Scientific Notes of Taurida V.I. Vernadsky National University
Series «Biology, chemistry». Vol. 26 (65). 2013. No 1. P. 146-152.

UDK 633/635:58

THE INVESTIGATION OF INFLUENCE OF NANOBIOSILVER ON WHEAT GERMINATION AND SILVER ACCUMULATION IN GRAINS

Omel'chenko A.V.[1], Yurkova I.N.[1], Bugara I.A.[1], Kotov S.F.[1], Pipiya O.M.[2]

[1]Taurida National V.I. Vernadsky University, Simferopol, Ukrain
[2]«Ecohleb-plus Ltd», Simferopol, Ukraine
E-mail: nanosilver@rambler.ru

The influence of various concentrations of nanosilver in water-soluble composition based on sodium alginate on wheat germination and silver accumulation in grains was investigated. It was shown that the concentrations of nanobiosilver up to 25,0 mg/L did not have any inhibitory effect on the processes of swelling and germination of seeds. The optimal range of the concentrations of nanobiosilver at which silver content in wheat seedlings is from 50,0 to 100,0 mg/kg dry matter was determined.
Keywords: nanobiosilver, the accumulation of silver, grains of wheat, germination.

INTRODUCTION

In recent acquired widespread views, eating of sprouted seeds of various plants that have useful properties, particularly the germinated seeds of wheat, are important in the provision of the human body by valuable nutrients [1]. Products from sprouted grains contain enough cellulose, carbohydrates, vitamins B group, vitamin E, minerals including iron, zinc and selenium, antioxidants, plant estrogens and other useful elements which are necessary for the body to maintain health. With regular use of bread on the base of sprouted wheat grains the body is cleared from toxins, carcinogens and toxic substances; metabolism is normalized, bowel motility is improved, cholesterol level in the blood and the risk of cardio-vascular system are reduced [1-5].

However, most of the valuable components of sprouted grains are rich nutrient environment for the development of many microorganisms, including mold fungi. Besides that most of the technological production lines of cereal bread have an open system. This leads to the secondary infection of bread that significantly affects the quality of products.

Known methods of soaking seeds before dispersing in different materials of antimicrobial activity have not always given a positive effect [6]. Mold spores are resistant to external influences and can quickly adapt to such substances in contrast to the natural antiseptic - silver, with a strong antibacterial, antifungal, antiviral action. In the literature, there are examples of successful use of «silver water» in baking of bread [7]. Unlike ionic silver, nanosilver has prolonged effect, which does not require high doses for achievement the desired fungicidal and bactericidal effect [8].

Water enriched by silver ions which are released from the surface of the nanoparticles penetrates during swelling of grain through its testa, filling the grain mass by silver ions, especially the exolayers of the grain. The presence of silver in food products allows to

destroy many pathogen bacteria and mold fungi. In this case, the silver concentration 0.05-0.1 mg/L is not harmful to human organism. At the same time it is known therapeutic and prophylactic effect of low silver doses that daily consumed by humans [9-12].

The purpose of this research was to study the effect of nanobiosilver on wheat germination and accumulation of silver in grains for decontamination of pathogens and prolongation the durability of products obtained from cereal seedlings.

MATERIALS AND METHODS

The objects of the research were wheat grain *Triticum aestivum* L. and water-soluble bactericidal nanobiocomposition of silver. For the synthesis of silver nanobiocomposition it was used silver nitrate («analytical grade») and sodium alginate (alginic acid sodium salt brown algae, BioChemika). All solutions were prepared by bidistilled water. Photoreduction of Ag^+ cations were performed in air environment at temperature 20 °C in 100 ml flasks made from Pyrex glass. The high-pressure mercury lamp DRSh-250 was used as light source [13, 14].

Experiments were carried out according to the description of the technological process of cereal bread production from sprouted wheat grain at the laboratory and at the enterprise «Ecohleb plus Ltd».

The wheat grains were pre-washed and for each option 300 g of them were placed into the cuvette and were poured with 600 ml of tap water (control) and nanobiosilver solutions with silver concentration 0,05, 1,0, 2,0, 3,0, 4,0, 5,0, 10,0, 15,0, 20,0 and 25,0 mg/L. The wheat grains were covered by the solution layer 1-2 cm. Swelling process was carried out during 24 hours at water temperature 22 ^oC. Then the solution was discarded and the swollen grains germinated during 10 hours at the same temperature. During the process of germination every 2 hours 100 seeds were collected and the number of hatched were counted. At the end of the germination process, the grains were dried in an oven at 105 °C for 10 hours and then 50 g of dry grains from each option were ground in the homogenizer.

The silver content in wheat biomass was determined by atomic absorption spectroscopy (Saturn-4 EPAV). For silver content determination 10 g of ground biomass were taken. Determination of silver was carried out after burning to ashes for 10 hours in the muffle furnace at temperature 400-450 °C. Further analysis was performed with electrothermal atomization.

An enlarged experiment was carried out in the enterprise «Ecohleb plus Ltd» in conditions of real manufacturing process with control option (without silver), soaking the grain in solution of nanobiosilver with concentrations 0,2 and 0,3 mg/L in three replicates. For each option 5 kg of wheat grains were taken.

For determination of cereal bread contamination by mold spores, cereal products were packaged in plastic bags, placed in the incubator at 25 °C for 3 days according to State standard [15].

The obtained data were processed by standard methods of mathematic statistic with using of computer program Microsoft® Excel 2007 and Statistica v.6.0. Stat Soft Inc.

RESULTS AND DISCUSSION

Grain germination process involves two steps: swelling (soaking in water) and germination (sprouting).

The swelling process is necessary for the enzymes activation and creation the conditions for biochemical reactions since dry seeds contain only bound water [16]. Therefore, considering the importance of swelling process, the initial aim was to study the effect of different concentrations of nanobiosilver on this process (fig. 1).

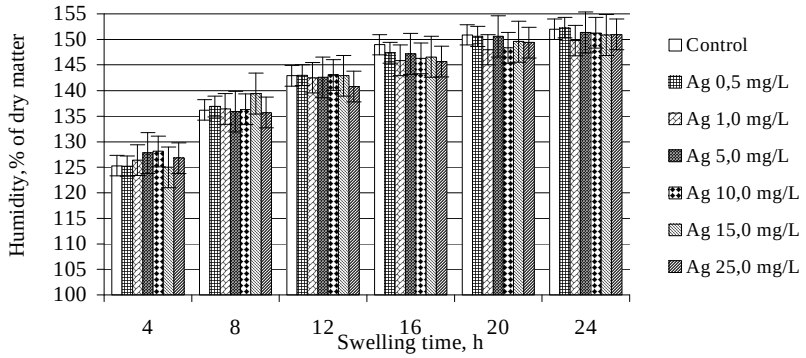

Fig. 1. The influence of different concentrations of nanobiosilver on grain wheat swelling

Significant increase of seed moisture was observed till 16 hours in all variants of the experiment (fig. 1), that corresponds to the maximum enzymatic activity [17]. Thus, nanobiosilver at concentration 0,5-25,0 mg/L did not inhibit the process of seeds swelling.

The results of investigation showed that high concentrations of nanobiosilver have not inhibitory effects on the process of seeds germination, which is important for the technology of cereal bread manufacture (table, fig. 2).

Table

Influence of different concentrations of nanobiosilver on germination of wheat grains

Variant	Germinated grains,%				
	2 h	4 h	6 h	8 h	10 h
Control (without Ag)	12,1±0,3	56,0±2,0	60,2±1,8	80,4±3,2	90,3±4,1
0,5 mg/L Ag	10,3±0,2	52,3±1,8	63,4±2,3	84,3±2,7	91,1±3,8
1,0 mg/L Ag	14,5±0,4	53,6±1,6	62,8±1,9	83,6±2,2	93,4±4,5
5,0 mg/L Ag	12,7±0,3	54,4±2,4	64,5±2,1	85,0±3,5	92,4±3,3
10,0 mg/L Ag	15,2±0,5	51,2±1,9	65,2±2,5	82,5±2,9	94,8±3,1
15,0 mg/L Ag	14,4±0,2	55,1±2,2	62,0±1,8	86,2±4,1	95,3±4,2
25,0 mg/L Ag	15,2±0,5	53,7±1,7	61,3±2,0	83,7±2,4	92,0±3,6

As seen from the results, nanobiosilver in wide concentration range (0,5- 25,0 mg/l) had no inhibitory effect on the processes of swelling and sprouting of wheat grains which used for production of cereal bread.

Fig. 2. Appearance of sprouted wheat grains (10 hour exposure).

Silver content in drinking water is regulated according to SanPiN 2.1.4.1074-01. «Drinking Water». In these regulations the silver content in nitrate form in drinking water is limited by concentration 0,05 mg/L [18].

According to the literature, harmless and therapeutic-prophylactic concentration of silver ions in water is 0,05-0,1 mg/L [7]. Therefore, the main aim of the research was to determine the concentration of nanobiosilver in the solutions for swelling grain in which the sorption of silver did not exceed 0,1 mg/kg in the established product.

The investigation of silver content in the grains after soaking in the solutions of nanobiosilver, were conducted in two stages. At the first stage, the concentrations of nanobiosilver were 5,0-25,0 mg/L. The obtained data showed that even at the lowest concentration of nanobiosilver 5,0 mg/L, high silver content (1,03 mg/kg) was observed in the biomass (fig. 3a).

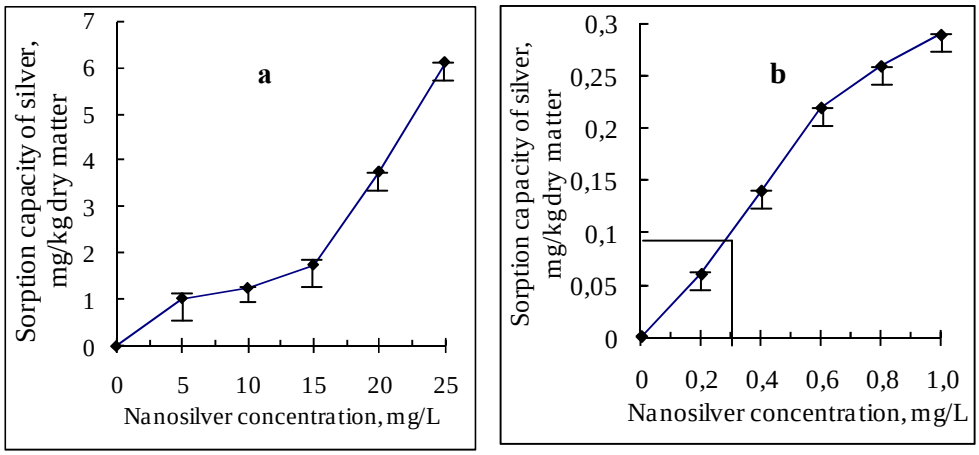

Fig. 3. Sorption capacity of sprouted wheat seeds in dependence from nanobiosilver concentration: a) 0,2-1,0 mg/L (silver concentration), b) 5,0-25,0 mg/L (silver concentration).

Therefore, on the next stage of research nanobiosilver concentration was reduced till 0,2-1,0 mg/L in the solution of soaked seeds. In this case, the accumulation of silver was

0,06-0,3 mg/kg of dry biomass (fig. 3b). According to the results of silver sorption in biomass of sprouted seeds it was shown that for achievement the silver concentration of 0,1 mg/kg, grains it is necessary to soak them in the nanobiosilver solution at the concentration 0,3 mg/L.

In conditions of soaking seeds in nanobiosilver solution with the concentration 0.3 mg/L in the close-up experiment on the plastic bag packaged cereal product in a thermostate at 25 °C, the formation of mold fungi colonies was observed on the 5th day whereas in the control (without addition nanobiosilver) – after 3 days.

Alternatively, one more product with antimicrobial properties was obtained by spraying the cereal product with nanobiosilver at the concentration less than 0,1 mg/kg: control (without silver), 2 ml of the nanobiosilver solution with the concentration 4,0 mg/L (corresponding to 50,0 mg/kg) and 2 ml of the concentration 8,0 mg/L (equivalent to 100,0 mg/kg) (fig. 4).

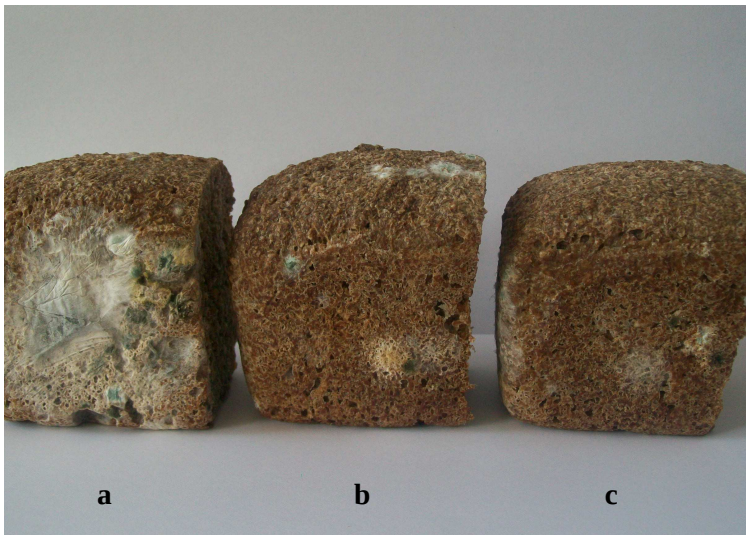

Fig. 4. The surface of cereal bread after treatment with nanobiosilver spray (5 days at 25 ^{o}C): a) control (without Ag); b) 50,0 mg/kg Ag^{o}; c) 100,0 mg/kg Ag^{o}.

Thus, the possibility of obtaining the bread from sprouted grains which possessed antimicrobial properties after treatment with nanobiosilver spray was investigated.

CONCLUSION

1. For the first time the influence of water-soluble composition of nanobiosilver on the germination of wheat was investigated. It was shown that high concentrations of nanobiosilver (up to 25,0 mg/L) did not have inhibitory effect on the processes of swelling and sprouting of seeds.
2. The optimal range of the initial concentrations of nanobiosilver in which the silver content in wheat seedlings ranged from 0,05 to 0,1 mg/kg of dry matter was detected.

3. The possibility of obtaining the cereal product with antimicrobial properties by treating its surface with nanobiosilver spray was investigated.

ACKNOWLEDGEMENTS

The authors thank members of «Ecohleb plus Ltd» for their help in carrying out the experiments.

References

1. Чубенко Н.Т. Применение зерна в хлебопечении / Н.Т. Губенко // Хлебопечение России. – 2004. – № 6. – С. 20.
2. Шаскольский В. Проростки – источник здоровья / В. Шаскольский, Н. Шаскольская // Хлебопродукты. 2005. – № 4. – С. 56-57.
3. Козубаева Л.А. Хранение и переработка сельхозсырья / Л.А. Козубаева, С.С. Кузьмина. – 2005. – № 5. – С. 49-50.
4. Лобачев Е.М. Как приготовить хлеб из пророщенной пшеницы / Е.М. Лобачев // Зерновое хозяйство. – 2003. – № 5. – С. 28-32.
5. Эльберт Г.К. Животворное зерно и хлеб / Г.К. Эльберт, Л.Г. Колесова // Хлебопродукты. – 1993. – № 7. – С. 34-35.
6. Горячева А.Ф. Сохранение свежести хлеба / А.Ф. Горячева, Р.В. Кузьминский. – М. : Легкая и пищевая промышленность, 1983. – 239 с.
7. Пат. 2058080 Россия, МКИ6 A 21 D 13/02. Способ производства зернового хлеба / Коротков Ю.А., Коваль И.В., Коваль Д.И. ; заявитель и патентообладатель Коротков Ю.А., Коваль И.В., Коваль Д.И. – № 930187313 ; заявл. 12.04.93; опубл. 20.04.96.
8. Бактерицидные и каталитические свойства стабильных металлических наночастиц в обратных мицеллах / Е.М. Егорова, А.А. Ревина, Т.Н. Ростовщикова [и др.] // Вестн. Моск. ун-та. Сер.2. Химия. – 2001. – Т. 42, № 5. – С. 332-337.
9. Кульский Л.А. Серебряная вода / Л.А. Кульский. – Киев : Наукова думка, 1987. – 138 с.
10. Furr J.R. Andrews A Antibacterial activity of Actisorb Plus, Actisorb and silver nitrate / J.R. Furr, A.D. Russel, T.D. Turner // J. Hosp Infect. – 1994. – Vol. 27, No 3. – P. 201-208.
11. Thomas S. A comparison of the antimicrobial effects of four silver-containing dressings on three organisms / S. Thomas , P. McCubbin // J Wound Care. – 2003. – Vol. 12, No 3. – P. 101-107.
12. Rasmussen S.B. Silver nanoparticles as a new generation of antimicrobials / S.B. Rasmussen, L.S. Reinert, S.R. Paludan // Biotechnol Adv. – 2009. – Vol. 27, No 1. – P. 76-83.
13. Пат. 10539 Украина, МКИ7 A 61 K 33/38, A 61 K 31/715. Способ получения водорастворимой бактерицидной композиции, содержащей наночастицы серебра / Юркова И.Н., Эстрела-Льопис В.Р., Рябушко В.И., Рябушко Л.И. ; заявник та власник патенту Таврійський національний університет імені В.І. Вернадського. – № u2001128682 ; заявл. 13.05.05 ; опубл. 15.11.05. Бюл. № 11.
14. Пат. 46366 Украина, МПК (2009) A 61 K 33/38, A 61 K 31/726. Спосіб отримання водорозчинної бактерицидної композиції, що містить наночастинки срібла / Юркова І.М., Рябушко В.І., Пархоменко Н.А.; заявник та власник патенту Таврійський національний університет імені В.І. Вернадського. – № u200901632 ; заявл. 12.05.09; опубл. 25.12.09. Бюл. № 24.
15. ГН 4.4.2.094-2002. Тимчасові гігієнічні нормативи вмісту мікроорганізмів в хлібі та хлібобулочних виробах. – Введ. 25.07.2002.
16. Обручева Н.В. Физиология инициации прорастания семян / Н.В. Обручева, О.В. Антипина // Физиология растений. – 1997. – Т. 44, № 2. – С. 287-302.
17. Рогожин В.В. Физиолого-биохимические механизмы прорастания зерновок пшеницы / В.В. Рогожин, Т.В. Рогожина // Вестник Алтайского аграрного университета. – 2011. – № 8 (82). – С. 17-21.
18. Питьевая вода. Гигиенические требования к качеству воды централизованных систем питьевого водоснабжения. Контроль качества. СанПиН 2.1.4.1074-01.

Омельченко А.В. Исследование влияния нанобиосеребра на прорастание зерна пшеницы и накопление в нем серебра / А.В. Омельченко, И.Н. Юркова, И.А. Бугара, С.Ф. Котов, О.М. Пипия // Ученые записки Таврического национального университета им. В.И. Вернадского. Серия «Биология, химия». – 2013. – Т. 26 (65), № 1. – С.146-152.

Исследованы различные концентрации наносеребра в водорастворимой композиции на основе альгината натрия на прорастание зерна пшеницы и накопление в нем серебра. Показано, что концентрации нанобиосеребра до 25,0 мг/л не оказывали ингибирующего действия на процессы набухания и проклевывания семян. Определен оптимальный диапазон концентраций нанобиосеребра, при которых содержание серебра в проростках пшеницы составляло от 50,0 до 100,0 мкг/кг сухого вещества.

Ключевые слова: нанобиосеребро, накопление серебра, зерно пшеницы, прорастание.

Омельченко О.В. Дослідження впливу нанобіосрібла на проростання зерна пшениці та накопичення в ньому срібла / О.В. Омельченко, І.М. Юркова, І.О. Бугара, С.Ф. Котов, О.М. Піпія // Вчені записки Таврійського національного університету ім. В.І. Вернадського. Серія „Біологія, хімія". – 2013. – Т. 26 (65), № 1. – С. 146-152.

Досліджено різні концентрації наносрібла у водорозчинній композиції на основі альгінату натрію на пророщування зерна пшениці та накопичення в ньому срібла. Показано, що концентрації нанобіосрібла до 25,0 мг/л не чинили інгібуючої дії на процеси набухання і прокльовування насіння. Визначено оптимальний діапазон вихідних концентрацій нанобіосрібла, при яких вміст срібла в проростках пшениці становило від 50,0 до 100,0 мкг/кг сухої речовини.

Ключові слова: нанобіосрібло, накопичення срібла, зерно пшениці, проростання.

Поступила в редакцию 25.02.2013 г.