SHREDDED CARROTS QUALITY PROVIDING BY TREATMENT WITH HYDROGEN PEROXIDE

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Abstract

Carrots are a vegetable crop providing a source of important nutritional compounds, but their shelf-life, especially for shredded carrots, is not so long. Minimally processed carrots are very common in developed countries and are gaining popularity due to their convenience and freshness. Hydrogen peroxide (H_2O_2) is also a well-studied oxidant agent, directly toxic to pathogens. It has both bacteriostatic and bactericidal activity.

The purpose of the present research was to investigate H_2O_2 influence on shredded carrots chemical composition. 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 major sugars (fructose, glucose and sucrose) were determined by applying the method of high performance liquid chromatography (HPLC). The total phenol content was determined spectrophotometrically according to the Folin-Ciocalteou method and antioxidant activity was determined using the $DPPH^{\bullet}$ (2.2-diphenyl-1-1-picrylhydrazyl radical) assay.

Significant influence of H_2O_2 water solution concentration and treatment time on analyzed shredded carrots quality parameters was found. It was detected that antioxidant activity decrease by approximately 28% in average in shredded carrots after treatment with disinfectant, total phenolic content by approximately 43% in average and total sugars by approximately 30% in average. For the maximal quality parameters preservation recommendable H_2O_2 water solution concentration is 1.5% and treatment time 30±1 s. As a result the content of total phenolic decreases by 35%, antioxidant activity by 20%, total sugars by 19%. As well, the content of fructose decreases by 8%, glucose by 18% and sucrose by 33%.

Keywords: total phenols, antioxidant activity, sugars, H_2O_2.

Introduction

Vegetables are an important part of our diet. They provide, not only the major dietary fiber component of our food, but also a range of micronutrients, including minerals, vitamins and antioxidant compounds, such as carotenoids and polyphenols. The nutritional value of fruit and vegetables is often associated with their antioxidant capacities (Singha et al., 2012).

Fresh-cut carrots (*Daucus carota* L.) could be finding on the market place as: whole peeled (baby), sticks, and sliced, shredded, grated and diced. Carrot is considered one of the vegetables whose consumption, both fresh and processed, has increased over the past years due not only to the nutritional and health benefits this vegetable provides, but also to the introduction of new carrot-derived products (Augspole, Rakcejeva, 2013).

One of the procedures involved in fresh-cut fruit and vegetable processing is cutting the product into smaller pieces. This cutting action essentially is causing the tissue to endure wounding stress. Wounding will cause some physiological effects to the tissue and it is understood that a more severe cutting process will elicit a greater wounding response decreasing the quality of the produce (Surjadinata, Cisneros-Zevallos, 2012).

Along with carotenoids, phenolic compounds also reveal antioxidative properties in vegetables. Phenolic compounds, especially flavonoids, show various types of biological activity, but the most important is the antioxidative activity. Still, it should be emphasized that phenolic compounds, beside coumarin compounds and lignins, are also synthesized in external carrot tissue as a result of mechanical injuries during harvest. This causes negative changes in the quality of storage roots, such as enzymatic browning or deterioration of taste and aroma as a result of enzymatic oxidation of pigments and unsaturated fatty acids (Smoleń, Sady; 2009). Phenolic compounds can act as antioxidants by interfering with oxidation processes through chain-breaking reaction activities (primary oxidation) or through scavenging of free radicals (secondary oxidation). The antioxidant properties of phenolic compounds stimulate the need to design strategies to enhance its content in plant tissues (Heredia, Cisneros-Zevallos, 2009).

The content of total phenolics in carrots ranges from 539.76 to 395.35 mg 100 g^{-1} (GAE) gallic acid equivalents of dry weight (Augspole et al., 2012). Phenolic acids and isocoumarins, predominant phenolics in carrots, contribute to plant tissue's defense mechanism against infections or injuries. Phenolic acid content in carrot peels may increase up to seven-fold when carrots are subjected to abiotic stress during postharvest handling and storage. Phenolic have also been associated with imparting bitter and sour tastes to stored and processed carrots and may result in color loss in processed strained carrots. The total content of phenolic acids in fresh carrots ranges from 77.2 mg kg^{-1} of fresh weight for yellow varieties to 746.4 mg kg^{-1} of fresh weight for purple varieties; in processed strained carrots the amount is between 24.9 and 156.2 mg kg^{-1} of dry weight (Socaciu, 2008).

Carrots, in particular, are noted for their rich antioxidants, especially β-carotene. In recent years, worldwide consumption of carrots has been steadily increasing because of their nutritional benefits. Carrots carry other potentially beneficial health effects, boasting anti-carcinogenic, anti-aging, antioxidant, and

immune-boosting properties, as well as the pro-vitamin activity of some carotenoids, all of which add to their importance in the diet (Yena et al., 2008). In the specific case of carrots, the most important micronutrient is β-carotene, which is a lipid-soluble carotenoid. It's typical chemical structure, consisting of a polyene chain with 11 conjugated double bonds and a β-ring at each end of the chain, gives β-carotene some health related properties (Knockaert et al., 2013).

Among the frequently used processes applied with the purpose of reducing/eliminating pathogens from food, heat-based treatments are the most efficient ones. Hydrogen peroxide (H_2O_2) is also a well-studied oxidant agent, directly toxic to pathogens. It has both bacteriostatic and bactericidal activity, also due to its capacity to generate other cytotoxic oxidizing species, such as hydroxyl radicals. At a concentration between 1 and 5%, hydrogen peroxide is generally used as sanitizer of some food contact surfaces and packaging materials in aseptic filling operations. The antimicrobial efficiency of hydrogen peroxide at higher concentrations (4–5%) is comparable to 100–200 ppm of chlorine treatment (Alexandre et al., 2012).

Fresh cut fruits and vegetables have been very popular for the bioavailability of numerous vitamins, minerals and other phytochemicals. However, they may naturally contain a wide variety of bacteria, fungi and yeast species. Commercial or homemade fresh cut fruits and vegetables are prepared by some simple treatments such as washing, cutting, grating, shredding and packaging. Among these steps, washing may be considered as the most critical step since it removes the soil particles and reduces the microbial load from the surface. Antimicrobial effects of chlorine based sanitizers on fresh cut produce have been previously reported, because they have been widely used as washing solution for fresh cut products to eliminate the microorganisms (Tornuka et al., 2012).

After summarizing data form scientific literature it was concluded, that hydrogen peroxide has mainly microbiological effect on treated food products. But not complete data was found about hydrogen peroxide influence on carrots chemical composition especially on antioxidant activity, phenols and sugars content. Therefore future experiments are needed. The purpose of the present research was as follow – to investigate hydrogen peroxide influence on shredded carrots chemical composition.

Materials and Methods

Materials

The research was accomplished on serotinous 'Nante' carrot (*Daucus carota* L.) hybrids 'Nante/Forto' harvested in Zemgale region (Latvia) in the first part of October 2012 and was immediately used for experiments.

Carrots were rinsed under the tap water, than peeled and shredded using Philips Comfort HR 7605 device.

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.

As a control sample non-treated carrots was analysed for comparison.

H_2O_2 water solution preparation

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 deionized water; solution was prepared one minute before the treatment process (Delgado et al., 2012; Watson et al., 2007).

Total phenolic content

The total phenolic content of carrots was determined by using Folin-Ciocalteu assay. An aliquot (1 mL) of extracts or standard solution of gallic acid 20, 40, 60, 80 and 100 mg L^{-1}) was added to 25 ml volumetric flask, containing 9 ml of distilled deionized water. Reagent blank using distilled deionized water was prepared. One milliliter of Folin-Ciocalteu's phenol reagent was added to the mixture and shaken. After 5 min, 10 mL of 7% Na_2CO_3 solution was added to the mixture. The solution was diluted to volume 25 mL with distilled deionized water and mixed. After incubation for 90 min at room temperature, the absorbance against prepared reagent blank was determined at 750 nm with spectrophotometer JENWAY 6300 (Baroworld Scientifid Ltd., UK). Total phenolic content of carrots was expressed as mg gallic acid equivalents (GAE) 100 g^{-1} in fresh weight (Baydar et al., 2006; Marinova et al., 2005). Then for result veracity a phenolic content of carrots was expressed as mg gallic acid equivalents (GAE) 100 g^{-1} in dry matter.

Antioxidant activity

The antioxidant activity was measured by the $DPPH^{\cdot}$ radical method according to A.L.K. Faller and E. Fialho, 2010 (Faller, Fialho; 2010). The antioxidant reaction was initiated by transferring 0.5 mL of carrot extract into a sample cavity containing 3.5 mL of freshly prepared $DPPH^{\cdot}$ methanol solution (0.004 g $DPPH^{\cdot}$ to 100 mL methanol). After 30 min of incubation in the dark at room temperature, the absorbance was measured at 517 nm with spectrophotometer JENWAY 6300 (Baroworld Scientifid Ltd., UK). Lower absorbance of the reaction mixture indicates higher free radical scavenging activity. Each determination was performed in triplicate, and the results were expressed as mean ± SD. Inhibition of $DPPH^{\cdot}$ in percentage (I%) of each carrot sample was calculated from the decrease of the absorbance according to the relationship:

$$I\% = \frac{A_{blank} - A_{sample}}{A_{blank}} \times 100 \qquad (1)$$

where A_{blank} is the absorbance of the control reaction (methanol–water with $DPPH^{\cdot}$), and A_{sample} is the absorbance of a carrot sample (Faller, Fialho, 2010).

Sugars

The content of glucose, fructose and sucrose of carrots grown in Latvia is determined by applying the method of high performance liquid chromatography (HPLC).

The method is based on the fact that the chromatographic separation of glucose, fructose and sucrose is based on their delayed time differences. To 5±0.01 g of sample 20 mL of water was added into a 50 mL volumetric flask, heated for 20 min at 60 °C in a water bath and cooled to ambient temperature (20±2 °C). Then, 1ml of Carrez I and 1 mL of Carrez II solutions were added and shaken. A volumetric flask was filled up with water till the mark and shaken well. First, solution was filtered through the paper filter. The obtained extract was filtered through a membrane filter with pore size of 0.2 μm. Second, extract was placed in a vial and tested by HPLC Prominence (Shimadzu, Japan) equipped with SypelcosilTM LC-NH2 column (250×4.6 mm, particle size – 5 μm) and autosampler SIL-20A. Sugars were detected with a refractive index detector RID-10A (Shimadzu); acquired data were processed using Shimadzu LabSolutions software (LCsolution Version 1.21 SP1). Acetonitrile: water (80:20 v/v) was used as eluent while column temperature was held at 30 °C. The flow rate was 1.0 mL min^{-1}. Injection volume of samples was 10 μL. Calibration curve was acquired after two repeated HPLC runs of seven standard solutions of reference compounds.

The chromatography data processing system fixes the composition of glucose, fructose, and sucrose in carrots by comparing the carrot chromatography with the chromatography of sugar standard-solution.

Mathematical data processing

Data are expressed as mean ± standard deviation; for the mathematical data processing p-value at 0.05 (Two Way analysis of variance, ANOVA), was used to determine the significant differences. In case of establishing statistically significant differences, homogeneous groups were determined by Tukey's multiple comparison test the level of confidence α=0.05. The statistical analyses were performed using Microsoft Excel 2007.

Results and Discussion

Total phenolic content

The presence of phenolic compounds in carrots contributes to their sensory qualities, like colour, bitterness, or aroma. Therefore, the response of phenolic compounds could be used as a good indicator to evaluate the vegetables quality during processing and storage. Major phenols in carrots include chlorogenic, caffeic, and p-hydroxybenzoic acids along with numerous cinnamic acid derivatives. The different carrot tissues have similar composition, but the individual phenolic content differs and it decreases from the exterior (peel) to the interior (xylem). Moreover, the reported data may vary with the extraction method, the way to express the results, and other factors such as cultivars, post-harvest and processing conditions (Gonçalves et al., 2010).

In the present research significant influence ($p<0.05$) of H_2O_2 water solution concentration and treatment time on total phenolic content amount was found. The content of total phenolic content decreases equally by 43%, comparing with initial content of total phenolic, amount in non-treated shredded carrots (Fig. 1).

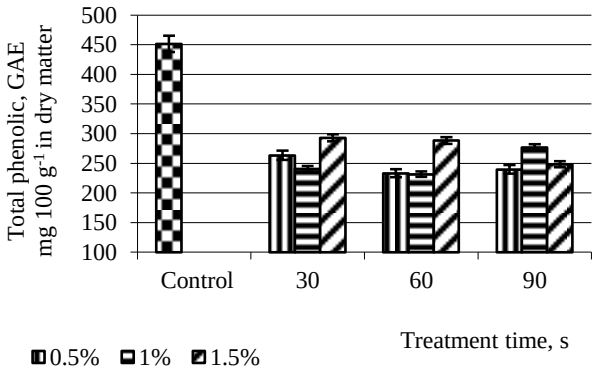

Figure 1. Total phenolic content in carrots

It was proved, that comparing the influence of different H_2O_2 concentrations on the content of total phenolic there are not significant differences ($p=0.962$). The similar tendency was observed analyzing the influence of treated time 30–90 s ($p=0.793$).

Acquired results mainly could be explained with possible intensive oxidation processes occurring in carrots. Very similar data was detected of shredded carrots samples treated with 1.5% H_2O_2 water solutions for 30 and 60 s (Fig. 1), however, the content of total phenolic was higher as 292.85±0.02 and 288.54±0.08 (GAE) mg 100 g^{-1} in dry matter respectively.

Antioxidant activity

Assessments of antioxidant properties of natural compounds are very important because of their uses in medicine, food and cosmetics (Mishra et al., 2012). During the last few years, the study of antioxidant capacity has received much attention, mainly due to the growing interest in the efficiency and function of natural antioxidants in food and biological systems. The antioxidative reactions of free radicals, which are molecules with unpaired electrons, are thought to contribute too many health problems, including cancer, cardiovascular diseases, and inflammatory problems and aging. Antioxidants are agents that, in one way or another, restrict the deleterious effects of these oxidant reactions, either scavenging free radical (eliminating them without generating more radical-induced damage) or other effects (i.e. preventing radical formation) (Fernandez-Orozco et al., 2011).

In the present experiments significant influence ($p<0.05$) of hydrogen peroxide concentration and shredded carrots processing time on antioxidant activity with disinfectant was established. It is indicate, that antioxidant activity decrease by 28% in average in shredded carrots after treatment with H_2O_2 disinfectant (Fig. 2).

After mathematical data processing was proved, that comparing the influence of different H_2O_2 concentrations and treated time 30–90 s on the antioxidant activity there are not significant differences significant difference ($p=0.617$) was not established in

antioxidant activity of with hydrogen peroxide treated shredded carrots. Obtained results are very similar with results on total phenolic contents (Fig 1).

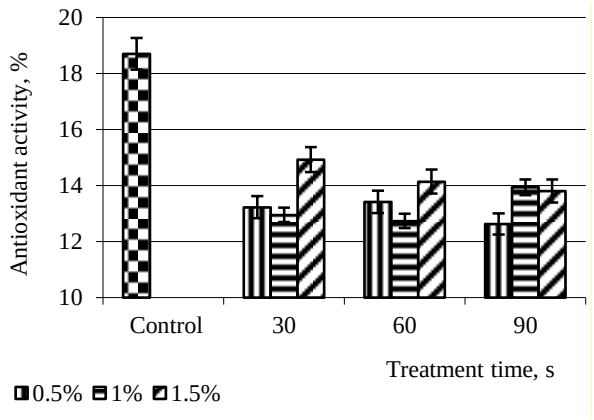

Figure 2. Carrots antioxidant activity

Maximally total antioxidant activity of shredded carrots is possible to maintain by processing with 1.5% hydrogen peroxide water solution for 30s, as a result antioxidant activity value was 14.92±0.03%, while, the total antioxidant activity of non-processed shredded carrots was 18.70±0.04%. Therefore, close interconnection (r=0.953) between total phenolic compounds and total antioxidant activity was detected in the present research.

Sugars

One of the most important qualities of vegetables is their sweetness, closely related to the soluble sugar content (Ozaki et al., 2009). Carrot is mainly constituted by water (approximately 90% of fresh weight) and carbohydrates, which account for 5% of carrot edible portion. In addition to terpenoids, carbohydrates have been reported to be one of the most important sensory indicators for consumer appreciation of this vegetable. As it is known, fructose, glucose and sucrose are the major sugars in carrot and extensive research has been published on their content in carrots of different variety and / or submitted to different processing and storage conditions (Soria et al., 2009).

In the present research negative influence of hydrogen peroxide on total sugar content and fructose, glucose and sucrose content in shredded carrots was detected (Table 1).

In the research obtained results demonstrate, there are not found significant differences ($p>0.05$) in total sugar content if shredded carrots was treated by 0.5% and 1.0% H_2O_2 water solution for 30–90 s. As a result the content of total sugars decreased by 33% in average compared to the control sample. Significant differences ($p<0.05$) in sugars content was found between shredded carrots samples treated with 1.5% H_2O_2 water solution for 30 and 90 s and for 60 and 90 s (Table 1). The content of total sugars decreased by 30% in average, if carrots were treated with different H_2O_2 concentrations for treated time 30–90 s compared to the non-processed shredded carrots. Mainly acquired results could be explained with possible oxidation processes influenced by hydrogen peroxide.

Table 1

Sugars content

H_2O_2 water solution, *%	Time, s**	Sugars, g 100 g^{-1}			Total sugars, g 100 g^{-1}
		Fructose	Glucose	Sucrose	
0	0	0.72±0.04	0.55±0.02	4.51±0.01	5.06±0.02
0.5	30±1	0.46±0.01	0.47±0.01	2.75±0.02	3.68±0.01
	60±1	0.26±0.01	0.45±0.02	2.69±0.01	3.40±0.02
	90±1	0.41±0.02	0.31±0.02	2.27±0.01	299±0.02
1.0	30±1	0.50±0.02	0.66±0.01	2.54±0.02	3.70±0.02
	60±1	0.44±0.04	0.13±0.01	2.45±0.01	3.02±0.01
	90±1	0.65±0.01	0.26±0.01	2.48±0.04	3.39±0.01
1.5	30±1	0.66±0.01	0.45±0.01	3.01±0.02	4,12±0.01
	60±1	0.26±0.01	0.19±0.03	2.76±0.06	3.21±0.01
	90±1	0.37±0.01	0.51±0.01	2.09±0.01	2.97±0.01

*concentration
**treatment time

Conclusions

In the present research significant influence ($p<0.05$) of H_2O_2 water solution concentration and treatment time on analyzed shredded carrots quality parameters was found.

It was detected that antioxidant activity decrease by approximately 28% in average in shredded carrots after treatment with disinfectant, total phenolic content by approximately 43% in average and sugars by approximately 30% in average compared to the non-processed shredded carrots.

For the maximal quality parameters preservation recommendable H_2O_2 water solution concentration is 1.5% and treatment time 30±1 s. In this treatment the

content of total phenolic compounds decrease by 35%, antioxidant activity by 20%, total sugars by 19%. As well, the content of fructose decreases by 8%, of glucose by 18% and of sucrose by 33%.

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