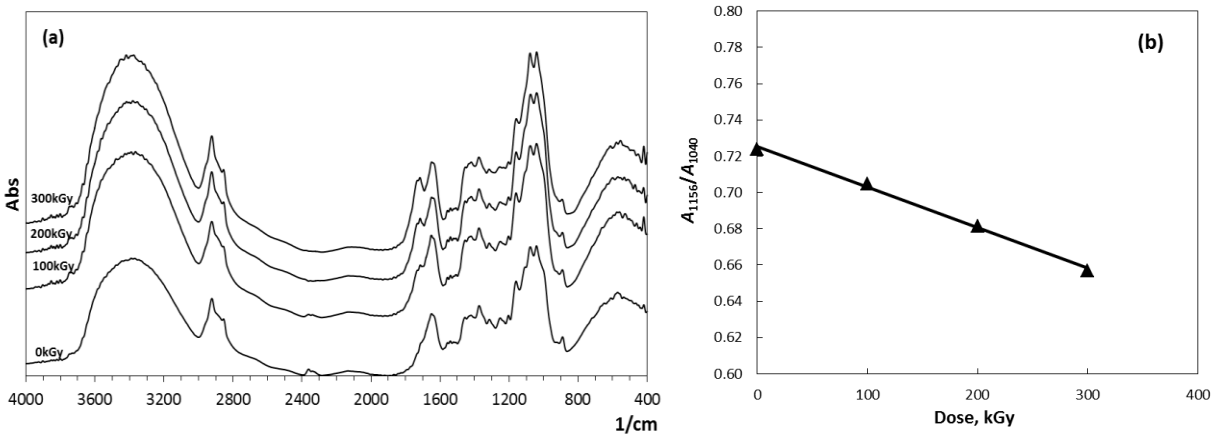

Fig. 2. The FTIR spectra (a) and the relationship between radiation dose and peak intensity (b) of *β*-glucan

Effect of irradiated β-glucan on immunity indexes in blood of mice

White blood cells (WBCs) are the cells of the immune system that are involved in protecting the body against both infectious disease and foreign invaders. All WBCs are produced and derived from a multipotent cell in the bone marrow known as a hematopoietic stem cell. Neutrophils defend against bacteria or fungal infection and they are usually first responders to microbial infection, while lymphocytes play very important roles for recognizing harmful particles or antigens and carrying out processes to deal with them. Different types of lymphocytes exist, known as T cells, B cells and natural killer cells, and their roles differ accordingly. T cells and natural killer cells destroy harmful cells and some T cells activate other immune cells. B cells produce antibodies, and both B and T cells create memory cells which remember threats.

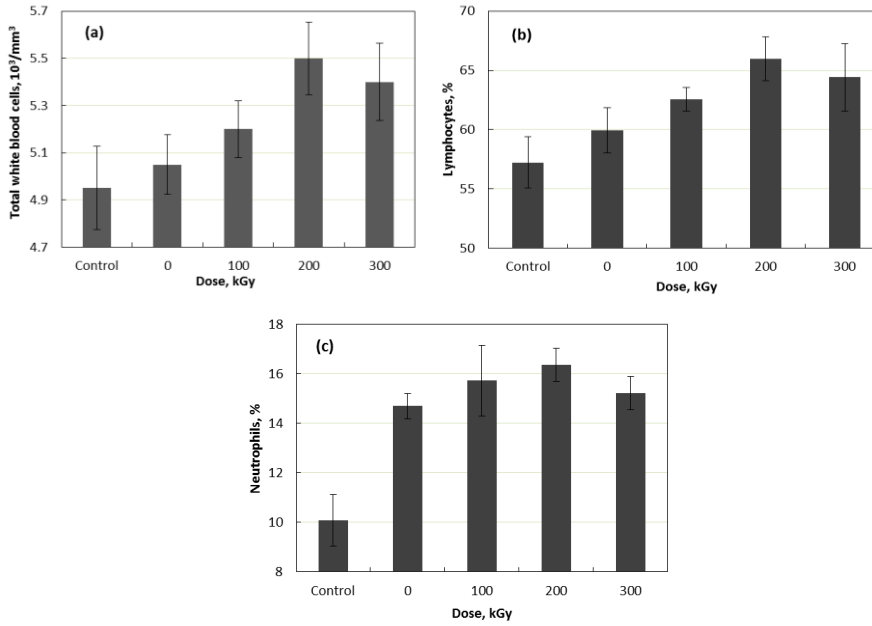

Fig. 3. The cellular immunity indexes in blood of mice after 28 days orally suppling by β-glucan irradiated at various doses (control: without supplementation with β-glucan); (a): WBCs, (b): Lymphocytes, (c): Neutrophils

In present study, the total white blood cells and the ratio of neutrophils and lymphocytes in blood of tested mice were analyzed after 28 days supplying with irradiated β-glucan. The results in Fig. 3a showed that the total WBCs in blood of mice supplied with β-glucan irradiated from 100 to 300 kGy are more than those of the control mice and of mice supplied with unirradiated β-glucan. The highest value of WBCs (5.5×10^3 cells/ml) was determined in the blood of mice supplied by 200-kGy-irradiated β-glucan. On the other hand, the rates of neutrophils in blood of mice supplemented with β-glucan irradiated at 200 (65.96%) and 300 kGy (64.42%) were higher than those in blood of control mice and mice supplied with unirradiated β-glucan or 100kGy-irradiated β-glucan (see Fig. 3b). This is very interesting result because lymphocytes play very important role for preventing the development of cancer and killing the tumor cells. Futhermore, β-glucans have been reported to drive neutrophil to accumulate during pathogenic, fungi-induced lung inflammation [24]. In our study, the results from Fig. 3c also displayed an increase of neutrophil count in blood of mice orally drenched with both unirradiated and irradiated β-glucan (14.70 - 16.37%) compared to that of control one (10.10%). Even so, the differentiations of neutrophil counts in blood of mice orally drenched β-glucan samples irradiated at 100 - 300 kGy were not significant.

The main function of immunoglobulins is to react against antigens in order to mediate their elimination. There are several types of immunoglobulins but immunoglobulin G (IgG) and M (IgM) play important roles for component activation (the complement system is very important in the defense mechanisms of the innate immunity and as a collaborator of some of the cytotoxic reactions mediated by antibodies) and neutralization (neutralization process allows antibodies to prevent viruses from infecting cells, due to the fact that antibodies coat the virus fragment needed for it to bind to the cell). In our results, the contents

of IgG and IgM (indicated by the value of OD at 405 nm in ELISA method) in serum of mice supplied by irradiated *β*-glucan was investigated to evaluate the effect of this material on humonal immune system of mice. The results in Fig. 4 showed that the values of OD_{405} of serums separated from mice suppied with 200kGy- and 300kGy-irradiated *β*-glucan were higher than that from control mice and mice supplied with 0kGy- and 100kGy-irradiated *β*-glucan.

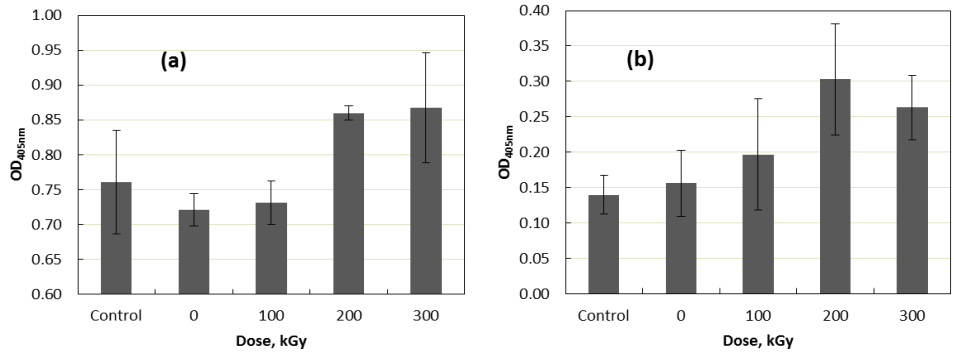

Fig 4. The enhancement of the contents of IgG and IgM in mice blood after supplementing 28 days of irradiated-*β*-glucan

IV. CONCLUSIONS

Gamma irradiation is a useful tool for degradation of β-glucan to induce water-soluble oligo*β*-glucan. The radiation degraded *β*-glucan stimulated innate immune system of mice including cellular indexes (white blood cell, neutrophils and lymphocytes counts) and humoral immunity indexes (IgA and IgM). The water-soluble oligo*β*-glucan with Mw ~ 24.9 kDa was found to have the highest immune stimulation effect and displayed as a very promising product for preparation of functional foods aiming at cancer prevention.

REFERENCES

[1]. A.T. Borchers, J.S. Stern, R.M. Hackman, C.L. Keen, M.E. Gershwin, “Mushrooms, tumors, and immunity”, Proc. Soc. Exp. Biol. Med., 221, 281-293, 1999.

[2]. G.D. Brow and S. Gordon, “Fungal beta-glucans and mammalian immunity”, Immunity, 19, 311-315, 2003.

[3]. S.M. Mantovani, F.M. Bellini, F.J. Angeli, R. Oliveira, F.A. Silva, R.L. Ribeiro, “*β*-Glucans in promoting health: Prevention against mutation and cancer”, Mut. Res., 658 (3): 154-161, 2008.

[4]. P. Methacanon, U. Weerawatsophon, P. Tanjak, P. Rachtawee, W. Prathumpai, “Interleukin-8 stimulating activity of low molecular weight *β*-glucan depolymerized by γ-irradiation”, Carbohydr. Polym., 86, 574-580, 2011.

[5]. N.N. Miura, Y. Adachi, T. Yadomae, Tamura, S. Tanaka, N. Ohno, “Structure and biological activities of b-glucans from yeast and mycelial forms of Candida albicans”, Microbiol. Immunol., 47 (3): 173-182, 2003.

[6]. D.B. Zekovic, S. Kwiatkowski, M.M. Vrvic, D. Jakovljevic, C.A. Moran, “Natural modifiel (1→3)-β-glucan in health promotion and disease alleviation”, Critical Rev. Biotechnol., 25, 205-230, 2005.

[7]. H.H. Sung, G.H. Kou, Y.L. Song, “Vibriosis resistance induced by glucan treatment in tiger shrimp (Penaeus monodon)”, Fish Pathol., 29, 11-17, 1994.

[8]. M. Suphantharika, P. Khunrae, P. Thanardkit, C. Verduyn, “Preparation of spent brewe’s yeast β-glucans with a potential application as an

immunostimulant for black tiger shrimp, Penaeus monodon", Bioresour. Technol., 88, 55-60, 2003.

[9]. J.B. Jorgensen, B. Robertsen, "Yeast b-glucan stimulates respiratory burst activity of Atlantic salmon (*Salmo salar* L.) macrophages", Dev. Comp. Immunol., 19, 43-57, 1995.

[10]. B. Robertsen, G. Rorstad, R. Engstad, J. Raa, "Enhancement of non-specific disease resistance in Atlantic salmon *Salmo salar* L., by a glucan from Saccharomyces verevisiae cell walls", J. Fish Diseas., 13, 391-400, 1990.

[11]. E.H. Byun, J.H. Kim, N.Y. Sung, J.I. Choi, S.T. Lim, K.H. Kim, H.S. Yook, M.W Byun, J.W. Lee, "Effects of gamma irradiation on the physical and structural properties of β-glucan", Rad. Phys. Chem., 77, 781-786, 2008.

[12]. F.A Moura, J.M. Pereira, D.O. Silva, E.R Zavareze, A.S Moreira, E Helbig, A.R.G. Dias, "Effects of oxidative treatment on the physicochemical, rheological and functional properties of oat β-glucan", Food Chem., 128, 982-987, 2011.

[13]. J.P. Roubroeks, R. Andersson., D.I. Mastromauro, B.E Christensenn Åman, "Molecular weight, structure and shape of oat (1-3),(1-4)-β-D-glucan fraction obtained by enzymatic degradation with (1-4)B-D-glucan-4-glucanohydrolase from *Tricoderma reesei*", Carbohydr. Polym., 46, 275-285, 2001.

[14]. A. Charlesby, "Crosslinking and degradation of polymers", Rad. Phys. Chem., 18, 59-66, 1981.

[15]. L.Q. Luan, N.H.P. Uyen, "Study on the application of irradiated β-glucan as a growth promoter for breeding", Vie. J. Sci. Technol., 22: 56-60, 2013.

[16]. L.Q. Luan, N.H.P. Uyen, "Radiation degradation of (1 → 3)-β-D-glucan from yeast with a potential application as a plant growth promoter", Inter. J. Biol. Macromol., 69, 175-180, 2014.

[17]. J. Sandula, G. Kogan, M. Kacurakova, E. Machova, "Microbial (1-3)- β-D-glucans, their preparation, physico-chemical characterization and immunomodulatory activity", Carbohydr. Polym., 3, 247-253, 1999.

[18]. H.J. Shin, D.C. Lee, "Study on the process to decrease the molecular weight of beta-(1,6)-branched beta-(1,3)-D-glucans" Kor. J. Biotechnol. Bioeng., 18, 352-355, 2003.

[19]. M.F. Moradali, H. Mostafavi, S. Ghods, G.A. Hedjaroude, "Immunomodulation and anticancer agents in the realm of macromycetes fungi (macrofungi)" Int. Immunopharmacol., 7, 701-724, 2007.

[20]. L.Q. Luan, N. Nagasawa, V.T.T. Ha, T.M. Nakanishi, "A Study of degradation mechanism of alginate by gamma-radiation", RadioIsotopes, 58, 3-12, 2009.

[21]. L. Q. Luan, N. Nagasawa, V.T.T. Ha, T. Kume, F. Yoshii, T. M. Nakanishi, "Biological effect of irradiated chitosan plant on plant *in vitro*", Biotechnol. Appl. Biochem., 41, 49-57, 2005.

[22]. E.G. Ershos, "Radiation-chemical degradation of cellulose and other polysaccharides", Russ. Chem. Rev., 67, 315-334, 1998.

[23]. M. Novák, A. Synytsya, O. Gedeon, P. Slepicka, V. Procházka, A. Synytsya, J. Blahovec, A. Hejlová, J. Copíková, "Yeast β-(1-3),(1-6)-D-glucan films: Preparation and characterization of some structural and physical properties", Carbohydr. Polym., 87, 2496-580, 2012.

[24]. R. Vassallo, J.E. Standing, A.H. Limper, "Isolated *Pneumocystis carinii* cell wall glucan provokes lower respiratory tract inflammatory responses", J. Immunol., 164, 3755-3763, 2000.