

EFFECT OF HYDROGEN PEROXIDE ON THE QUALITY PARAMETERS OF SHREDDED CARROTS

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Abstract

The main purpose of the present experiments was to investigate the effect of various hydrogen peroxide (H_2O_2) concentrations and for various lengths of treatment on the total carotenoid, β -carotene content, colour intensity and microbiological safety on the fresh shredded carrots. Shredded carrots were dipped in 0.5, 1.0 and 1.5% H_2O_2 water solution for $30 \pm 1s$, $60 \pm 1s$ and $90 \pm 1s$. Negative effect of H_2O_2 on β -carotene content and colour parameters of analyzed shredded carrots samples was not detected. In carrots treated with H_2O_2 ($p = 0.008$) for 60 – 90s the total content of carotenoids significantly decreased during treatment compared to untreated carrot samples. There was significant difference ($p < 0.05$) observed between treated and non-treated shredded carrot samples on the total bacteria count. It was possible to reduce significantly ($p < 0.05$) the content of yeasts and mould up to 99.99% by shredded carrots treatment with 1.5% hydrogen peroxide water solution for $30 \pm 1s$. In the non-processed carrots *E.coli* was detected; however, it was possible to destroy *E.coli* by treating carrots with 0.5% H_2O_2 water solution for $30 \pm 1s$. Considering all experimentally obtained results, we have concluded that fresh shredded carrots could be treated in water with the addition of hydrogen peroxide 1.5% for $30 \pm 1s$ to maintain quality.

Key words: hydrogen peroxide, carotenoid, β -carotene, colour, microbiology.

Introduction

Carrot (*Daucus carota* L.) is among the top-ten most economically important vegetable crops in the world, in terms of both area of production and market value. Carrot improvement today includes several academic, private and government research programs around the world that work in concert with local, regional, and global industries (Prohens and Nuez, 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. Shredded carrots are an increasingly popular product, but their sales are restricted due to rapid deterioration during storage (Barry-Ryan et al., 2000). Strained carrots sold in retail markets exhibit a diverse range of colour and taste characteristics. From a single processor, this variability may be influenced by differences in raw product, growing conditions, processing parameters and the degree of physiological stress. However, much of this variability is avoided by selecting carrot cultivars for desirable colour, taste and aroma characteristics (Talcott et al., 2001). Carrots have the highest β -carotene, a precursor of vitamin A, content among human foods (Hsieh and Ko, 2008). Nutritionally the most important elements in carrots are phenolic compounds, carotenoids, soluble dry matter, β -carotene, sugars and others. Carotenoids are fat soluble compounds that are associated with the lipid fractions. This class of natural pigments occurs widely in nature. Furthermore, some of them are

involved in the cell communication and xanthophylls have shown to be effective as free radical scavengers (Rodríguez-Bernaldo and Costa, 2006). Carotenoids, the main pigments that are responsible for the colour of carrots, are of importance to food and nutrition due to their pro-vitamin A and antioxidant activity. β -carotene constitutes a large portion (60 – 80%) of the carotenoids in carrots, followed by α -carotene (10 – 40%), lutein (1 – 5%) and the other minor carotenoids (0.1 – 1%) (Sun and Temelli, 2006).

The global processing and storage design to achieve high-quality minimally processed foods requires a combination of different strategies and technologies that would help reduce degradative processes in fresh-cut vegetables. In the design of new processes to obtain pre-cut fruits and vegetables with improved nutritional and health-promoting characteristics, processing and storage technology is selected on the basis of how they improved the nutritional constituents and antioxidant characteristics of the plant products (Martín-Belloso et al., 2011). The cutting process leads to cellular damage, which coupled with increased adhesion and leakage of intracellular material, can lead to increased growth rate of spoilage microorganisms or pathogens. Minimally processed fruit and vegetables are often washed in water or water containing chemicals. The wash water can help distribute bacteria into the damaged sites on the processed fruit or vegetables (Watson et al., 2007). It is important therefore, that processors use the best practice available to supply fruit and vegetables with the minimum risk to consumers through digestion of pathogens and with as long a shelf life as possible to aid distribution channels, minimise waste and increase

profits for processors. 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. The US Food and Drug Administration (FDA) have published lists of GRAS compounds that can be used in many food processing applications where they have been declared safe by expert panels. Regarding the FDA list as a reference, among the chemicals used in this study, chlorine dioxide (ClO_2), hydrogen peroxide, citric acid ($\text{C}_6\text{H}_8\text{O}_7$), and ethanol (EtOH) are listed as GRAS (Generally Recognized As Safe) substances (Loredo et al., 2013; Vardar et al., 2012). The 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 (H_2O_2) is also a well studied oxidant agent (Rodrigues et al., 2012; Sahin et al., 2012; Yildiz et al., 2009), directly toxic to pathogens. Hydrogen peroxide (oxygenated water) is characterized by containing a pair of oxygen atoms ($-\text{O}-\text{O}$), which are highly oxidative with the release of O_2 in aqueous solutions, and this create antimicrobial activity, mainly for Gram-positive and Gram-negative bacteria, due to its capacity to generate other cytotoxic oxidizing species, such as hydroxyl radicals (Alexandre et al., 2012; Delgado et al., 2012; Demirkol et al., 2008; Loredo et al., 2013; Malik et al., 2013; Rodrigues et al., 2012; Ruelas et al., 2007; Tornuk et al., 2011). 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; Ruelas et al., 2007). Hydrogen peroxide is a stable, partially reduced form of oxygen, and its rapid turnover is characteristically mediated by enzyme action. H_2O_2 plays a dual role in plants. H_2O_2 provides a host of benefits by cleansing water from harmful substances such as spores, dead organic material and disease-causing organisms while preventing new infections from occurring. H_2O_2 is of great use in hydroponics and soilless gardening and is sometimes used for root initiation in cuttings (Khandaker et al., 2012).

Further studies on the effectiveness of hydrogen peroxide treatment on the carotenoid, β -carotene content and colour intensity of fresh shredded carrots are needed. The main purpose of the present experiments was to investigate H_2O_2 effect on the carotenoid, β -carotene content, colour intensity and microbiological safety on the fresh shredded carrots.

Materials and Methods

Experiments were carried out at the Department of Food Technology of the Latvia University of Agriculture. The object of the research was carrots (*Daucus carota* L.) 'Nante' cultivar carrot hybrid 'Nante/Forto' grown in Latvia and harvested in Zemgale region in the first part of October 2012 and immediately used for experiments. Meteorological data were obtained from “Latvian Environment, Geology and Meteorology Centre”. Meteorological conditions of 2012 were characterised by relatively high temperatures in the first two months of the summer of 2012 in Latvia. In June and July the average monthly air temperature was + 18.2 °C. In summer of 2012 the average rainfall was 312 mm, respectively, close to optimal precipitation. The autumn of 2012 in Latvia was warm and relatively dry - it was warmer and drier than normal. The autumn temperature was 3 degrees above normal. The quantity of autumn precipitation was 61 mm (87% of normal).

Serotinous 'Nante' carrot hybrid Nante/Forto was analyzed. Shredded carrots were treated with 0.5, 1.0 and 1.5% H_2O_2 water solution for 30 ± 1 s, 60 ± 1 s and 90 ± 1 s. The scheme of experiments is shown in Figure 1.

Preparation of hydrogen peroxide solutions - to prevent degrading of the hydrogen peroxide solutions were prepared by mixing food grade concentrated hydrogen peroxide 30 g 100 g⁻¹ (Peróxidos do Brasil Ltda, Curitiba, Brazil) with sterile deionised water; solution was prepared one minute before the treatment process (Augspole et al., 2013; Delgado et al., 2012).

The total carotenoids were analyzed with spectrophotometric method (used the UV/VIS spectrophotometer Jenway 6300) at 440 nm. A sample of 1g of homogenized shredded carrots was placed in 100 ml conic flask and 30 ml of 96 g 100 g⁻¹ ethanol was added. The sample was stirred on a magnetic stirrer for 15 min, then 25 mL of petrol ether were added and stirring was continued for one more hour. After 1 hour when both layers were completely divided, the top yellow layer was used for further detection of carotenoids at 440 nm. Carotene equivalent (KE) was found, using a graduation curve with $\text{K}_2\text{Cr}_2\text{O}_7$. The content of carotenoids (mg 100g⁻¹) was calculated by equation (Kampuse et al., 2012; Biswas et al., 2011).

$$X = \frac{12.5 \times 100 \times KE}{36 \times a} \times 100 \quad (1)$$

Where 12.5 and 36 – coefficients for relationship between $\text{K}_2\text{Cr}_2\text{O}_7$ and carotenoids;
KE – carotene equivalent by graduation curve;
a – sample weight, g.

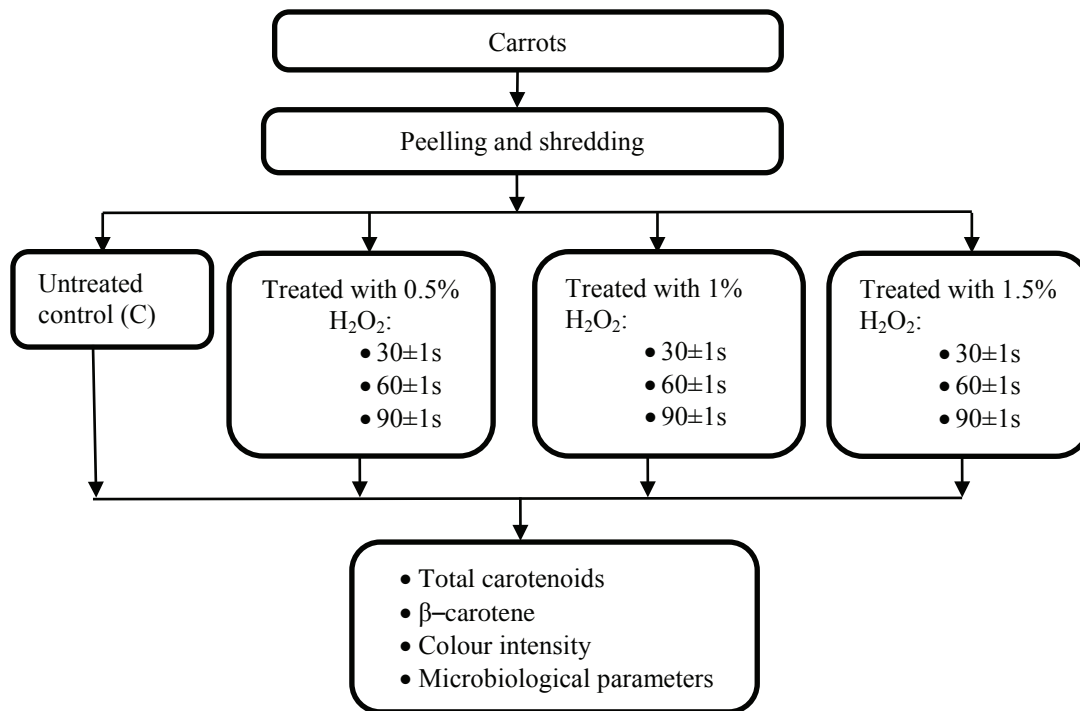


Figure 1. The scheme of experiments.

β -carotene content was analyzed with spectrophotometric method. For extraction, a representative portion of this sample (1 g) was accurately weighed in a glass test tube. Then 5 ml of chilled acetone was added to it, and the tube was held for 15 min with occasional shaking at 4 ± 1 °C, vortex at highspeed for 10 min, and finally centrifuged at $1370 \times g$ for 10 min. Supernatant was collected into a separate test tube, and the compound was re-extracted with 5 ml of acetone followed by centrifugation once again as before. Both supernatants were pooled together and then passed through the Whatman filter paper No. 42. The absorbance of the extract was determined at 449 nm wave length in a UV-Vis spectrophotometer (Rakcejeva et al., 2012; Biswas et al., 2011). A working standard containing $32 \mu\text{g mL}^{-1}$ was prepared from the 1 mg mL^{-1} stock solutions kept at 4 °C. From this working standard different dilutions were made to spike the samples. Blank samples of 1.0 g were spiked with working standards to obtain the final concentrations 16.000, 8.000, 4.000, 2.000, 1.000, 0.500, 0.250, 0.125, 0.062, 0.031 and $0.015 \mu\text{g g}^{-1}$ of β -carotene and extracted as described previously. Calibration curves were plotted by taking Optical Density value to the respective in concentrations by back extrapolation methods. These curves were used to quantify the β -carotene content in the samples analyzed (Rakcejeva et al., 2012).

Colour of the carrot samples was evaluated by measuring CIE L^* , a^* , and b^* parameters by means

of “ColorTec-PCM/PSM” (ColorTec Associates, Clinton, USA). L^* , a^* , and b^* indicate whiteness/darkness, redness/greenness, and blueness/yellowness values, respectively. The maximum value for L^* is 100, which would be a perfect reflecting diffuser. The minimum for L^* would be zero, which would be black. The values of a^* and b^* axes have no specific numerical limits. Positive a^* is red and negative a^* is green. Positive b^* is yellow and negative b^* is blue (Rakcejeva et al., 2012).

Samples for microbiological testing were prepared by dilution method in conformity with standard LVS EN ISO 6887-1:1999 and 6887-4:2044. TPC (total plate count) – determined in conformity with standard LVS EN ISO 4833:2003A; yeast and mould plate count – determined in conformity with standard LVS ISO 21527-2:2008. Plate counts evaluated as decimal logarithm of colony forming units (CFU) per gram of a product ($\log \text{cfu g}^{-1}$).

Statistical analysis. The results were processed by mathematical and statistical methods. Statistics on completely randomized design were determined using the General Linear Model (GLM) procedure SPSS, version 16.00. Two-way analyses of variance ($p \leq 0.05$) were used to determine significance of differences between different samples.

Results and Discussion

Food processing has both positive and negative effects on the levels of carotenoids in food, but overall it is more evident that processing may be beneficial through disruption of matrix (cell walls) which facilitates their release and solubilisation as free or esterified/glycosylated forms in appropriate solvents, or after long heating times, leading to chemical changes (Patras et al., 2009). The results of this study indicate the needs for further insights into the carotenoid role of H₂O₂ treatment.

Colour is an important quality parameter for fresh and processed carrots (Patras et al., 2009). The colour intensity of carrots is considered a reliable indicator of higher nutritive value (Goncalves et al., 2010). The colour of shredded carrots is especially important when carrots for fresh-cut industrial use are prepared. Interesting results were obtained in relation to product colour (Table 1). It was found that colour values were retained unchanged when samples were treated with H₂O₂ solutions. The colour of carrot roots results from pigment accumulation in the shredded carrots tissue. The samples of the investigated carrots differed in L*, a* and b* colour parameters. The highest L* value, related to the lightness, was found for carrot samples of 1% H₂O₂ 90 ± 1s (56.53 ± 1.04) and 1% H₂O₂ 60 ± 1s (56.18 ± 1.08) (Table 1).

In the case of a* and b* parameters, related to the redness and yellowness, respectively, the highest values were obtained for 1.5% 60 ± 1s (20.41 ± 0.84) and 0.5% 90 ± 1s (46.57 ± 0.98), respectively (Table 1). The L*, a* and b* colour parameters of the carrot samples were not significantly influenced by H₂O₂ treatment compared to control samples. All colour parameters were fairly consistent. There were no significant differences found in the L* (p = 0.058)

colour parameter, a* colour parameter (p = 0.610) and b* colour parameter (p = 0.247). Therefore it is possible to conclude, that several concentrations and treatment times of H₂O₂ do not significantly influence colour changes of fresh shredded carrots. The results obtained in the present research conform to results cited in scientific literature, namely, Watson et al. (2007) reported that no colour changes were found of carrots treated with H₂O₂ in different concentration and treating times.

Carotenoids in fresh-cut products are highly susceptible to oxidative deterioration due to the enhanced susceptibility under acute abiotic stress. In general, H₂O₂ negative impact wasn't reported on total carotenoid, β-carotene content of the shredded carrots. In the present study it was detected, that the total content of carotenoids significantly decreased in carrots treated with H₂O₂ (p = 0.008) comparing to untreated carrot samples. In our experiment we applied hydrogen peroxide treatment, which is the recommended form for carrot processing. It was found that the effect of different doses of hydrogen peroxide had insignificant effect on total carotenoid content accumulation in shredded carrots (Table 1). In the present research it was determined, that more advisable H₂O₂ concentration for maximal carotenoid content preservation in fresh shredded carrots 1.5% is recommendable with treating time of 30s. However, the 1.5% H₂O₂ concentration and 60 – 90s long treating time were not suitable for preservation of carotenoids. In scientific literature it has been reported, that food processing has both positive and negative effects on the levels of carotenoids in food, but overall it is more evident that processing may be beneficial through disruption of matrix (cell walls) which facilitates their release and solubilisation

Table 1

Results of colour intensity, total carotenoids and β-carotene content for shredded carrots

Concentration of H ₂ O ₂ , %	Treatment time, s	Colour intensity			Total carotenoids, mg 100g ⁻¹	β-carotene, mg 1g ⁻¹ DW
		L*	a*	b*		
0.0	-	56.81±0.61	19.98±1.06	46.55±1.43	185.14±0.16	164.44±0.00
0.5	30±1	54.88±1.57	18.18±1.03	46.17±1.14	172.89±0.11	164.18±0.19
	60±1	52.69±1.18	17.39±1.03	41.23±1.09	167.86±0.41	155.18±0.15
	90±1	52.61±1.58	18.28±0.52	46.57±0.98	132.70±0.10	156.90±0.25
1.0	30±1	54.47±1.23	18.09±0.75	42.99±0.80	153.58±0.16	151.25±0.27
	60±1	56.18±1.08	18.42±0.76	45.35±1.28	157.05±0.20	155.36±0.17
	90±1	56.53±1.04	18.61±0.23	46.17±0.94	153.10±0.12	162.72±0.15
1.5	30±1	53.47±0.84	19.60±0.65	41.72±0.67	166.14±0.06	163.20±0.21
	60±1	55.43±0.75	20.41±0.84	35.96±0.54	132.52±0.15	156.12±0.10
	90±1	54.00±1.06	18.02±0.61	43.65±0.79	146.82±0.10	150.23±0.10

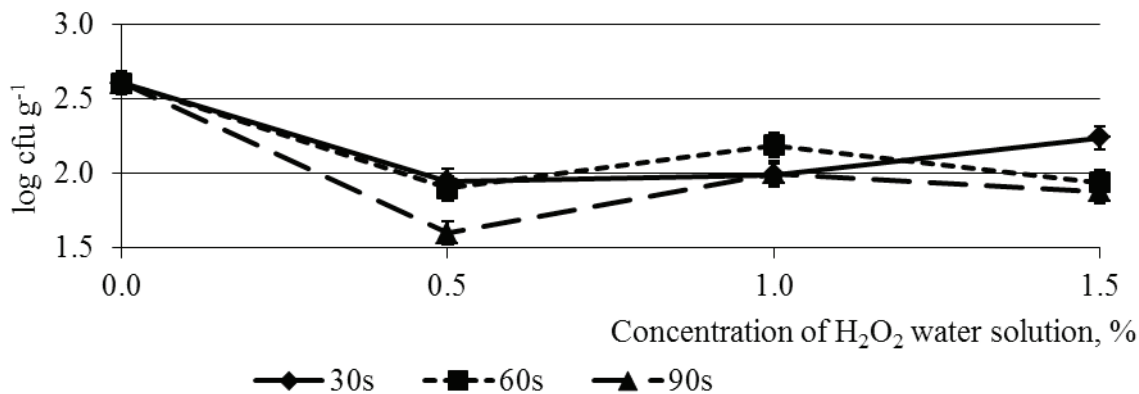


Figure 2. The dynamics of hydrogen peroxide treatment on the total bacteria count.

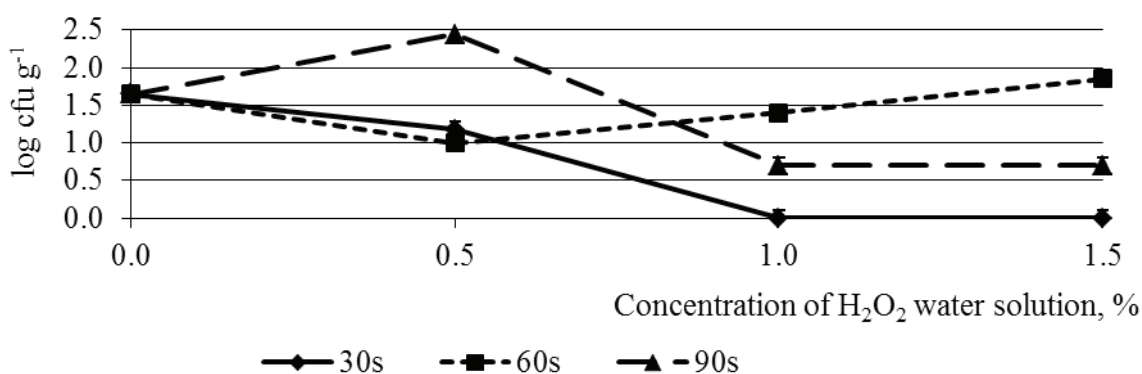


Figure 3. The dynamics of hydrogen peroxide treatment on yeasts and moulds count.

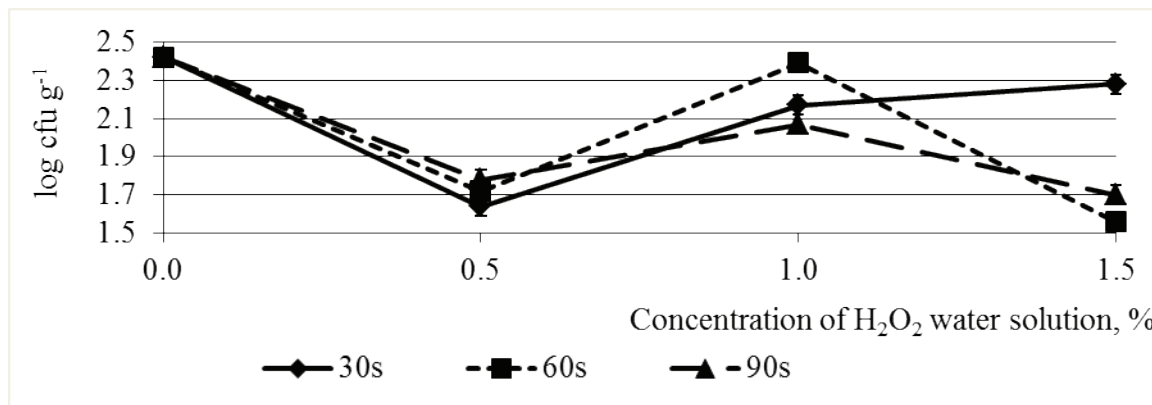


Figure 4. The dynamics of hydrogen peroxide treatment on the *E.coli*.

as free or esterified/glycosylated forms in appropriate solvents, or after long heating times, leading to chemical changes (Patras et al., 2009). However, the negative effect of H₂O₂ on β-carotene content was not observed in this study, too. There was no significant difference (p = 0.240) between analysed carrot samples (Table 1). The results of this study indicate the need for further insight into the total carotenoid and β-carotene role of H₂O₂ treatment.

Microbiological safety is the main quality parameter of food. Therefore for the determination

of shredded carrots, the microbiological parameters were evaluated as main quality indicator. In the present research possible hydrogen peroxide disinfection functions were proved. There was significant differences (p<0.05) between the total mesophilic aerobic bacteria count of treated and non-treated shredded carrot samples. The present study showed, that hydrogen peroxide solutions of 0.5% and treatment time of 90 ± 1s are more effective (Figure 2).

However, it was possible significantly reduce ($p < 0.05$) the content of yeasts and mould up to 99.99% by shredded carrots treatment with 1.5% hydrogen peroxide water solution by 30 ± 1 s (Figure 3).

Minimally processed carrots were microbiologically spoiled by bacteria rather than yeasts and mould. It was reported that microbial growth increased due to the increase in surface by peeling and cutting, high pH, and the moisture content of minimally processed carrots (Augspole et al., 2013).

In the non-processed carrots *E.coli* was detected, however, it was possible to destroy *E.coli* by carrot treatment with 0.5% hydrogen peroxide water solution and treatment time of 30 ± 1 s. Therefore excellent microbiological effect of hydrogen peroxide was proved (Figure 4).

Conclusions

1. The negative effect of H_2O_2 on total carotenoids, β -carotene content and colour parameters of analyzed shredded carrot samples is not detected.
2. The results of physicochemical property measurements of total carotenoids, β -carotene

content and colour value show that samples treated with hydrogen peroxide could be suitable for the market.

3. There is a significant difference ($p < 0.05$) between the total mesophilic aerobic bacteria count of treated and non-treated shredded carrot samples. It was possible significantly reduce ($p < 0.05$) the content of yeasts and moulds for up to 99.99% by shredded carrots treatment with 1.5% hydrogen peroxide water solution for 30 ± 1 s. It is possible to destroy *E.coli* by treating carrots with a minimal concentration of hydrogen peroxide water solution and treatment for 30s.
4. Fresh shredded carrots treated in water with the addition of hydrogen peroxide 1.5% for 30 ± 1 s can provide the maintenance of the quality.

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