Decontamination of Cut Carrot by Persteril® Agent Based on the Action of Peroxyacetic Acid

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Abstract

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The use of cleaned and cut fresh vegetables for direct consumption without cooking is limited by the short shelf life caused by the fast growth of contaminating microflora. With the aim of reducing the contamination, we tested the possible use of peroxyacetic acid (brand name Persteril) as an additive. Peroxyacetic acid breaks down quickly into oxygen and acetic acid; with the latter quickly vaporising through the packaging. Tests were carried out on a model of pre-washed, cut, and re-washed carrots, which were left naturally contaminated to resemble real grocery store conditions. Four decontamination regimens were applied: (1) rinsing with ordinary tap (drinking) water, (2) rinsing with a 0.2% solution of Persteril, (3) rinsing with a 0.2% solution of Persteril + the addition of concentrated Persteril into the packaging before sealing, and (4) rinsing with a 0.2% solution of Persteril + the addition of concentrated Persteril into the packaging before sealing + another addition of concentrated Persteril after 24 hours. The total number of aerobe mesophilic microorganisms (TNM) and the numbers of yeasts and molds were monitored in the samples taken during 28-days of storage. The last decontamination regimen reduced the initial contamination by TNM by about 1×10^4 CFU/g or 4 log units and no further microbial growth was observed during storage. Yeasts and molds were reduced by about 3.16×10^3 CFU/g or 3.5 log units. No statistically significant changes in colour, texture or taste were noted during storage. There was a slight change immediately after the application in the odour of samples treated with concentrated Persteril; however, the odour returned to original levels during storage.

Keywords: carrot; decontamination; Persteril; peroxyacetic acid; texture; sensory quality; colour

The increased interest of consumers in the consumption of fresh vegetables has led to a trend towards minimally processed vegetable products, the procedure consisting in the removal of non-edible parts and processing into a ready-to-eat form (i.e. direct consumption in raw state). When using leaf and root vegetables for direct consumption (i.e. vegetable salads, sandwiches with vegetables etc.), we encounter an issue involving high initial contamination by various bacteria, yeasts and molds. This concerns particularly the products nearing the end of their shelf life, where the growth becomes exponential and reaches numbers that are not permitted under current regulations. Healthy unprocessed vegetables are sufficiently protected against microbial activity but the processed and cut vegetables loose the natural barrier and thus represent a suitable medium for the microbial growth. With insufficiently sanitary processing and inappropriate storage, the vegetables may become a source of microbial infections. Decontamination options for fresh vegetables are lim-

ited by technology, regulations, or both. The use of steam or anabiotic agents on fresh vegetables is, generally, forbidden. Washing or rinsing with tap water (note, in this discussion it is assumed that tap water is potable) is a basic and commonly used procedure. The washing efficiency depends on many factors, namely on the initial microbial concentration and on the washing manner (length, speed of the water stream, and water temperature). It is evident that rinsing or washing in nothing more than tap water is often insufficient and thus, the decontamination is ineffective. The literature describes more advanced methods of decontamination, e.g. rinsing with (*i*) ozonated water (Beltrán *et al.* 2005), (*ii*) warm water (Delaquis *et al.* 2004; Baur *et al.* 2005), (*iii*) chlorinated water (Behrsing *et al.* 2000; Weissinger *et al.* 2000; Baur *et al.* 2005), (*iv*) ultrasound (Seymour *et al.* 2002), and (*v*) solutions of hydrogen peroxide (Sapers & Jones 2006) etc.

We have found literature data describing various methods of vegetables decontamination; for example, decontamination of iceberg lettuce by free chlorine (Delaquis *et al.* 2004). Iceberg lettuce was cut, washed in water (0–100 mg of chlorine/l, water temperature 50°C, duration 1 min), and this achieved the reduction in TNM by one order of magnitude in comparison with rinsing in 4°C water. Chlorine concentrations of up to 100 mg/l had no marked effect on the elimination of microorganisms from salad surfaces relative to water temperature. The differences in aroma at rinsing water temperatures of 50°C and 4°C were not significant ($P > 0.05$) after either 1 day or 7 days of storage at 1°C. When rinsing with chlorinated water (100 mg/l) at 50°C, the aroma was significantly reduced ($P < 0.05$) during storage on both day 1 and day 7; which was not the case for rinsing water temperature of 4°C.

Behrsing *et al.* (2000) studied the reduction of contaminating *E. coli* by rinsing vegetables in chlorinated water. Rinsing salad leafs in chlorinated water (chlorine concentrations of 50 mg/l and more) for 30 s or longer reduced *E. coli* by 1.9–2.8 log units from the initial count of 6.8 log units. For broccoli, the reduction was 1.7–2.5 log units depending on the time and chlorine concentration. On rinsing for 2 min at a concentration of 100 mg/l and at 4–25°C, the reduction was approximately 2.4 log units across the whole range of temperatures. Zhang *et al.* (1996) studied the reduction of *Listeria monocytogenes* on green let-

tuce and cabbage. When rinsing with chlorinated water (concentration 200 mg/l, 4°C) for 10 min, they reduced *L. monocytogenes* by 1.3 log units, and with 22°C water the reduction was 1.7 log units. In the case of cabbage, the reduction was by 0.9 log units and 1.2 log units, respectively, under the same conditions. Rinsing cut lettuce in 4°C water containing 80 mg/l chlorine at pH = 6.5 for 3 min yielded a reduction of mesophilic microorganisms by 2.1 log units as compared to rinsing in tap water (Beltrán *et al.* 2005).

Klaiber *et al.* (2004) rinsed whole carrots in water with chlorine concentration of 200 mg/l, temperature 4°C, pH = 8, for 2 min, and achieved an average reduction of total mesophilic counts by 0.5 log units. When using grated carrots, the reduction was by one order of magnitude.

Seymour *et al.* (2002) reduced *Salmonella typhimurium* in iceberg lettuce by 1.7 log units by washing it in chlorinated water (chlorine concentration unknown).

Baur *et al.* (2005) studied decontamination of iceberg lettuce under various conditions. They changed the concentration of chlorine and water temperature over a constant rinsing period of 60 seconds. They recorded the following results: the reduction of TNM by 1.65 log units, pseudomonas by 1.79 log units, enterobacter by 2.26 log units – using chlorinated water (200 mg/l, 4°C). When using the same concentration of chlorinated water but at a different temperature (50°C), they reduced total counts by 2.16 log units, pseudomonas by 1.83 log units, and enterobacter by more than 2.96 log units. Chlorinated water at a concentration of 0.3 mg/l, and at 50°C yielded a total count reduction by 1.63 log units, pseudomonas by 1.55 log units, and enterobacter by more than 1.85 log units.

Decontamination of fresh tomatoes was investigated by Sapers and Jones (2006). Following inoculation with *E. coli* NRRL B-766, they reduced its number by 1.7 log units by applying chlorine (200 ppm) for 3 min at 20°C. *Salmonella* inoculation was reduced by 1.78 log units when using chlorine (200 ppm) for 2 min at 20°C. After storage at 4°C for 48 h (after inoculation and treatment) *E. coli* was reduced by 1.16 log units. *Salmonella* was reduced by 1.34 log units after 24 h of storage at 4°C.

Chlorine-based formulations are moderately effective as shown above, however, they are not suitable in view of the possible hazard of undesirable by-products during reactions with the sub-

stances present on/in food. The most significant by-products are trihalomethanes, some of which have been reported to be potent mutagens. The most common organic compound of this group is chloroform which emerges after the reaction with organic matter. It is a highly lipophilic compound with a high affinity for foods rich in fat – Velíšek (1989 – cited from Ševčík 2006). Moreover, free chlorine reacts very quickly in water solution with nitrogen compounds which results in a variety of chlorine compounds that have a small or no antimicrobial effect (Ševčík 2006).

There is evidence that peroxide-based formulations are the most suitable. For example, peroxyacetic acid (brand name Persteril) contains no carcinogens and is thus suitable for agricultural and food industry uses. It is well known that the peroxyacetic acid-water solutions are used for decreasing microbial contamination of vegetables blanched before freezing and canning. It is applied in very low concentrations; however, it is rather unstable at very low concentrations and therefore needs to be prepared immediately prior to the application (Ševčík 2006).

Rodgers *et al.* (2004) used peracetic acid (80 mg/l) for decontamination of fruit and vegetables and in this way reduced *E. coli* O157:H7 and *L. monocytogenes* by 4.4 orders of magnitude. Ruiz-Cruz *et al.* (2007) tested, among others agents, peracetic acid (40 mg/l) solution on grated carrots inoculated with the strains of *E. coli* O157:H7, *L. monocytogenes*, and S*almonella*.

Vandekinderen *et al.* (2008) described the results obtained with grated carrots contaminated with natural microflora after the application of various decontaminating agents including peracetic acid. This agent has also been shown to be better suited for disinfection of sewage water than chlorine compounds (Baldrya *et al.* 1995). On the other hand, Rossoni and Gaylarde (2000) who tested the aptness of *E. coli*, *Pseudomonas fluorescens,* and *Staphylococcus aureus* to be rinsed off stainless steel surfaces with peracetic acid and sodium hypochlorite, achieved better results with the latter. As the literature provides contradictory data, the aim of this study was to test the efficacy of Persteril on carrots contaminated by natural microbial flora and not to introduce deliberate contamination with selected strains of pathogenic microbes. The second aim of our study was to test the efficacy of peroxyacetic acid vapours gener-

ated by injecting the tested agent into the packed, previously decontaminated carrots.

MATERIAL AND METHODS

Vegetables. Carrots (Nandryn variety) were brought from a fruit and vegetables processing company, in a cooled transport van. The carrots were not washed, thus they had soil remains on their surface. We selected samples that averaged 30 mm in thickness; they were washed under 14°C running water in order to remove the surface dirt. The carrots were then cut into cylinders and halved along the long axis. All cut up samples were rinsed under running water to eliminate crushed tissues and juice that could act as a growth medium for the contaminating microflora.

Sample size: length 31.2 ± 1.0 mm, diameter 30.8 ± 1.5 mm, weight 12.4 ± 1.5 g, number of samples per bag 13 (5 pieces for microbial testing, 8 pieces for the measurements of texture, colour, and for sensorial evaluation).

Decontamination agent. The decontamination agent was Persteril brand of peracetic acid (OVERLACK Ltd., Plzeň, Czech Republic). The concentration used was 0.2% (5.6 ml of 36% Persteril per 1 liter of water).

Treatment regimens. Raw carrot samples were treated using four different decontamination regimens:

Regimen 0 – rinsing with clean water and sealing into Stomacher® bags (Intersicence, Paris, France) which were closed by heat welding.

Regimen A0 – soaking in a water solution of 0.2% Persteril (5.6 ml 36% Persteril per l of water) for 5 min followed by sealing into Stomacher® bags (closed by heat welding). The temperature of the soaking solution was 14°C.

Regimen A0P – soaking in a water solution of 0.2% Persteril for 5 min with subsequent addition of concentrated 36% Persteril (100 µl) prior to sealing in a Stomacher® bag. The temperature of the soaking solution was 14°C.

Regimen A0PP – soaking in a water solution of 0.2% Persteril for 5 mins with subsequent addition of concentrated 36% Persteril (100 µl) prior to sealing in a Stomacher® bag. The temperature of the soaking solution was 14°C. An additional 100 µl of Persteril was added after 24 h and the bag was resealed.

Storage. The carrot samples, in sealed bags, were stored in a refrigerator at an average temperature of 7.1°C for 28 days. The samples for microbial testing, measurements of colour and texture, and for sensorial evaluation were removed at the start of the experiment which was repeated at 7-day intervals.

Microbial testing. All samples were tested for total number of aerobe mesophilic microorganisms (TNM) and for yeasts + molds (Y+M) in two parallel sets. The samples were put into a nine-fold volume of (w/w) physiological solution and homogenised in a blender (Stomacher®) for three minutes. This time had been confirmed by preliminary tests as sufficient for releasing all microorganisms present.

The analyses were carried out according to the valid microbiological norms: ČSN 56 0080: 1983, ČSN 56 0081: 1983, ČSN 56 0084: 1986, ČSN ISO 4832: 1995 (56 0085), ČSN ISO 7954: 1994 (56 0087), ČSN ISO 6887-1: 1999 (56 0102) idt ISO 6887-1: 1999; idt EN ISO 6887-1: 1999.

The media used were manufactured by Oxoid CZ Ltd. (Brno, Czech Republic).

Homogenisation solutions: PV: 10 g peptone+ 8 g NaCl + 1 ml TWEEN 80, pH = 7.2 ± 0.2; physiological solution FR: 0.9% NaCl.

Texture measurement. We used a Texture Analyser, type TA-XT2i (Stable Micro Systems, Survey, UK). A cylindrical probe of 3 mm in diameter was used. The maximum force (first local maximum) needed for penetration was measured. The measurement was carried out on the outer cylindrical aspect and in the sample centre (flat aspect of half-cylinder). We repeated the measurement five times on different samples for statistical evaluation – Student's *t*-test (95% reliability interval)

(ŠTĚPÁNEK 1975). The probe speed in the measurement was 1 mm/s, the resolution of the power sensor was 0.1 grams.

Colour measurement. A Minolta CE-300 colorimeter (Japan) was employed for this measurement. The colour was measured at the sample center (flat aspect of half-cylinder). The measurement was repeated five times for statistical evaluation – Student's *t*-test (95% reliability interval). The Cielab colour system *L*, a*, b** was applied. The *L** coordinate expresses the brightness of the colour tone, the +*a** coordinate describes the measure of red colour, and +*b** coordinate the measured yellow colour. The cylindrical aspect of the samples was not measured due to a poor contact with the sensor; the measurements were only made on the flat aspects (surfaces).

Sensory evaluation. The sensory evaluation was performed by means of a graphic unstructured scale using a 100 mm line with a mark at the position adequate to the perception of the given phenomenon. The left end of the line was verbally described as the "best quality", the right end as the "worst quality". At least five assessors evaluated the appearance and odour of the samples. The data were statistically evaluated using the Student's *t*-test (95% reliability interval).

RESULTS AND DISCUSSION

Microbial quality

All the resulting microorganism counts are recalculated and refer to the outer sample aspect. We assumed that natural contamination occurs usually on the surface only and not inside the carrots. The
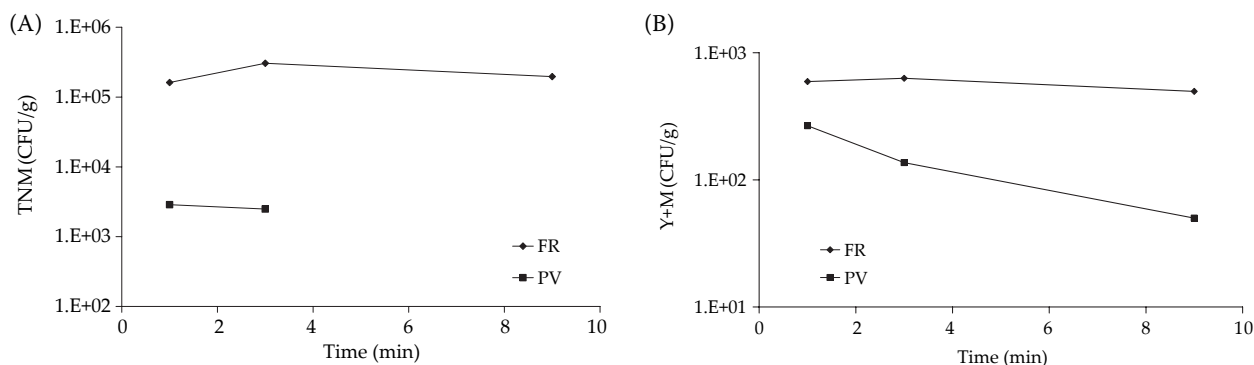
FR – physiological solution; PV – FR with peptone and tween

Figure 1. Microorganism release (TNM) (A) and (Y + M) (B) from the carrot surface in a stomacher in relation to the homogenisation length and diluting solution
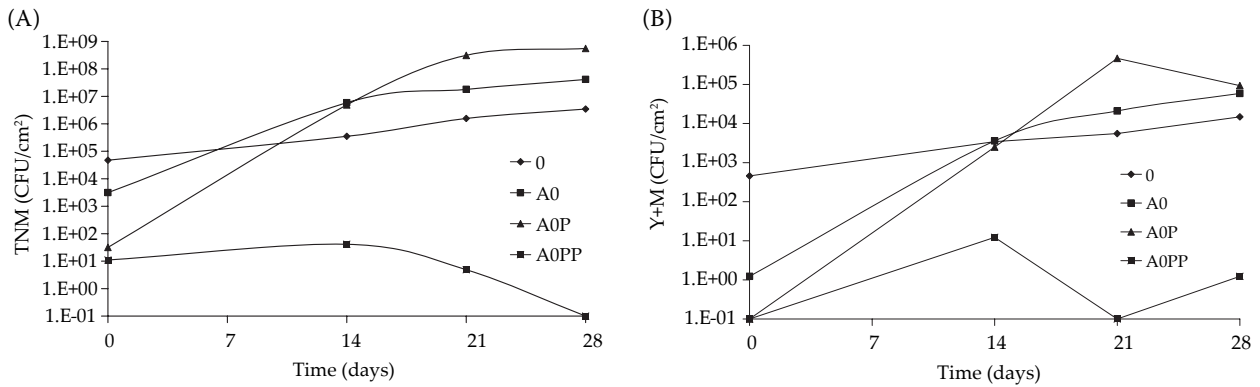
(A)

(B)

Figure 2. Influence of treatment on TNM (A) and Y+M (B) in washed carrot

efficiency of rinsing microorganisms off the carrot surface was tested by differing, the lengths of homogenisation, and by the use of two different homogenisation solutions. Figure 1 illustrates the influence of these parameters on the release of microorganisms from the carrot surfaces (TNM and Y+M) and relative to the homogenisation length and diluting solutions: FR is physiological solution, and PV–FR is physiological solution saline combined with peptone and Tween. The result is that FR is more suitable for this purpose than PV–FR, and that optimum homogenisation time was three minutes. Figure 2a shows how the treatment regimens influenced TNM in washed carrots. It is evident that rinsing with 0.2% Persteril solution (A0 method) reduced total counts in comparison with untreated samples by approximately 1.2 log units, and the A0P method by 3.2 log units. Unfortunately, after 7 days and 10 days of storage respectively, TNM counts were the same as in the untreated samples; and the counts were increasing with time. The A0PP treatment regimen yielded the best results. The initial reduction was by about 3.6 log units which was similar to the A0P regimen.

No unacceptable growth occurred during storage, and after 14 days, the TNM counts actually started to decline. With regard to yeasts and molds (Figure 2b) the treatment with the A0 regimen achieved a reduction of 2.6 log units whereas the A0P and A0PP regimens yielded the reduction of 3.7 log units. After 14 days, the growth had reached the levels of untreated samples stored for 14 days and the growth continued in the A0- and A0P-treated samples. The A0PP regimen showed oscillations during storage, however, after 28 days the difference between the untreated samples at the beginning of storage was about 2.6 log units. We can therefore conclude that the application of concentrated Persteril combined with the disinfectant effect of the vapours is more efficient than mere rinsing in a diluted solution.

Texture

In the present study, the texture was defined by means of maximum force (first local maximum) upon the penetration into the cylindrical trunk. Fig-
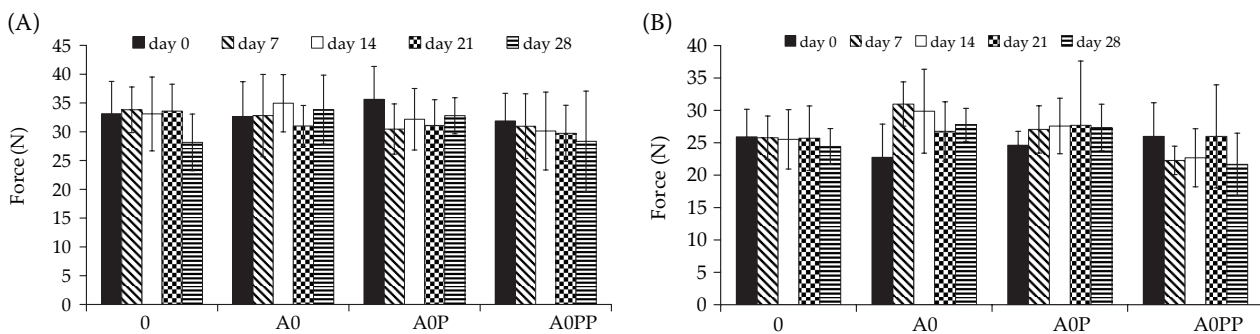
(A)

(B)

Figure 3. Dependence of penetration force on storage duration in variably treated carrot (A) outer cylindric aspect and (B) centre
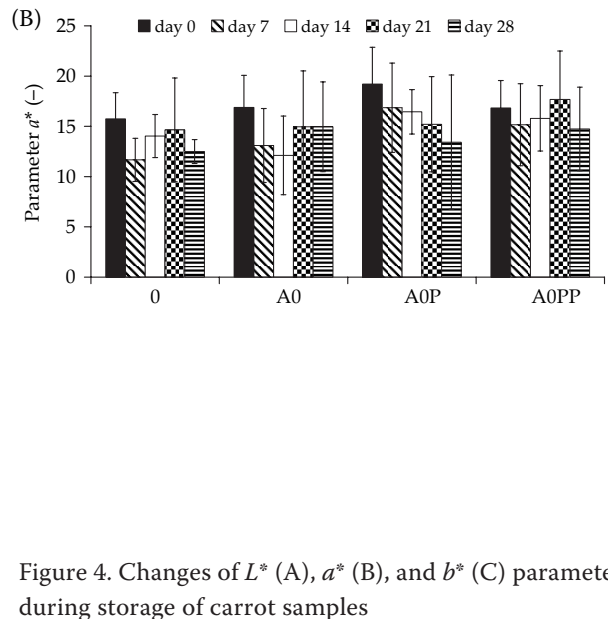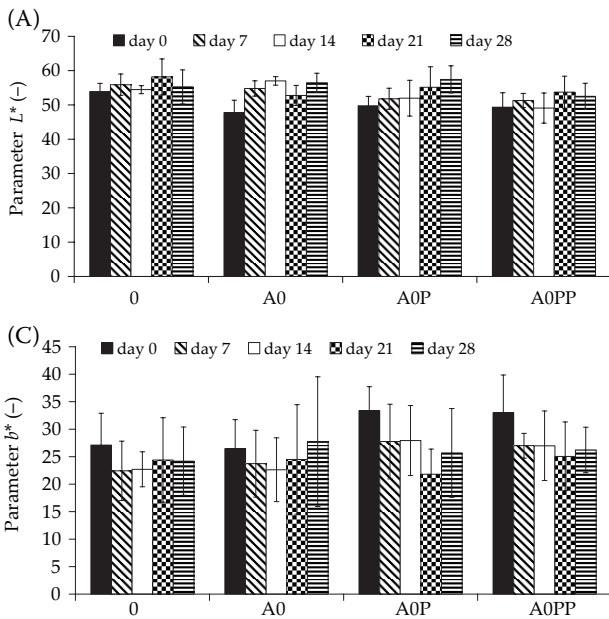
(A)

(B)

(C)

Figure 4. Changes of L^* (A), a^* (B), and b^* (C) parameter during storage of carrot samples

ure 3 present the results of the texture measurements in different samples of treated carrots depending on storage duration (95% reliability interval). The results imply that the changes in texture defined by the penetration force were statistically insignificant over the entire storage interval with all treatment regimens. This is valid for both the outer cylindrical surface and the sample centre. Therefore, the application of Persteril had no influence on the carrot texture during the storage period.

Colour

Figure 4 shows the respective parameters in relation to the treatment regimen and storage duration (95% reliability interval). The results of the brightness parameter L^*: there was evidence for a statistically significant increase during storage between day 0 and day 28 in the A0 and A0P

regimens which did not occur with the 0 and A0PP regimens. Parameters a^* (red colour) and b^* (yellow colour) showed no statistically significant changes regardless of the treatment regimen and storage time.

Sensory quality

The changes in the odour of the samples during storage are charted in Figure 5a (95% reliability interval). A statistically significant change in odour was evident in the samples treated using the A0P and A0PP regimens. The addition of concentrated Persteril to the bag on day 0 led to an unpleasant acidic odour which was caused by Persteril dissociation. However, the odour gradually disappeared in the course of storage of the A0PP-treated samples. Eventually, it decreased to levels comparable to the 0 and A0 treatments.
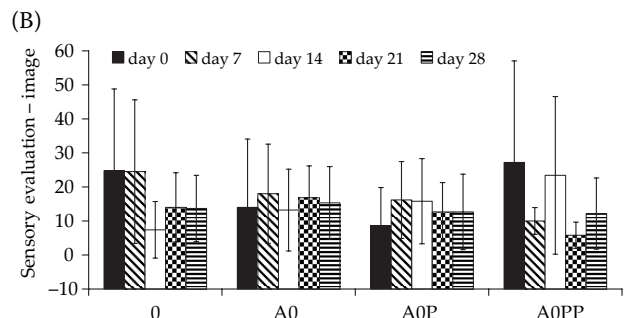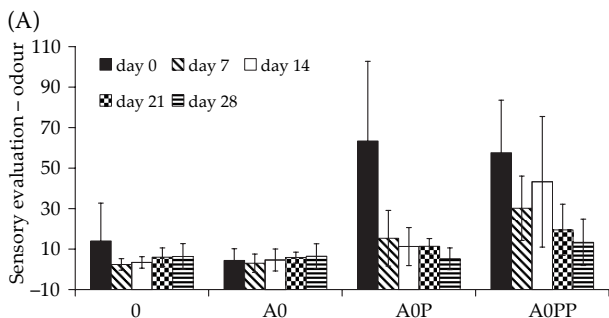
(A)

(B)

Figure 5. Sensory evaluation – odour (A) and image (B) of carrot samples during storage underr various regimens of treatment

Odours in the A0PP samples were slightly more intensive due to the double dose of concentrated Persteril. This difference was, however, statistically insignificant. Finally, Figure 5b shows the changes in the appearance of the samples during storage. No statistically significant change was observed during the course of storage with any type of treatment. The limited sensory evaluation of the taste and acidity done by experienced person after two days of storage and near the end of the observation time provided the evidence that no effect of Persteril was apparent.

CONCLUSION

The results suggest that Persteril can be a very effective decontaminating agent for the treatment of carrots, if a suitable regimen for the treatment is chosen. Mere dipping or rinsing is not as effective as the application of concentrated Persteril in two phases with a time delay between the applications. In the first phase, microbial strains are reduced but spores can still germinate. The bacteria resulting from germination are then reduced during the second phase of the treatment. The manufacturer's website www.persteril.cz presents the application options for this agent. For fruits and vegetables, dipping is recommended for 10 s into a 0.05–0.15% solution (made from water and 36% Persteril). In absolute units, this represents a solution of 0.02% to 0.05% concentration. Further, subsequent dipping in water is advised after 15 minutes. The recommended concentration is 4–10 times lower than that tested in our study. Our results suggest that the treatment proposed by the manufacturer may not be totally effective on bacterial bio-films present on the surface of carrots, as a representative of root vegetables. Our results are in accordance with the conclusions of Rossoni and Gaylarde (2000), who found that mere rinsing with Persteril had little effect. Our results suggest that the use of Persteril, as a gaseous agent, is a suitable means for the prolongation of the shelf life of root vegetables.

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