Food Microbiology 26 (2009) 882–888

Contents lists available at ScienceDirect

Food Microbiology

journal homepage: www.elsevier.com/locate/fm

Optimization and evaluation of a decontamination step with peroxyacetic acid for fresh-cut produce

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ARTICLE INFO

Article history:
Received 24 July 2008
Received in revised form
4 June 2009
Accepted 5 June 2009
Available online 12 June 2009

Keywords:
Minimal processing
Peroxyacetic acid
Decontamination
Native microflora

ABSTRACT

Since several disadvantages are associated with the use of sodium hypochlorite as a decontamination agent, the attention for alternative agents such as peroxyacetic acid (PAA) is increasing. In this study the effectiveness of PAA to remove the native microflora was tested in four types of fresh-cut vegetables: grated carrots, fresh-cut white cabbage, iceberg lettuce and leek. Furthermore, the influence of varying PAA concentrations (0, 25, 80, 150 and 250 ppm) and varying contact times (1, 5 and 10 min) was described by means of a linear model. The efficiency of PAA to remove the native flora was highly dependent on the type of fresh-cut produce: the highest microbial reductions were obtained for carrots (0.5–3.5 log cfu/g) and white cabbage (0.5–3.5 log cfu/g) followed by iceberg lettuce (0.4–2.4 log cfu/g). The obtained efficiency was the lowest for fresh-cut leek (0.4–1.4 log cfu/g). Furthermore, all the treated samples, regardless of the type of vegetable and the contact time and concentration of the PAA treatment, were acceptable for consumption.

1. Introduction

Peroxyacetic acid (PAA) is known as a strong oxidant. It is commercially available as a quaternary equilibrium mixture containing acetic acid, hydrogen peroxide, PAA and water as is shown by model (1) (Kitis, 2004). Frequently, a stabilizing agent is added to inhibit PAA decomposition processes.

$$CH_3CO_2H + H_2O_2 \leftrightarrow CH_3CO_3H + H_2O \quad (1)$$

PAA solution is produced from the reaction of acetic acid or acetic anhydride with hydrogen peroxide in the presence of sulfuric acid, which functions as a catalyzing agent (Kitis, 2004). Typical non-food applications of PAA are its use as bleaching agent in the textile and the paper industry, the reduction of off-odours, sludge debulking and its role in the epoxidation of olefins (Kitis, 2004; Zhao et al., 2008). The most important application area of PAA is the domain of disinfection, in waste water plants, cooling towers, the medical and pharmaceutical industry as well as in the food-processing and beverage industry (Kitis, 2004).

The disinfection efficiency of PAA towards micro-organisms can be ranked as follows: bacteria > viruses > bacterial spores > protozoan cysts (Baldry, 1983; Kitis, 2004; Koivunen and Heinonen – Tanski, 2005; Oh et al., 2005). Its antimicrobial action primarily relates to the production of reactive oxygen species, which can result in DNA and lipid damage (Small et al., 2007). It also relies on the denaturation of proteins and enzymes and the increase of cell wall permeability by oxidizing sulfhydryl and disulfide bonds (Hilgren et al., 2007; Small et al., 2007). PAA is also involved in the disruption of cell membranes and the blockage of enzymatic and transport systems in micro-organisms (Koivunen and Heinonen – Tanski, 2005).

Supplementing PAA with other decontamination agents is a possibility to improve its fungicidal and sporicidal properties. Adding additional hydrogen peroxide to PAA induced a synergistic antimicrobial effect. In this way the sporicidal effect could be increased by two to eight times when compared with the individual activity of the biocides, peracetic acid and hydrogen peroxide (Alasri et al., 1993). The combined use of peroxyacetic and octanoic acid realized an improvement of the fungal reduction in recycled vegetable process water when compared with the use of only PAA (Hilgren and Salverda, 2000).

Commercially available PAA based sanitizers contain a considerable amount of hydrogen peroxide that also possesses antimicrobial properties (Wagner et al., 2002). Hydrogen peroxide is widely used as disinfectant in the concentration ranges of 3–90%

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doi:10.1016/j.fm.2009.06.004

(v/v) and is considered as environmental friendly because water and oxygen are its sole reaction products (Small et al., 2007). Hydrogen peroxide alone is not widely used as a decontamination agent due to its slow disinfection action and its low disinfection efficiency (Koivunen and Heinonen – Tanski, 2005). However, when compared with hydrogen peroxide, PAA has some advantages over hydrogen peroxide such as its effectiveness at lower concentrations (<0.3%, v/v). Consequently when PAA is used as a disinfection agent, its disinfection power predominates over the antimicrobial effect of the accompanying hydrogen peroxide. PAA is also not susceptible to peroxidases and it retains in a better way its activity in the presence of organic loads or food residue when compared with chlorine (Fatemi and Frank, 1999; Hilgren et al., 2007; Small et al., 2007) and in a broad temperature range (Hilgren et al., 2007). PAA can be used over a broad spectrum of pH (3.0–7.5) (Kunigk and Almeida, 2001). Moreover the use of PAA does not cause the formation of harmful chlorinated by-products; the only decomposition products reported seem to be acetic acid and oxygen (Monarca et al., 2002). Disadvantages of the use of PAA are its instability at higher concentrations (15%) and the higher cost when compared with traditionally used sanitizers like sodium hypochlorite (Kunigk and Almeida, 2001).

Since PAA has good antimicrobial properties at low temperatures in the pH range of 3–7.5 (Kitis, 2004) and the disinfection by-products produced by PAA were mainly non-mutagenic (Monarca et al., 2002), it can be suitable as disinfectant in the food industry where it can replace the traditionally used sodium hypochlorite. One of the possible applications is the decontamination of fresh-cut fruits and vegetables of which the initial microbial load is relatively high (Nguyen-the and Carlin, 1994). In the USA, fresh produce was linked to the largest number of foodborne illnesses associated with outbreaks, constituting 22% of all illnesses occurring between 1990 and 2005 (Smith – De Waal and Bhuiya, 2007). Moreover, during this period Norovirus was the major cause of produce outbreaks, accounting for 40%, whereas *Salmonella* and *Escherichia coli* O157:H7 were responsible for respectively 18 and 8% of the produce-related outbreaks. Up till now several publications were dealing with the use of PAA based sanitizers with the objective to remove pathogens like *Listeria monocytogenes*, *E. coli* O157:H7, *Enterobacter sakazakii* and *Salmonella* spp. from fresh-cut vegetables and fruits in order to increase the safety of these products (Beuchat and Schouten, 2002; Lukasik et al., 2003; Beuchat et al., 2004; Gonzalez et al., 2004; Kim et al., 2006; Wang et al., 2006, Yuk et al., 2006; Ruiz-Cruz et al., 2007). On the other hand studies dealing with the effect of PAA on the native microflora on fresh-cut fruits and vegetables are rather limited (Nascimento et al., 2003; Ruiz-Cruz et al., 2007). Consequently, the aim of this research was to determine the disinfection efficiency of PAA in 4 types of fresh-cut vegetables with varying PAA concentrations and treatment times and to form a model that estimates the microbial count reduction for each vegetable during the operation by means of a response surface methodology.

2. Materials and methods

2.1. Plant material

Carrots (*Daucus carota* L.), iceberg lettuce (*Lactuca sativa* var. *capitata* L.), leek (*Allium porrum* L.) and white cabbage (*Brassica oleracea* var. *capitata* L.) were obtained from a local wholesale business (Van Landschoot, Gent, Belgium). They were transported to the laboratory within 30 min and upon their arrival they were manually processed. The carrots were peeled and grated in sticks of 0.15 × 0.15 × 3.5 cm by means of a food processor (Compacto Kitchen Cutter, Philips, Eindhoven, The Netherlands). The outer leaves of the white cabbage, leek and iceberg lettuce were manually removed. Then the cabbage was cut by means of a food processor (Compacto Kitchen Cutter) in 2–5 mm thick pieces. The other vegetables were cut in 1 cm pieces by means of a sharp knife. After processing each batch of vegetable was homogenized.

These four vegetables were included because of their difference in initial microbial load, the different topology of the vegetable tissue and their economical relevance in the fresh-cut produce industry. Furthermore they represented different vegetable groups like leafy vegetables (iceberg lettuce), Brassicaceae (white cabbage), vegetables necessary to be heated before consumption (leek) and root vegetables (carrots). Moreover, iceberg lettuce and white cabbage are known to be susceptible to browning so that also the worse case situation can be simulated concerning the effect of PAA on visual quality.

2.2. Decontamination procedure

During this research a commercially available peroxyacetic acid based sanitizer, called Chriox 5, was used (Christeyns N.V., Ghent, Belgium). Chriox 5 is a stabilised mixture of peroxyacetic acid (PAA) (4.6–5%), hydrogen peroxide (23–25%), acetic acid (8–9%), stabiliser (<1%) and water (60–65%). Before each experiment the concentration of the active PAA in the Chriox 5 product was determined by iodometric titration (Vandekinderen et al., 2008). First the sample was diluted in a solution (50/50, v/v) of potassium iodide (10 g/L, Sigma–Aldrich, Steinheim, Germany) and methanol (Fluka, Buchs, Switzerland) at −10 °C. The liberated iodine was titrated with a standardized 0.01 N sodium thiosulfate solution (Sigma–Aldrich). Using these conditions the hydrogen peroxide reacts not significantly with the iodide and the PAA determination is performed without significant interference of hydrogen peroxide. Furthermore appropriate dilutions based on the active PAA concentration were made. To prepare the different solutions tap water was used. An overview of the tested conditions is given in Table 1. All the proposed conditions were tested in triplicate. The PAA concentrations and the contact times were chosen after a screening of the relevant literature and taking into account practical considerations.

A mass of 100 g of vegetables was immersed in 1 L PAA solution at 17 ± 1 °C under continuous agitation (150 tpm) on an orbital shaker (Ika, Staufen, Germany) during the specific treatment time. Afterwards the excess of decontamination agent was removed by using a manual kitchen centrifuge (Zyliss, Bern, Zwitserland) during 1 min. For each combination of concentration, contact time and type of vegetable three replicates were performed. Fresh-cut, but unwashed vegetables were used as control to determine the initial microbial load before washing.

2.3. Microbiological analysis

A 30 g sample of the (un)treated fresh-cut vegetable was aseptically taken and transferred into a sterile stomacher bag. A tenfold dilution was made in Peptone Physiologic Salt solution (PPS; 8,5 g/L NaCl (VWR, Fontenay Sous Bois, France) and 1 g/L neutralised bacteriological peptone (Oxoïd, Hampshire, England))

Table 1
Overview of the tested peroxyacetic acid concentrations with their pH and the tested contact times.

Concentration (ppm)	pH	Treatment time (min)
0	7.69	1, 5 and 10
25	6.80	1, 5 and 10
80	5.74	1, 5 and 10
150	4.90	1 and 5
250	4.42	1 and 5