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Role of commercial sanitizers and washing systems on epiphytic microorganisms and sensory quality of fresh-cut escarole and lettuce

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Abstract

Increasing concerns about the possible formation of carcinogenic compounds and the emergence of new, more tolerant pathogens, have raised questions on the use of chlorine in fresh-cut produce. There is a growing need to investigate the efficacy of new commercial sanitizing and other alternative technologies. In the present study, the effectiveness of chlorine and other commercial sanitizer agents (Sanova, Sanoxol 20, Tsunami 100, Purac FCC 80, Citrox 14W and Catallix) was evaluated on epiphytic microorganisms and their influence on the sensory quality of fresh-cut escarole and lettuce. Different sanitizer concentrations (manufacturer's recommended dose and half of this dose) and application systems (submersion and spray) were also compared. The antimicrobial efficacy of the treatments was evaluated, initially after washing, and after 8 days of storage simulating a commercial shelf-life (3 days at 4 °C + 5 days at 8 °C). All the tested washing solutions were more effective in reducing the microbial load than water washes, particularly in fresh-cut escarole. However, the microbial load of fresh-cut escarole and lettuce after 8 days of storage was very similar for most of the treatments despite the different application systems and concentrations of the sanitizers. Clearly epiphytic microorganisms of fresh-cut escarole and lettuce were able to grow rapidly during storage under low temperature, reaching similar or higher values than the unwashed fresh-cut produce at the day of production. The overall visual quality of fresh-cut salad leaves was scored as good or very good ($\geq$6) after 8 days of storage, except for the product washed with Purac. Thus, despite the high number of mesophilic bacteria present in the product, between 6 and 8 log cfu g^{-1}, it was not associated with a detrimental quality. Therefore, the determination of the initial epiphytic reductions of fresh-cut products after washing with different sanitizing agents provides little information about the microbial or sensory quality of the product at the time of consumption.

Keywords: Leafy vegetables; Natural microflora; Storage; MAP; Minimally processing

1. Introduction

The increasing public health concern related to the microbial safety of fruit and vegetables has resulted in increased numbers of studies that analyze the efficiency of different methods for reducing the microbial load of fresh produce (Parish et al., 2003). This research direction corresponds with the large majority of published research studies on the safety of fresh-cut products (Lukasik et al., 2003; Beuchat et al., 2004; Gonzalez et al., 2004; Inatsu et al., 2005; Ukuku et al., 2005; Allende et al., 2007; Gómez-López et al., 2007; Selma et al., 2008). These conclude that the simple practice of washing raw fruit and vegetables removes a portion of pathogenic and spoilage microorganisms, decreasing their initial levels and microbiological activity. Because bacteria cells attach in a relatively short time period to the surface of fruit and vegetables and they tend to locate in protected binding sites, they may escape contact with washing or sanitizing agents, which makes it difficult to remove all cells by vigorous washing or treatment with chlorine (Takeuchi et al., 2000; Sapers, 2001; Mandrell et al., 2006). Thus, the success of the washing depends on different factors such as target microorganisms, characteristics of produce surfaces, attachment of cells to produce surfaces, formation of resistant biofilms and internalization of microorganisms, type of washing, exposure time, dose, pH, temperature, etc. Additionally, the remaining microbial load could grow rapidly, reaching similar values to those of the unwashed products. Hence, maintenance of this reduction during storage is as important as initial microbial reductions after washing (Ragaert et al., 2007).

It has been also reported that the presence of competing microorganisms contributes to the reduction of pathogens (Liao and Fett, 2001). Carlin et al. (1996) reported that reducing

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microflora on salad leaves by chemical disinfection permitted, in some cases, better growth of *Listeria monocytogenes*. Therefore, it is important to note that techniques aimed at decreasing the activity of spoilage microorganisms might possibly enhance the growth of pathogens due to a reduction in competing flora (Parish et al., 2003).

To examine the techniques, it is generally accepted that an ideal sanitizing agent should have two important properties: a sufficient level of antimicrobial activity and a negligible effect on the sensory quality of the product. It should be taken into account that the concentration/level of sanitizers or other intervention methods may be limited by unacceptable sensory impact on the produce (Brackett, 1992). Therefore, the sensory quality of the product should be also evaluated when selecting the optimal sanitizing technique (Martínez-Sánchez et al., 2006). In fact, undesirable effects may occur, such as softening of plant tissue (Ruiz Cruz et al., 2006) and these could contribute to the proliferation of surviving microorganisms (Ragaert et al., 2007). Thus, rather than a high microbial population being responsible for deterioration of fresh produce, deterioration may be responsible for high microbial populations (Zagory, 1999).

Currently, chlorine is the sanitizing agent most used by the fresh-cut industry mainly due to its antimicrobial activity and low cost. However, increasing public health concerns about the possible formation of chlorinated organic compounds and the emergence of new more tolerant pathogens, have raised doubts in relation to the use of chlorine by the fresh-cut industry (Singh et al., 2002). The use of alternative sanitizing agents instead of chlorine has been adopted by few fresh-cut companies as a marketing strategy to attract consumers. Additionally, the safety and efficacy of chlorine might eventually be the reason of the implementation of restrictions by regulatory agencies (Sapers, 2001). Therefore, there is an increasing need to investigate the efficacy of new commercial sanitizers and other alternative technologies.

The aim of this work was the study of the efficacy of commercial sanitizers and washing applications in the reduction of epiphytic microorganisms and maintenance of sensory quality of fresh-cut escarole and lettuce after washing and storage, simulating a commercial shelf-life of 3 days at 4 °C and 5 days at 8 °C.

2. Materials and methods

2.1. Plant material

Escarole (*Cichorium endivia*) and iceberg lettuce (*Lactuca sativa* L.) were selected for this study as the main characteristic leafy vegetables for fresh-cut salads. Plant material was purchased from a local wholesale market in Murcia (Spain) at the day of harvest and transported within 10 min under refrigerated conditions to the laboratory. Once in the laboratory, escarole and lettuce heads were stored in darkness overnight at 4 °C and 95% relative humidity (RH).

The core and outer leaves of whole heads were removed and discarded. Internal leaves were cut into 3 cm pieces with a sterile stainless steel knife. Shredded leafy vegetables were well mixed to obtain a homogeneous sample, and divided into different batches of 2 kg each per washing treatment. The whole process was conducted in a processing room at 8 °C under sanitary conditions. Two replications of each experiment, separately in time, were carried out between January and April. Results from one replication of each experiment are presented in this work, since similar tendencies were observed in both trials.

2.2. Washing of fresh-cut escarole using commercial sanitizers following the manufacturer's recommendations

Shredded escaroles were washed using eight different solutions following the manufacturers' recommended doses: (1) tap water, (2) chlorine, prepared from sodium hypochlorite (10%, w/v) (Panreac, Montcada i Reixac, Barcelona, Spain) adjusted to pH 6.5 with hydrochloric acid (total chlorine concentration was determined using the DPD method (APHA, 1992)), (3) Sanova (Ecolab®, Barcelona, Spain), (4) Sanoxol 20 (Adyabac®, Prevención Bio Ambiental S.A.), (5) Tsunami 100 (Ecolab®, Barcelona, Spain), (6) Purac FCC 80 (PURAC bioquímica, Spain), (7) Citrox 14W (Citrox Limited, Middlesbrough, UK), and (8) Catallix (TMI Europe S.A., France). Tap water at 5 °C was used for the preparation of each wash. Table 1 shows the specific characteristics of each washing solution (commercial names, active compounds, concentrations, pH and washing duration). When a rinsing step was recommended by the manufacturer, the product was sprayed with tap water at 5 °C for 1 min to remove the chemical residue, as it was the case of chlorine and Sanova (Fig. 1A). After washing, fresh-cut escarole was spin-dried in a hand-held salad spinner (Dynamic model E-20, Vence, France) for 1 min.

2.3. Washing of fresh-cut lettuce using different sanitizer concentrations and application systems

Shredded lettuce was washed at 5 °C in cold tap water containing different sanitizing solutions: (1) Chlorine, (2) Sanova, (3) Sanoxol 20, (4) Purac FCC 80 and (5) Citrox 14W, most of them at two concentrations, the manufacturers' recommended dose (Table 1) and half of this dose. Additionally, two sanitizers (proposed by the manufacturer to be applied by both submersion and spray), Tsunami 100 and Sanoxol 20, were tested to evaluate differences in the application system. In the spray applications, an initial wash treatment was used to remove the bulk of field soil from produce followed by washing with the sanitizing solutions using a spray nozzle at approximately 10–15 cm from the lettuce surface (FDA, 2007). Fig. 1 shows the scheme for submersion (A) and spray (B) applications.

2.4. Packaging and storage

Fresh-cut escarole and lettuce samples of 150 g taken at random were packed in individual bags (210 mm × 130 mm) of oriented polypropylene (OPP, 35 μm) under a passive modified atmosphere. The O_2 transmission rate of the film was 5.17×10^{-15} mol s^{-1} m^{-2} Pa^{-1} at 25 °C (data provided by the