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# THE EFFECT OF HYDROGEN PEROXIDE, OZONISED WATER AND *NATURESEAL*® AS5 SOLUTION ON THE MICROBIOLOGICAL PARAMETERS OF FRESH-CUT CARROT

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**Abstract**

Innovative (ozonised water, hydrogen peroxide) treatment solutions is one of the newest treatment agents used to decrease the count of microorganisms in vegetables. *NatureSeal*® AS5 is a commercial solution used for the treatment of vegetables worldwide. The aim of this work was to study the effect of the various treatment solutions – hydrogen peroxide, ozonised water and *NatureSeal*® AS5 (in various concentrations and for different treatment time) with the main purpose to ensure the microbiological safety of fresh–cut carrots. Non–treated carrots were used as control sample. The following treatment regimes were investigated: ozonised water treatment time 60; 120; 180±1 s and concentrations 1.0; 2.0; 3.0 mg $L^{-1}$; hydrogen peroxide solutions, treatment time 30±1 s, 60±1 s 90±1 s and concentrations 0.5%; 1.0% 1.5%; *NatureSeal*® AS5 water solution 2.5% 5 min ± 1 s were prepared for treatment of shredded carrots. *E.coli* in fresh carrots was not detected. Mathematical processing of the obtained results revealed that various concentrations of hydrogen peroxide ($p=0.019$; $\alpha=0.05$) and treatment times ($p=0.049$; $\alpha=0.05$) significantly influenced the TPC colony forming units in carrots. The obtained results prove the treatment agent properties of hydrogen peroxide. The comparison of chosen treatment regimes detected a significant ($p=0.003$; $\alpha=0.05$) influence of the ozonised water on the decrease in the count of TPC colony forming units in carrots after treatment. During experiments it was proved that *NatureSeal*® AS5 solution could be recommended as treatment agent for the treatment of fresh shredded carrots.

***Keywords***: hydrogen peroxide, ozone, *NatureSeal*® AS5, microbial contamination, carrot

## Introduction

Carrot is one of the important root vegetables rich in bioactive compounds like carotenoids and dietary fibers with appreciable levels of several other functional components having significant health–promoting properties. The consumption of carrot and its products is increasing steadily due to its recognition as an important source of natural antioxidants having anticancer activity (Sharma et al., 2012).

Minimally processed vegetables (MPV) are prepared and handled to maintain their fresh nature while providing convenience to the consumer, as ready-to-eat. Producing MPV involves cleaning, trimming, peeling, coring, slicing, shredding, washing, etc. (Augspole et al., 2014; Ayhan et al., 2008). Fresh–cut carrots can be found in the market place as: whole peeled (baby), sticks, or sliced, shredded, grated and diced. There is a shelf-life limitation for minimally processed carrots from 4 to 5 days due to high respiration rate, development of off–flavour, acidification, and loss of firmness, discolouration, and microbial spoilage (Augspole, Rakcejeva, 2013). The only step for reducing microorganisms during processing is washing. A variety of disinfectants (hydrogen peroxide and ozone) have been used to reduce bacterial populations on fruit and vegetables (EU Scientific Committee on Food, 2002). *Alegree et al*. (2013) reported that these treatment agents have proved incapable of completely removing or inactivating microorganisms on fresh produce.

One of the new approaches is the use of "generally recognized as safe" (GRAS) compounds due to minimal concerns about their environmental impact and low residues in the treated commodity (Loredo et al., 2013). The US Food and Drug Administration (FDA, 2011), under the ruling 21 C.F.R. 173.315, has approved the use of hydrogen peroxide as plant protection agent in the processing of fresh fruits and vegetables (Rodrigues et al., 2012). Hydrogen peroxide is a strong oxidizing agent proposed as an alternative for decontamination of fruits and vegetables due to its low toxicity and safe decomposition products (Alexandre et al., 2012; Loredo et al., 2013). Hydrogen peroxide has been shown to inactivate a wide variety of infective biological agents ranging from the vegetative cells and spores of bacteria and fungi, protozoa and their cysts, viruses and even prions (Malik et al., 2012; Delgado et al., 2012; Loredo et al., 2013).

The US Food and Drug Administration (FDA, 2011), under the ruling 21 C.F.R. 173.315, has approved the use of ozone as plant protection agents in the processing of fresh fruits and vegetables. The fresh produce industry is showing interest in $O_3$ applications due to growing consumer preference for minimally processed foods, frequent outbreaks of food–related illnesses, identification of new food pathogens, and the passage of legislation governing food quality and safety (Rodrigues et al., 2012; O'Donnell 2012). Within the food industry, ozone has been used routinely for washing and storage of fruits and vegetables. Ozone can be bubbled through water into which it will partially dissolve. This ozonised water then can be used for washing and/or in transfer flumes to reduce the microbial loads of berries and other fruits and vegetables. Controlled studies report that ozonised water may actually provide greater than 90% reduction of total bacterial counts for some vegetables. Such

treatments also have been shown to reduce fungi populations and, subsequently, reduce fungal decay (Farid, 2010). Ozone is a naturally occurring substance found in our atmosphere and it can also be produced synthetically. The characteristic fresh, clean smell of air following a thunderstorm represents freshly generated ozone in nature (Farid, 2010). Therefore, $O_3$ technology is a good option for the wash–water disinfection for the fresh-cut industry, because it will reduce the need for water replacement and for high sanitizer concentration.

A commercially available anti-browning agent *NatureSeal*™ is a calcium ascorbate powder used extensively in the fresh cut industry. Ascorbic acid functions as reducing agent to deter surface browning and $CaCl_2$ treatment provides tissue firming and has been reported to reduce browning (Saha et al., 2009; Rößle et al., 2009). This is the first commercial antioxidant product of its kind that doesn't have a bad aftertaste or residue (Asrey et al., 2008). Initially developed for apples and pears, the technology has been expanded for use on 19 different produce items including fresh-cut carrots (Agricoat, 2010). Using *NatureSeal*™ preparations carrot processing can adjust the pH of the product − reducing, hindering the development of microflora. *NatureSeal*® is sold commercially to food retailers who use the coating to treat fresh–cut fruits and vegetables. It uses a special blend of vitamin salts and minerals to extend the shelf life of sliced fruits for up to 21 days under refrigeration, without detectable changes in colour, flavour or texture (Asrey et al., 2008).

There are several processing steps in the fresh–cut produce production chain and many points for potential microbial contamination exist in each of these steps. The objective of the current research was to evaluate the effect of the various treatment solutions – hydrogen peroxide, ozonised water and *NatureSeal*® AS5 (in different concentrations and different treatment times) with the main purpose to ensure the microbiological safety of fresh–cut carrots.

## Materials and Methods

### *Materials*

Experiments were carried out in Department of Food Technology at the Latvia University of Agriculture. The research was accomplished on 'Nante' type 'Forto' variety carrots (*Daucus carota* L.) grown in Latvia and harvested in Zemgale region in the first part of October, 2015.

### *Preparation of shredded carrots*

Fresh, whole, non–damaged and washed (with drinking water) carrots were used for the research purpose. Carrots were peeled with a Baumann vegetable peeling knife made of non–corrosive steel and covered with a special coating – ceramic layer protecting the product from sticking. Peeled carrots were washed in running water and dried at temperature of +20±2 °C in air ambience for 3±1 min. Carrots were shredded using a kitchen combine (Philips Comfort HR 7605, Austria) with the power capacity 350 W. The volume of a shredded carrot chip was: cross–section 1.5±3.0 mm and length 35–50 mm.

### *Treatment with $H_2O_2$*

30%, 34.01 g $mol^{-1}$ hydrogen peroxide was used for experiments (Czech Republic). 0.5%; 1.0% and 1.5% hydrogen peroxide solutions in deionised water 0.055 μS $cm^{-1}$ at temperature of 20±2 °C were prepared for treatment of shredded carrots. Solution was prepared one minute before the treatment of shredded carrots to avert the decomposition of hydrogen peroxide. Shredded carrots were immersed in the hydrogen peroxide water for 30±1 s, 60±1 s and 90±1 s. After treatment carrots were placed on a non-corrosive steel sheave (grid diameter 0.3 mm) to draw off the excessive water (3±1 min). The influence of hydrogen peroxide on the carrots quality was analysed immediately after treatment and during storage.

### *Treatment with ozone*

Ozonised water was obtained in high concentration ozone and oxygen generator SOZ–YMS (BNPOZONE Company, China) equipped with the water pump, where the ozone was dissolved in water by means of the ejector. The maximum ozone concentration could be up to 12.0 mg $L^{-1}$ per one circulation time. The ozone concentration in water mg $L^{-1}$ was measured with a portative ozone detector DO3 (Eco Sensors Division of KWJ Engineering Inc., USA). The amount of ozone in the container was measured over the water surface. The measurement was done manually by taking of 10 mL ozonised water in the bottle and measuring it with the portative measurer DO3 (Eco Sensors Division of KWJ Engineering Inc., USA). The electrochemical T–Series sensor (3ET1PO3) with sensibility ±0.05% (0–0.05 mg $L^{-1}$) was used in the device; the maximum detected concentration was up to 5.0 mg $L^{-1.}$ After determination of ozone concentration (1.0 mg $L^{-1}$, 2.0 mg $L^{-1}$ and 3.0 mg $L^{-1}$), shredded carrots were immersed in the prepared solution (2.0 L) at temperature of 20±2 ºC for 60; 120 and 180±1 seconds. After treatment carrots were placed on a non-corrosive steel sheave (grid diameter 0.3 mm) to draw off the excessive water (3±1 min). The influence of ozonised water on the carrots quality was analysed immediately after treatment and during storage.

### *Treatment with NatureSeal®AS5 solution*

*NatureSeal*® AS5 solution – a blend of dry vitamin and mineral substances patented in the USA, a safe product with the active compounds of ascorbic acid and calcium. 2.5% *NatureSeal*® AS5 water solution was used for the treatment of carrots. 2.5% *NatureSeal*® AS5 water solution was prepared right before the use where shredded carrots were immersed at temperature of 20±2 °C for 5 min±1 s. After treatment carrots were placed on a non–corrosive steel sheave (grid diameter 0.3 mm) to draw off the excessive water (3±1 min). The influence of *NatureSeal*® AS5 on the carrots

quality was analysed immediately after treatment and during storage.

*Microbiological analysis*

Microbiological evaluation of carrot was performed according to the standard 'Microbiology of food and animal feeding stuffs' LVS EN ISO 7218:2007. All microbiological evaluations were conducted with threefold repetition. Plate counting method was used for microbial detection. Total plate count of mesophilic aerobic and facultative anaerobic microorganisms was investigated on Nutrition agar (dilutions 1:1000; 1:10000) in conformity standard method LVS EN ISO 4833:2003. Yeast plate count was investigated on Malt extract agar (dilutions 1:100; 1:1000) in conformity standard method ISO 21527–1:2008. *E.coli* (LVS ISO 7251). Counting of colonies formed and calculating the number of CFUs was accomplished by automatic colony counter Acolyte.

**Results and Discussion**

The influence of two alternative treatment methods – hydrogen peroxide ($H_2O_2$) and ozonised water on the quality parameters of fresh–cut carrots was analysed in the present research. The commercial *NatureSeal*® AS5 solution (control sample) was used to treated carrots for the comparison purposes.

Pre–treatment (washing, peeling, shredding) of carrots damages the carrots tissue and results the growth of microorganisms. Therefore, hydrogen peroxide ($H_2O_2$) was used as a treatment agent for the treatment of fresh–cut carrots. The use of hydrogen peroxide is recommended for treatment of vegetables thanks to its low toxicity and safe decomposition of $O_2$ and $H_2O$.

However, microbial safety is one of the most important factors to be considered for the preservation of minimally processed foods (Bico et al., 2009). *E.coli* in fresh carrots was not detected.

A significant ($p=0.09$; $\alpha=0.05$) difference in the dynamics of TPC between the control carrots sample and carrots treated with $H_2O_2$ was found after analysing the influence of $H_2O_2$ on the quality parameters of carrots using different concentrations of $H_2O_2$ and treatment times (Figure 1).

The TPC of control sample was 2.61 log cfu $g^{-1}$. Higher TPC (2.25 log cfu $g^{-1}$) was found in carrots treatment with the 0.5% $H_2O_2$ for 30±1 s (decrease by 13.97% in comparison with non–treated carrots sample); however, lower TPC (1.65 log cfu $g^{-1}$) was detected – after carrots treatment with the 1.5% $H_2O_2$ for 90±1 s (the TPC decrease by 36.78%). After evaluation of $H_2O_2$ treatment activity, it may be concluded that it is possible to decrease significantly (by 27.20%) TPC of carrots during treatment with the 1.0% $H_2O_2$ for 30±1 s. Mathematical processing of the obtained results revealed that various concentrations of $H_2O_2$ ($p=0.019$; $\alpha=0.05$) and treatment times ($p=0.049$; $\alpha=0.05$) significantly influenced the TPC count in carrots (Figure 1).

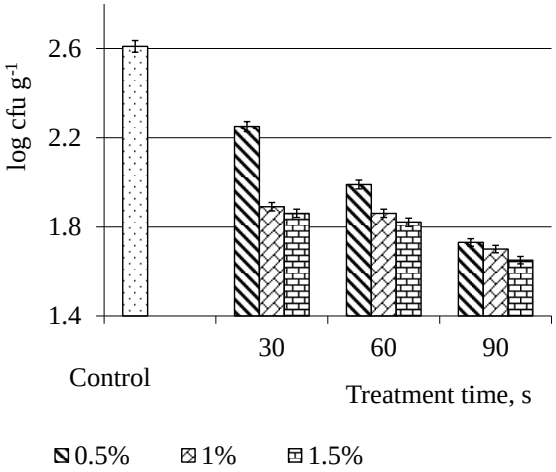


**Figure 1. Influence of $H_2O_2$ treatment regime parameters on the TPC units in carrots**

The obtained results prove the treatment agent properties of $H_2O_2$.

A significant ($p<0.05$) decrease in the yeast count was detected after treatment of fresh-cut carrots with the 1.0% and 1.5% $H_2O_2$ for 90±1 s – yeasts were not found after treatment (Figure 2).

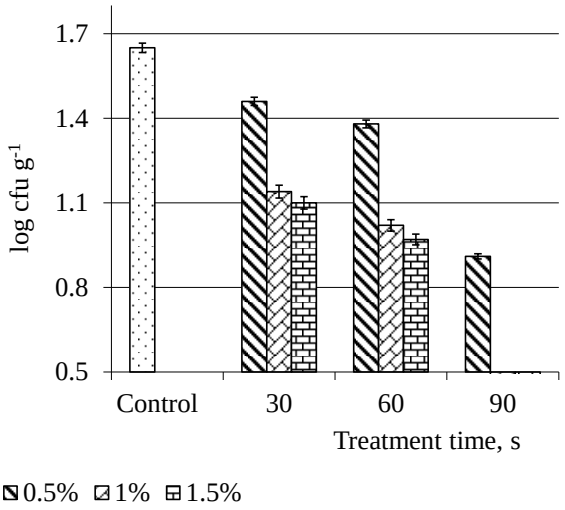


**Figure 2. Influence of $H_2O_2$ treatment regime parameters on the yeast units in carrots**

Yeasts (1.47 log cfu $g^{-1}$) were found in carrots treated with the 0.5% $H_2O_2$ for 30±1 s. Therefore, a longer (90 s) treatment time and higher concentration of treatment agent (1.0% and 1.5%) is more suitable for the carrots microbiological safety providing.

Ozone ($O_3$) is one of the newest treatment agents in the world used to decrease the count of microorganisms in vegetables. Ozone is safe and friendly (GRAS – Generally recognized as safe) in the food. Inhibition of the microorganisms' growth is the main advantage of ozone treatment; as a result fresh–cut carrots keep their freshness and quality for longer time.

Different treatment regimes as ozonised water concentration and treatment time were investigated to evaluate their influence on the quality of shredded

carrots. The following treatment regimes were investigated based on the data presented in scientific literature: treatment time – 60; 120; 180±1 s and concentrations 1.0; 2.0; 3.0 mg $L^{-1}$. The comparison of chosen treatment regimes detected a significant ($p=0.003$; $\alpha=0.05$) influence of the ozonised water on the decrease in the count of TPC in carrots after treatment (Figure 3).

An essential decrease in the TPC count from 2.34 to 1.13 log cfu $g^{-1}$ and 1.10 log cfu $g^{-1}$, i.e. – by 51.71% and 52.99% respectively – was obtained after carrots treatment with 2 mg $L^{-1}$ and 3 mg $L^{-1}$ of ozonised water for 180±1 s.

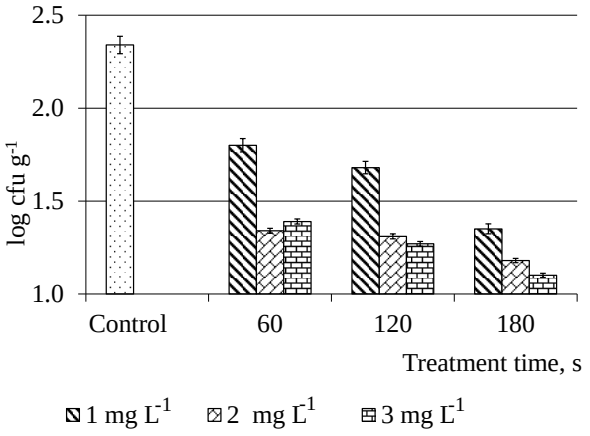


**Figure 3. The TPC units in carrots after treatment with the ozonised water**

Essential decreases (by 96.36%) in the yeast content (Figure 4) were obtained after treatment of carrots with 2 and 3 mg $L^{-1}$ of ozonised water for 180±1 s, yet not so–pronounced ones (by 50.63%) – after treatment of carrots with 1 mg $L^{-1}$ of ozonised water for 60±1 s.

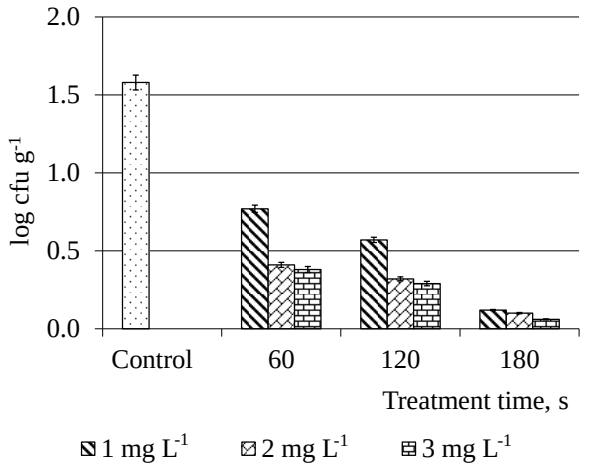


**Figure 4. The yeast units in carrots after treatment with the ozonised water**

Therefore, a longer (180 s) treatment time and a lower treatment agent concentration (2 mg $L^{-1}$ and 3 mg $L^{-1}$) are advisable to ensure the microbiological safety of carrots.

*NatureSeal*® AS5 is a commercial solution used for the treatment of vegetables worldwide. Ascorbic acid and calcium are the active components of *NatureSeal*® AS5. It is the first commercial product, which does not create a non–acceptable aftertaste and does not remain in the product. Therefore, it is possible to control the pH value (decrease) of a product by the treatment of products with the mentioned solution; as a result the growth of microorganisms could be prevented similarly as it is done using other treatment agents (Bhagwat et al., 2004). The recommended treatment time for the use of the commercial *NatureSeal*® AS5 solution (AgriCoat *NatureSeal* Ltd, England) is 5 min±1 s and preparation concentration in the water solution – 2.5%. The present experiments were done to verify the possible influence of *NatureSeal*® AS5 on the microbiological indicators of 'Nante' type 'Forto' variety carrots grown in Latvia and to compare this influence with alternative treatment methods ($H_2O_2$ and ozonised water) of carrots. The initial count of TPC in non–treated carrots was 2.62 log cfu $g^{-1}$, which decreased up to 1.28 log cfu $g^{-1}$ after treatment with *NatureSeal*® AS5 (Figure 5).

Comparing the results of the microbiological analyzes of shredded carrots after treatment with *NatureSeal*® AS5 preparations (Figure 5) and 120 ± 1 s to 2 mg $L^{-1}$ ozonated water (Figure 3), it found that treatment with *NatureSeal*® AS5 preparation gives similar TPC count decrease in carrots.

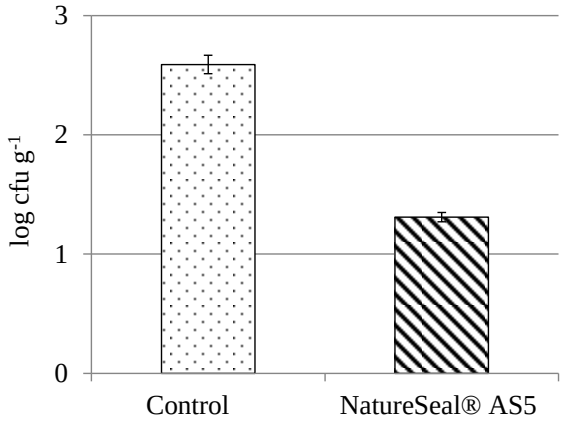


**Figure 5. The TPC units in carrots after treatment with the *NatureSeal*® AS5**

During experiments it was proved that *NatureSeal*® AS5 solution could be recommended as treatment agent for the treatment of fresh–cut carrots.

## Conclusions

The study confirmed that commercial *NatureSeal*® AS5 preparations and ozonised water may be recognized as an effective treatment agents to inactivate undesirable microflora in fresh–cut carrots. Therefore, ozonised water should be alternative for commercial *NatureSeal*® AS5 preparations. However, in the present research obtained results demonstrate hydrogen

peroxide low potential as disinfectant for fresh–cut carrots safety providing.

## References


1. Agricoat (2010) FreshBites NatureSeal. Spring 2010. England: AgriCoat NatureSeal Ltd, p. 1–4.
2. Alegre I., Viñas I., Usall J., Anguera M., Altisent R., Abadias M. (2013) Antagonistic effect of Pseudomonas graminis CPA–7 against foodborne pathogens in fresh–cut apples under simulated commercial conditions. *Food Microbiology*, Vol. 33, p. 139–148.
3. Alexandre E.M.C., Brandão T.R.S., Silva C.L.M. (2012) Assessment of the impact of hydrogen peroxide solutions on microbial loads and quality factors of red bell peppers, strawberries and watercress. *Food Control*, Vol. 27, p. 362–368.
4. Asrey R., Sasikala C., Barman K., Koley T.K. (2008) Advances in postharvest treatments of fruits – a review. *Annals of Horticulture*, Vol. 1 (1), p. 1–10.
5. Augspole I., Rakcejeva T. (2013) Effect of hydrogen peroxide on the quality parameters of shredded carrots. **In:** *Proceedings of Annual 19th International Scientific Conference Research for Rural Development*, Jelgava, Latvia, p. 91–97.
6. Augspole I., Rakcejeva T., Dukalska L., Kozlinskis E. (2013) Hydrogen peroxide effect on the quality of shredded carrots during storage. **In:** *FaBE 2013: International conference on Food and Biosystems Engineering*, Skiathos Island, Greece, p. 312–321.
7. Augspole I., Rakcejeva T., Dukalska L., Skudra L. (2014) Providing Quality of Shredded Carrots during Storage by Treatment with Ozonised Water. *Material Science and Applied Chemistry*. Vol. 30, p. 10–17.
8. Ayhan Z., Esturk O., Tas E. (2008) Effect of Modified Atmosphere Packaging on the Quality and Shelf Life of Minimally Processed Carrots. Turkish *Journal of Agriculture and Forestry*, Vol. 32, p. 57–64.
9. Bhagwat A.A., Saftner R.A., Abbott J.A. (2004) Evaluation of wash treatments for survival of foodborne pathogens and maintenance of quality characteristics of fresh-cut apple slices. *Food Microbiology*, 21, p. 319–326.
10. Bico L.S., Raposo M.F.J., Morais R.M.S.C., Morais A.M.M.B. (2009) Combined effects of chemical dip and/or carrageenan coating and/or controlled atmosphere on quality of fresh–cut banana. *Food Control*, Vol. 20, p. 508–514.
11. Delgado D.A., Sant'Ana A.S., Granato D., Massaguer P.R. (2012) Inactivation of Neosartorya fischeri and Paecilomyces variotii on paperboard packaging material by hydrogen peroxide and heat. *Food Control*, No. 23, p. 165–170.
12. EU Scientific Committee on Food (2002) Risk profile of the microbiological contamination of fruits and vegetables eaten raw. Available at:http://europa.eu.int/comm/food/fs/sc/scf/out123_en.pdfs (accessed 05.05.09.).
13. Farid M.M. (2010) Mathematical Modeling of Food Processing. Taylor and Francis Group, LLC, Boca Raton, p. 607–622.
14. Loredo A.B.G., Guerrero S.N., Alzamora S.M. (2013) Impact of combined ascorbic acid/CaCl2, hydrogen peroxide and ultraviolet light treatments on structure, rheological properties and texture of fresh–cut pear (William var.). *Journal of Food Engineering*, Vol. 114, p. 164–173.
15. Malik D.J., Shaw C.M., Rielly C.D., Shama G. (2013) The inactivation of Bacillus subtilis spores at low concentrations of hydrogen peroxide vapour. *Food Engineering*, Vol. 114, p. 391–396.
16. O'Donnell C., Tiwari B.K., Culen P.J., Rice R.G. (2012) Ozone in food processing. First Edition. John Wiley & Sons, Ltd, The Atrium, 308 p.
17. Rodrigues S., Fernandes F.A.N. (2012) Advances in Fruit Processing Technologies. Taylor & Francis Group, LLC; Boca Raton, 454 p.
18. Rößle C., Gormley T.R., Butler F. (2009) Efficacy of Natureseal® AS1 browning inhibitor in fresh–cut fruit salads applications, with emphasis on apple wedges. *Horticultural Science and Biotechnology*, Special Issue, p. 62–67.
19. Saha K., Paulsingh S., Harte B. (2009) Effect of Transport Vibration on Quality of Minimally Processed and Packaged Fresh–cut Cantaloupe. *Applied Packaging Research*, Vol. 3, No 1, p. 15–26.
20. Sharma K.D., Karki S., Thakur N.S., Attri S. (2012) Chemical composition, functional properties and processing of carrot a review. *Journal of Food Science and Technology*, Vol. 49 (1), p. 22–32.