Influence of Washing Method on the Quality of Prepacked Iceberg Lettuce

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ABSTRACT

The contamination of minimally processed fresh products such as fresh-cut Iceberg lettuce with microorganisms especially *Escherichia coli* and Salmonella spp. is a serious safety problem in the fresh food business. Therefore, the elimination of such microorganisms on sliced Iceberg lettuce by washing with water, ozonated water (1.5 ppm ozone concentration) and combinations of both was investigated. After washing and after six days of storage at 4°C the quality of the lettuce slices was determined by sensory evaluation, microbiological analysis, and cell sap pH, Vitamin C- and sugar content measurements. Washing lettuce slices with tap water, ozonated water, ozonated water followed by tap water always resulted in a decrease in microbial populations of one log unit per 500 gram sample. Washing with water followed by washing with ozonated water resulted in a half log reduction of cfu. After storage a three log higher growth of the microorganisms in the water-washed samples was observed compared to the treatment with tap water followed by washing with ozonated water. The Vitamin C and sugar contents were not affected by ozonated water. The application of ozone is helpful for lettuce sanitation and harmless for the contents of lettuce.

Keywords: Iceberg lettuce, ozonated water, wash process, quality, microbial contamination

1. INTRODUCTION

1.1 Background

During recent years, the consumption of prepacked and 'ready to eat' produces has increased dramatically. These products are prepared for restaurants, fast food outlets and retail markets. Consequently, there is an increasing demand for the development of improved methods that guarantee a high produce quality until the end of shelf live. The projected shelf life varies greatly between the different fresh-cut products. For prepacked mixed salads a maximum of six days of storage at 6°C is recommended in Germany (DGHM 2003). Produce quality is a very complex term, which includes both produce appearance, texture, feeling and taste, and it's contamination with microorganisms. The consumer can only assess the sensory appearance. The producer must guarantee a low microbial load. Vigorous washing of fruits and vegetables with fresh tap water typically reduces the number of microorganisms by 10 – 100 fold (Beuchat 1998). The application of chlorine as a sanitizer is a usual practice in several countries. However, chlorination causes the formation of hazardous by-products, such as carcinogenic trihalomethane (THM) in food (Brungs 1973, Page *et al*. 1976, Wei *et al.*

1985). Consequently, the use of chlorine is restricted by German law (LMBG 1997). Ozone may be suitable as an alternative sanitizer. It is a highly effective oxidant, which is approximately four times more reactive than chlorine. Ozone has been in use for over 100 years as a preservative for foods and food ingredients, for purification in the brewing industry, for odour control and for medical therapy (Kim *et al.* 1999). The bactericidal, virucidal and fungicidal effects have been well documented in a number of studies (Bott 1991, Rice 2001).

Microflora commonly found on lettuce leaf surfaces include species of genera such as Lactobacillus and Pseudomonas. A number of reports describe the species most commonly associated with minimally processed produce. In particular, bacteria such as Salmonella spp., *E. coli* and, Listeria have been associated with outbreaks of food borne diseases (CDC 1997, Burnett and Beuchat 2000; Khadre *et al.* 2001, Tauxe 2002).

It is well known that the storage temperature regime plays a major role in the preservation of produce quality (Riva *et al*. 2001, Garcia-Gimeno and Zurera-Cosano 1997). High temperatures encourage the growth of many microorganisms, which promote the spoilage of lettuce. However, some human pathogens such as *Listeria monocytogenes* can easily survive at refrigeration temperatures (Carlin *et al.* 1995). Consequently, the washing process should be highly optimised to guarantee a minimal number of spoilage microorganisms and human pathogens on the lettuce surface.

1.2 Objectives

This study is part of a series of investigations that will clarify the effect of ozonated water on relevant microorganisms on the lettuce, starting from the effects of ozonated water on *E. coli*, *L. monocytogenes*, Salmonella spp. and *Bacillus cereus* in solutions (Molloy *et al.* 2003) up to an industrial scale, which means that the effect of ozonated water was tested in a lettuce washing process of a manufactory where prepacked salads are produced (Hassenberg, Idler, *et al.* unpublished). The obtained microorganisms were selected because the DGHM recommended standard guidelines for these bacteria (DGHM 2003). In this study, four different washing procedures for cut Iceberg lettuce were investigated; i) the conventional washing using tap water, ii) washing with ozonated water, iii) a two step wash process using tap water followed by ozonated water and iv) a two step wash process, washing with ozonated water followed by tap water washing.

2. MATERIALS AND METHODS

2.1 Preparation of Lettuce

Immediately prior to the experiment, 18 unwashed Iceberg lettuces, packed in PVC bags and delievered by the same manufacturer, were purchased from a local retail market, where they had been stored at ambient temperature (approx. 20°C). The core and the outer leaves were removed from the lettuce heads and discarded. The inner leaves were cut into bite-sized slices (approx. 3 x 4 cm). Due to the extensive sample size two days were required to complete the investigations.

2.2 Washing Solutions

Batches of lettuce samples were washed with one of the following treatments:

i: water,

ii: ozonated water,

iii: water and ozonated water,
iv: ozonated water and water.

The ozonated water (ozone concentration approx. 1.5 ppm) was generated using a 'Bewazon 1' (BWT Wassertechnik GmbH, Germany) ozone generator. The concentration of the dissolved ozone was measured photometrically with a LASA^R 2plus photometer (Bruno Lange, Germany).

2.3 Treatment of Lettuce

For each treatment 5 kg of lettuce slices were placed in a stainless steel sieve and dipped into 5 litre of water (method i) or ozonated water (method ii) for two minutes. For method iii (water and ozonated water) and method iv (ozonated water and water) the lettuce was dipped into the water for one minute and into the ozonated water for one minute. After washing, the lettuce was dried using a kitchen salad centrifuge. After each treatment, the lettuce samples were split into four subsets. Two subsets were analysed directly after washing while the other two samples were packed in 0.5 kg batches in polyethylene bags and stored at 4°C for six days before analyses.

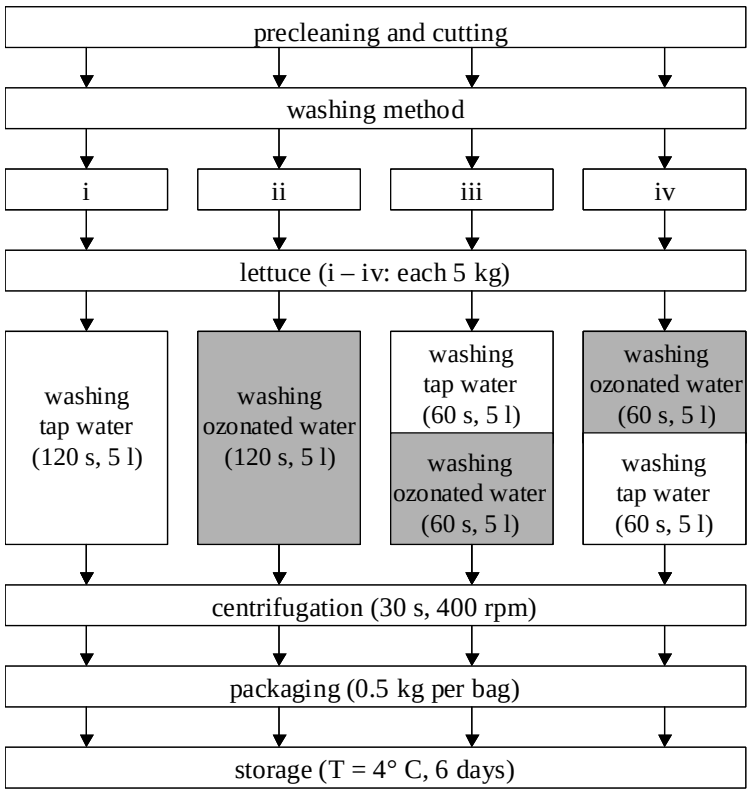

Figure 1. Flow diagram indicating the preparation of cut, packaged Iceberg lettuce samples by different washing methods

2.4 Analysis of Vitamin C and Sugar

Lettuce slices (~ 15 g) were frozen with liquid nitrogen before grounding because frozen cells are easy to destroy to achieve the cell sap. Then the frozen samples were grounded using a pestle and mortar. During grounding the lettuce melt, so it is possible to obtain the sap. Then the samples were centrifuged at 5000 rpm for 10 min. The supernatant was filtered and the

filtrate was used for Vitamin C analysis with the 'Reflectoquant' test kit (Merck, Germany). For the analysis of sugar contents, the filtrate was diluted with distilled water (1:1). Sugar contents were measured with a HPLC system comprising a LCD 202 integrator (GAT, Germany), a RI – 71 detector (Shodex, Japan), a GINA 50T auto sampler (Dionex GmbH, Germany) and a LC 1110 pump (ICI Instruments, GB). Using an injection volume of 10 µl, the analyses were performed on a Eurokat H column (300 x 8 mm, 10 µl; mobile phase: 0.01 N H_2SO_4; flow: 0.8 ml/min; pressure: 63 bar; temperature: 20° C).
The determination performed twice for each method.

2.5 Microbiological Analysis

The samples of lettuce (25 g) were mixed with 225 ml buffered peptone water (pH = 7, Oxoid, CM 1049B) in a stomacher bag and homogenized for 2 min in a stomacher. The mixture was serially diluted and plated for the determination of aerobic mesophilic plate count and *E. coli*. Media and conditions were as follows: DEV nutrient agar (Merck, Germany) incubated at 25°C for 72 h for aerobic mesophilic plate count; TBX agar (Merck, Germany) incubated for 4 h at 30°C and 18 h at 44°C for *E. coli*. Salmonella spp. were detected followed the DIN-EN ISO 6579 2002-12. The instruction includes the enrichment of Salmonella spp. to guarantee the finding also of a small number of Salmonella spp. Normally, the occurrence of Salmonella spp. is rarely associated with prepacked lettuce and statistical information are not available. But the recommended standard guideline by DGHM for Salmonella spp. (Salmonella spp. should not detectable in 25 g lettuce) (DGHM 2003), causes in the decision to analyse Salmonella spp. The determination performed twice for each variant.

3. RESULTS AND DISCUSSION

The initial aerobic mesophilic plate count on the lettuce was similar at the two days of experiments (unwashed control for method i and ii: $2.6 \cdot 10^5$ cfu/g; unwashed control for method iii and iv: $3.5 \cdot 10^5$ cfu/g) (Table 1). Hence, it is reasonable to compare the results. After washing the inactivation of the microorganisms varied with the procedure. The best result, a one log reduction, was obtained after washing with fresh tap water (method i). This observation is consistent with findings of Adams *et al.* (1989), who reported a 92.4% reduction of the lettuce leaf microflora just by washing with tap water. Furthermore, Baur *et al.* (2004) achieved a reduction of the initial counts by ~0.5 log cfu/g using tap water. For methods ii, iii and iv a half log reduction or less was found. The findings of the reduction of the aerobic mesophilic plate count agree with the reduction of *E. coli*. Here, we found also the best results for method i, a 1.5 log reduction. Salmonella were never found in the samples. After six days of storage at 4°C, a marked difference in growth of microorganisms was observed between the washing treatments. The initial count of microorganisms of method i was $3.7 \cdot 10^4$ cfu/g. By the end of the experiment the amount of microorganisms had increased to $1.5 \cdot 10^8$ cfu/g, which is nearly a four log increase. A 2 to 2.5 log increase was observed for method ii (initial count: $6.6 \cdot 10^4$ cfu/g, final count: $4.8 \cdot 10^6$ cfu/g) and iv (initial count: $7.0 \cdot 10^4$ cfu/g, final count: $2.1 \cdot 10^7$ cfu/g). However, washing with both, tap water followed by ozonated water (method iv), resulted in the least growth of microorganisms. The initial washing with tap water removes larger debris such as soil particles but also cellular fluids, which leak from cells damaged by cutting the leaves into bite-sized slices. These pollution and cellular fluids would cause a decrease of the ozone concentration in the washing water, because ozone reacts also with inorganic and organic matter. The second step removes biological contaminants by ozone. The initial count of microorganisms increased during

storage from $1.8 \cdot 10^5$ to $2.6 \cdot 10^6$ cfu/g. This increase of microbial count corresponds by approximately one log unit. The great importance of a low organic load in the washing water was also highlighted by Baur *et al.* (2004). A minimisation of the amount of damaged cells resulting from cutting the leaves may be achieved by using very sharp blades (Watada and Qi 1999). On this background it becomes clear why an initial washing with ozonated water followed by a washing with tap water was much less successful. Under this condition, most of the dissolved ozone was consumed by the oxidation of water debris and cellular fluids. Hence, the expected sustainable effect decreased.

A comparison of all methods shows that the use of ozone resulted in a lower increase of the aerobic mesophilic bacterial plate count, so the maximum permissible value, recommended by DGHM (2003), is not exceeded. Only the final bacterial count of method i exceeded the threshold of $5 \cdot 10^7$ cfu/g and reached $1.5 \cdot 10^8$ cfu/g.

In view of the threshold for *E. coli* recommended by DGHM (2003) all treatments were insufficient. The maximum permissible value of $1 \cdot 10^3$ cfu/g was maintained only by method iv at the day of treatment, the other determined counts exceeded this value. Nevertheless, all treatments resulted in a successful suppression of growth of *E. coli* during storage in comparison with the water washed variant (method i). The lowest increases were found for methods iii (initial count: $6.5 \cdot 10^3$ cfu/g, final count: $3.7 \cdot 10^4$ cfu/g, factor: 5.7) and iv (initial count: $1.0 \cdot 10^3$ cfu/g, final count: $4.3 \cdot 10^3$ cfu/g, factor: 4.3). For method i the highest increase was obtained (initial count: $8.0 \cdot 10^3$ cfu/g, final count: $3.6 \cdot 10^5$ cfu/g, factor: 45).

Table 1. Microbiological analysis of samples taken at the day of washing and at the end of storage at 4°C (n=2)

Washing technique	aerobic mesophilic plate count [cfu/g]		E. coli [cfu/g]	
	day of treatment	after six days storage	day of treatment	after six days storage
unwashed control for method i and ii	$2.6 \cdot 10^5$	n.d.	$1.3 \cdot 10^5$	n.d.
method i	$3.7 \cdot 10^4$	$1.5 \cdot 10^8$	$8.0 \cdot 10^3$	$3.6 \cdot 10^5$
method ii	$6.6 \cdot 10^4$	$4.8 \cdot 10^6$	$1.9 \cdot 10^4$	$5.7 \cdot 10^5$
unwashed control for method iii and iv	$3.5 \cdot 10^5$	n.d.	$4.5 \cdot 10^3$	n.d.
method iii	$1.8 \cdot 10^5$	$2.6 \cdot 10^6$	$6.5 \cdot 10^3$	$3.7 \cdot 10^4$
method iv	$7.0 \cdot 10^4$	$2.1 \cdot 10^7$	$1.0 \cdot 10^3$	$4.3 \cdot 10^3$

The pH-value of the lettuce was monitored before and after washing. Here a small decrease of approx. 0.2 was obtained independent of the washing method used (Table 2). After storage no consistent results were found. The pH-value did not chance for method i and iii and decreased for method ii and iv compared to the untreated sample. This result agrees with observations from several other authors. Bolin and Huxsoll (1991) reported a constant pH during storage of salad-cut lettuce at 2°C for 21 days. Furthermore, Abdul-Raouf *et al.* (1993) found a decrease in pH of shredded lettuce during 14 days of storage. The decline of the pH was higher as the storage temperature increased.

The Vitamin C and sugar contents of lettuce were not affected by washing independently of the washing method. Small differences in the content may be due to inhomogeneous samples.

It is known that the outer green leaves contain more vitamins than the inner leaves (Lee and Kader 2000). During storage the Vitamin C content did not change but the sugar contents decreased. The reduction of sugar contents were also observed by Lopez-Galvez *et al.* (1997) who reported a decrease between 12% and 20% during 15 days of storage at 5°C. The above-average decrease in sugar contents in method iii during storage can be caused by heterogeneous samples. A decrease caused by the effect of ozone (contact time: one minute) is not plausible in comparison with method ii, where the contact time between the lettuce and the ozonated water was two minutes and the decrease of sugar contents was similar to method i.

Table 2. Analysis of the pH, and the Vitamin C and sugar contents of samples at the day of washing and at the end of storage at 4°C (n=2)

Washing technique	pH		Vitamin C [mg/100g]		sugar [g/l]	
	day of treatment	after six days storage	day of treatment	after six days storage	day of treatment	after six days storage
unwashed control for method i and ii	6.32±0.13	n.d.	23.5±0.5	n.d.	24.96±1.48	n.d.
method i	6.11±0.10	6.39±0.21	27.5±0.5	28.7±1.5	22.69±1.68	17.98±1.09
method ii	6.11±0.04	6.03±0.05	25.5±2.5	22.0±1.5	22.45±2.28	17.93±0.67
unwashed control for method iii and iv	6.28±0.07	n.d.	28.0±1.0	n.d.	19.17±0.18	n.d.
method iii	6.06±0.05	6.32±0.15	23.5±0.5	31.3±1.5	19.15±0.38	13.77±0.45
method iv	6.10±0.08	6.04±0.03	28.0±1.0	40.3±2.0	19.88±0.42	17.63±0.26

4. CONCLUSIONS

Washing with ozonated water alone did not achieve the expected sustainable cleaning. This may be due to a degradation of the ozone by debris and cellular fluids. Therefore, an initial reduction of the level of such contaminations is highly recommended. This can be achieved by trimming the lettuce before washing to remove the more highly contaminated outer leaves (Adams *et al.* 1989, Baur *et al.* 2004) and by using very sharp blades for preparing slices (Watada and Qi 1999). A two-step washing process combining an initial washing with tap water followed by a washing step using ozonated water guaranteed the best sustained results.
The use of ozonated water (method ii, iii and iv) resulted in a lower increase of the aerobic mesophilic bacterial plate count during six day storage in comparison with the water washed variant (method i) and in the compliance of the maximum permissible values, recommended by DGHM. This was not observed for method i. Here the final bacterial count exceeded the guidance level of $5 \cdot 10^7$ cfu/g. The guidance level for *E. coli* recommended by DGHM was exceeded in all variants. Nevertheless, all treatments using ozonated water resulted in a successful suppression of growth of *E. coli* during storage in comparison with the water washed variant (method i).

The Vitamin C and sugar contents of lettuce, were not affected by using ozonated water at a concentration of 1.5 ppm and treatment times up to 2 minutes.

In summary, the use of ozonated water is helpful for the compliance of the maximum permissible values, but for final comprehensive recommendation for an effective, economically priced procedure further investigations are needed.

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