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Effectiveness of chlorine washing disinfection and effects on the appearance of artichoke and borage

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Aim: Optimal conditions for chlorine application to obtain a reasonable decrease in the microbial counts without damaging the appearance of artichoke and borage have been established.

Methods and Results: The influence of chlorine concentration (0–200 mg l^{-1}), pH, addition of organic acids, contact time and presence of protective structures on the microflora and vegetal appearance were studied. When pH was not controlled the effect of chlorine depended on its concentration until the pH increase caused by addition of chlorine reached 8·8. Any further increase in chlorine concentration was nullified by the pH increase. When pH was adjusted to 4·5 with acetic acid, the effectiveness increased with concentration. However, the use of citric acid to control pH caused a sharp decrease in effectiveness at concentration about 250 mg l^{-1}. The higher effectiveness of chlorine on homogenized plant extracts compared with the whole plant showed the impact of the vegetal structures on the resistance of the microorganisms. For artichoke, a relationship between the effectiveness of chlorine disinfection and its structures was also found. Extended washing times did not affect the total counts. However, in both vegetables, the appearance was affected by the extended contact times.

Conclusions: The solutions rendering the highest microbial reduction with minimum damages were: 50 mg l^{-1} free chlorine without pH control for artichoke and 100 mg l^{-1} free chlorine at pH 7·0 for borage.

Significance and Impact of the Study: Specific conditions for chlorine disinfection of artichoke and borage were determined to reduce the microorganisms in minimally processed artichoke and borage without damaging their appearance.

INTRODUCTION

Minimally processed fresh (MPF) fruits and vegetables are fresh, raw fruits or vegetables processed in order to supply a ready-to-eat or ready-to-use product. The fruits or vegetables are trimmed, peeled, cut, washed and disinfected. The products are packaged in sealed polymeric film or tray. A shelf life of several days under refrigeration is necessary to obtain feasible transport and retail of the final products. MPF fruits and vegetables are an important and quickly developing type of food (Wiley 1994). The consumers value these products given their similarity to the fresh products and their ease of use.

The spoilage of the MPF fruits and vegetables is characterized by a brown discoloration, necrosis, loss of texture, exudation and/or production of off-odours or off-flavours. The spoilage development is usually concomitant with the growth of microorganisms in MPF products, but this does not necessarily mean that all the spoilage is of microbial origin, and other factors, such as the enzymatic activities, are significant (Nguyen-the and Carlin 1994). The tissue disruption caused by the preparation procedures results in a high respiration rate, which can lead to a rapid deterioration (Bolin and Huxsoll 1991; Brecht 1995; Saltveit

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1997). In addition, the cut tissues release nutrients that support the growth of the microflora present on the raw products (Brackett 1987; Magnuson *et al*. 1990; Nguyen-the and Carlin 1994).

The relationship between the initial microbiological counts and the shelf life (Barriga *et al*. 1991) and the effect on the sensorial quality of the products are important aspects to consider. Moreover, MPF fruits and vegetables can act as vehicles for many different foodborne pathogenic microorganisms (Brackett 1994; Beuchat 1996; Tauxe *et al*. 1997). In some cases, MPF fruits and vegetables are consumed without cooking, thus making the presence of pathogens a concern. There have been numerous reports of foodborne outbreaks associated with these contaminated foods (Nguyen-the and Carlin 1994; Ackers *et al*. 1996; Beuchat 1996; CDC 1997; Odumeru *et al*. 1997). Therefore, it is very important to use a good sanitation technology in order to reduce sufficiently the microorganisms in MPF fruits and vegetables.

The washing with chlorinated water (50–200 mg l^{-1}) is widely used to decontaminate fresh fruits and vegetables on a commercial level. Chlorine is the disinfectant most widely used, in the form of liquid or hypochlorite salt, due to its low cost and ease of holding. However, the efficiency of the decontamination depends on the vegetable (Garg *et al*. 1990), the microflora composition and the growth stage, together with the application conditions (Izumi 1999). Factors such as the chlorine concentration, the pH, the organic material load, the temperature or the contact time affect the effectiveness of the washing procedures for vegetable decontamination (Mazollier 1988; Adams *et al*. 1989). All these factors also affect the spoilage process of the vegetables (Nguyen-the and Carlin 1994). Although many studies on the effect of chlorine on the microbial counts and the foodborne pathogens have been performed, very few of them have linked the microbial results with the effect of chlorine on the sensorial aspects of vegetables (Bolin and Huxsoll 1991; Li *et al*. 2001). However, it is well established that the appearance is the most important selection factor for the consumers (Shewfelt 1987).

Drastic disinfection treatments have succeeded in reducing the counts of microorganisms but the appearance of the vegetables become affected (Karapinar and Gönül 1992; Bari *et al*. 1999; Li *et al*. 2001). For chlorine disinfections, it must be kept in mind that a high chlorine concentration may provoke irritation of the eyes, the skin or the lungs of the workers and be corrosive to the metal in the equipment, specially with a low pH (Beuchat 1998) and cause tainting. Besides, chlorine and hypochlorites give rise to health concerns, since they generate dangerous residues such as chloroamines and trihalomethanes (Simons and Sanguansri 1997).

Artichoke (*Cynara scolymus* L) is a well-known vegetable. In Spain, artichoke is cultivated widely with an annual production of 350 000 tons. Borage (*Borrago officinalis* L) is a vegetable very appreciated in the Ebro valley (with an annual production of 15 000 tons) but less known in other regions. Borage is an annual herb half a metre high. It is covered in a pubescent and even prickly epidermis, which must be removed during its preparation. After peeling, the boiled fleshy stems are very pleasant to eat. The presentation of both vegetables as MPF products, ready to be cooked, would be very convenient for their commercialization to reduce transport costs, storage space and preparation time.

However, the characteristics of both vegetables imply a different response regarding their preparation as MPF vegetables. Thus, while borage needs a harsh treatment with high liquid losses, the main problem in artichoke is its tendency to suffer rapid enzymatic browning. It is necessary to design a specific procedure for each vegetable.

The aim of this work is to establish the optimal conditions for chlorine application to obtain a reasonable decrease in the microbial counts without damaging the appearance of the artichoke and the borage as a first step in their processing as MPF products.

MATERIALS AND METHODS

Preparation of the vegetables

After the manual removal of the leaves, stalks and outer bracts, fresh artichokes (var. 'Blanca de Tudela') were washed with chlorinated water at 4 ± 2 °C (10 l kg^{-1}) by immersion for 5 min. After that, the artichokes were rinsed until the free chlorine levels were below 0·3 mg l^{-1}. Then, the excess water was removed by centrifugation. The borage was manually peeled, cut and processed under the same conditions.

Washing solutions

Liquid sodium hypochlorite was added to the washing water to obtain 25, 50, 75, 100, 150 and 200 mg l^{-1} of free chlorine. A batch with tap water without chlorine addition was used as control.

These chlorine concentrations were tested under four different conditions:

(a) without pH adjustment
(b) adjusting the pH of the washing solution to 4·5 by citric acid addition
(c) adjusting the pH of the washing solution to 4·5 by acetic acid addition
(d) adjusting the pH of the washing solution to 7·0 by sodium phosphate addition

Table 1 Final concentrations of acid in the chlorine washes and stock solutions

Chlorine (mg l^{-1})	Citric acid (mg l^{-1})	Acetic acid (mg l^{-1})	Sodium phosphate (mg l^{-1})
25	125	18	105
50	150	25	210
75	175	36	280
100	200	42	370
150	265	63	550
200	325	81	740
2000 (stock solution)	5000	920	10 000

The final concentrations of acid in the chlorine solutions are shown in Table 1.

The washing solutions were tested on whole vegetables and on the homogenized preparations of artichoke and borage, respectively.

Determination of the effect of chlorine on bacterial suspensions

Twenty-five grams of vegetables were homogenized for 1 min in 200 ml of a sterile solution of 0·1% (w/v) soy peptone (Difco, Detroit, MI, USA) using a stomacher (IUL, Barcelona, Spain) and distributed in sterile tubes at 9 ml each. To each tube, a 2000 mg l^{-1} stock chlorine solution was added to give final concentrations of 0, 25, 50, 75, 100, 150 and 200 mg l^{-1}. Sterile water was added to give a final volume of 10 ml. After 5 min, decimal dilutions were prepared with soy peptone (0·1% w/v) and mesophilic microorganisms were enumerated.

Four 2000 mg l^{-1} stock chlorine solutions were used: without pH adjustment, with pH 4·5 by citric acid addition, with pH 4·5 by acetic acid addition, and with pH 7·0 by sodium phosphate addition. The final concentrations of acid in the stock chlorine solutions are shown in Table 1. The solutions were prepared immediately before their use.

Microbiological analysis

Samples (25 g) of vegetables were homogenized for 1 min in 225 ml of a sterile solution of 0·1% (w/v) soy peptone (Difco) using a stomacher (IUL). Further decimal dilutions were prepared with the same diluents. To analyse whole artichokes, 25 g were taken after chopping the vegetable under sterile conditions.

Mesophilic microorganisms were enumerated on plate count agar (Difco) following the pour plate method and incubated at 31 ± 1 °C for 72 h (ICMSF 1978).

Spore-forming bacteria were enumerated on plate count agar (Difco) following the pour plate method, after thermal shock (10 min at 80 °C). The plates were incubated at 31 ± 1 °C for 72 h (ICMSF 1978).

Table 2 Sensorial attributes evaluated in artichoke and borage

Artichoke	Colour	Surface
		Cuts
		Presence of spots
		General browning
	Texture	Firmness
		Consistency
	General appearance	
Borage	Colour	Intensity of green
		Necrotic ends
		General browning
	Texture	Firmness
		Friability
	General appearance	

Sensorial evaluation

A jury of seven members evaluated the sensorial characteristics of washed vegetables. The panellists were selected on the basis of their interest, time available, liking for the vegetables and aptitude to describe the sensory characteristics of food products.

With the panellists' help, a simple scorecard was devised. The intensity of the attributes was quantified on a scale from 1 to 5 (from undesirable to very desirable). Table 2 shows the chosen attributes for artichoke and borage.

Samples were presented on coded plastic dishes. The order of presentation of the samples was randomised. Testing sessions were conducted under normal light conditions (ISO/DIS 8589).

Other determinations

The pH was measured with a Crison model 2002 pH meter (Crison Instruments, Barcelona, Spain). The free chlorine was determined by colorimetric reaction with DPD (*N*,*N*-diethyl-1,4-phenylenediamine) (Merk, Darmstadt, Germany).

Statistical analysis

The experiments were carried out three times for each condition tested and all the analyses were performed in duplicate. Variance analysis was performed using the SYSTAT program for Windows; Statistics version 5·0 (Evanston, Illinois 1992). Tukey's test was performed to compare the means using the same program. Significance was defined as $P < 0.05$. The plate count data were transformed to logarithms before their statistical treatment.

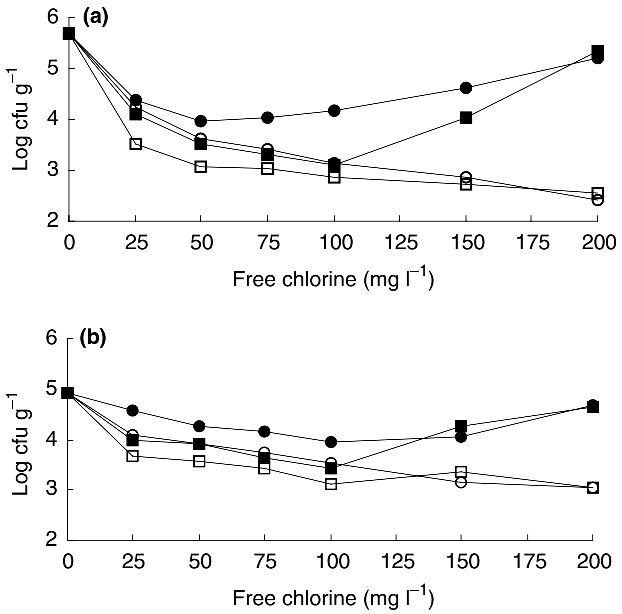

Fig. 1 Mesophilic counts obtained after washing with different chlorine concentrations. (a) Artichoke, (b) borage. ●, Without pH control; ■, pH 4·5 with citric acid; ○, pH 4·5 with acetic acid; and □, pH 7·0

RESULTS

Effect of the chlorine concentration and the pH on the washing solutions

The initial mesophilic counts were slightly higher for artichoke than for borage (6·8 and 6·2 log cfu g^{-1}, respectively). After removal of the outer bracts, the mesophilic counts for artichoke stood at 6·1 log cfu g^{-1}. Peeling the borage reduced its counts to 5·6 log cfu g^{-1}. Washing with tap water had a limited effect: total counts of artichokes and borage were reduced by 0·4 and 0·7 log cfu g^{-1}, respectively.

Figure 1a,b shows the microbial counts obtained after washing with different chlorine solutions. The lowest counts were obtained after washing with 200 mg l^{-1} chlorine pH 4·5 adjusted with acetic acid, or 200 mg l^{-1} chlorine pH 7·0 (2·4 log cfu g^{-1} for artichoke, and 3·1 log cfu g^{-1} for borage).

As expected, the addition of chlorine to the washing solutions where the pH was not controlled led to an increase in the pH value (Table 3). The pH increase was slightly lower in borage than in artichoke. Borage washing solutions had a higher presence of organic solubles, which showed a buffer effect. When the pH was not controlled, the chlorine disinfectant effect depended on its concentration, until the pH value reached 8·8 (75 mg l^{-1} for artichoke and 100 mg l^{-1} for borage). A further increase in the chlorine concentrations was nullified by the increase in the pH.

No significant decontamination differences were found between washing solutions at pH 4·5 and 7·0 either in

Table 3 pH values in washing solutions with different chlorine concentrations

Chlorine (mg l^{-1})	Artichoke	Borage
0	6·9 ± 0·2	6·9 ± 0·2
25	8·2 ± 0·1	8·0 ± 0·1
50	8·6 ± 0·1	8·4 ± 0·0
75	8·8 ± 0·1	8·7 ± 0·2
100	9·2 ± 0·2	8·9 ± 0·2
150	9·3 ± 0·1	9·2 ± 0·2
200	9·3 ± 0·1	9·2 ± 0·1

artichoke or in borage. When citric acid was used as a pH control, a decrease in the effectiveness of chlorine was found in 150 and 200 mg l^{-1} chlorine solutions. With these chlorine concentrations, high amounts of citric acid were necessary to reach a value of pH 4·5 (above 250 mg l^{-1} citric acid) (Table 1). This effect was not observed when acetic acid was used to obtain the same pH value.

Figure 2a,b shows the scores obtained in the sensorial evaluation of artichoke and borage after washing. The effects of the washing conditions were very different for artichoke and for borage. According to the panellists, washing

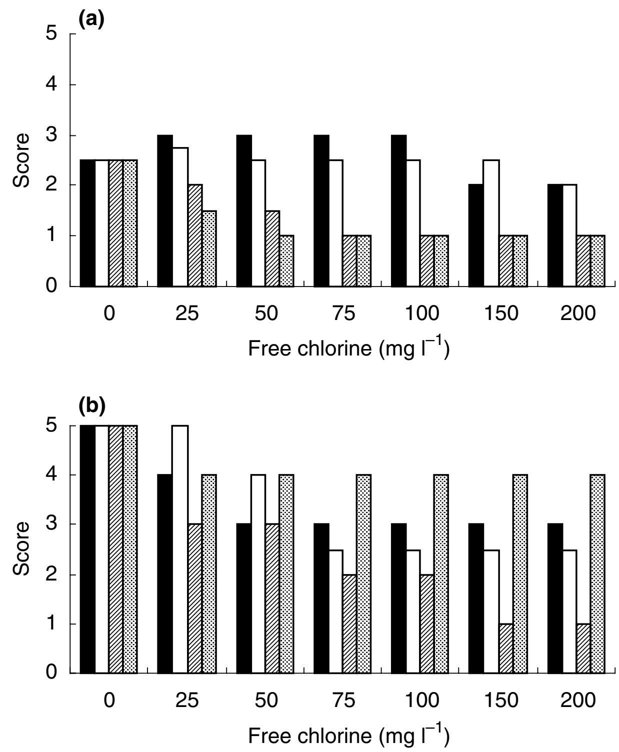

Fig. 2 Scores obtained after sensorial evaluation of washed vegetables with different chlorine concentrations. (a) Artichoke, (b) borage. ■, Without pH control; □, pH 4·5 with citric acid; ▨, pH 4·5 with acetic acid; and ▦, pH 7·0

adversely affected the appearance of the artichoke under all the conditions tested, and colour was the parameter most affected. The presence of a brown-black colour in the cuts and the damaged sites due to the enzymatic browning was the most important cause of spoilage.

For borage, different causes of spoilage were identified by the panellists: loss of its typically brilliant green colour, loss of texture and presence of necrotic sites. In general, chlorine disinfection had a lower effect on the sensory quality of borage and the panellists gave higher scores than for artichoke. With lower chlorine concentrations, the appearance was improved by citric acid addition. However, when the chlorine concentration was 75 mg l^{-1} or higher, the amount of acid citric necessary to reach a pH of 4·5 gave rise to a loss in green intensity. The higher scores were reached at pH 7·0 with chlorine concentrations above 75 mg l^{-1}. Washing with solutions with acetic acid produced a very clear colour and a loss of texture leading to a low sensory score.

According to the results obtained, the washing conditions giving the higher microbial reductions without damaging the appearance of the vegetables were selected: 50 mg l^{-1} chlorine without pH control for artichoke and 100 mg l^{-1} chlorine with pH 7·0 for borage.

Effect of the artichoke morphology on the disinfection by chlorine

The effectiveness of chlorine might be conditioned by the complexity of the artichoke morphology. The washing conditions which gave the highest microbial reduction with less damage on the appearance were tested. Thus, Fig. 3 shows the effects of the washing with 50 mg l^{-1} of chlorine solution on different areas of the artichoke: outer, medium and inner bracts and stems. For this study, the different parts were washed and analysed separately.

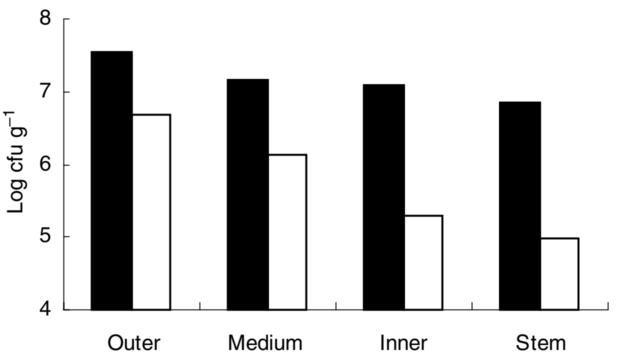

Fig. 3 Mesophilic counts of different artichoke structures before (■) and after (□) washing with 50 p.p.m. free chlorine solution

The highest microbial counts were found in the outer bracts (7.5 log cfu g^{-1}) and the lowest in the stems (6.8 log cfu g^{-1}), probably due to the protective effect of the leaves. After the washing, the inner bracts and stems showed the highest microbial reduction (1·79 and 1·85 log units, respectively). These counts were significantly different to those found for the outer and medium areas, which only showed a reduction of 0·85 and 1·03 log units, respectively.

Effect of chlorine on bacterial suspensions

The effect of chlorine on bacterial suspensions of artichoke and borage, without structural protection, is shown in Fig. 4a,b. Chlorine had a greater effect on bacterial suspensions than on whole vegetables. The microbial reduction obtained on the bacterial suspensions was <1 log cfu g^{-1} for artichoke and 1·5 log cfu g^{-1} for borage.

Note the complete loss of disinfectant activity in the chlorine solutions whose pH was adjusted to 4·5 by citric acid addition.

Effect of the contact time

In order to establish the effect of the contact time on the microbial counts and the appearance of the vegetables, the washing times were extended from 5 min to 30 min.

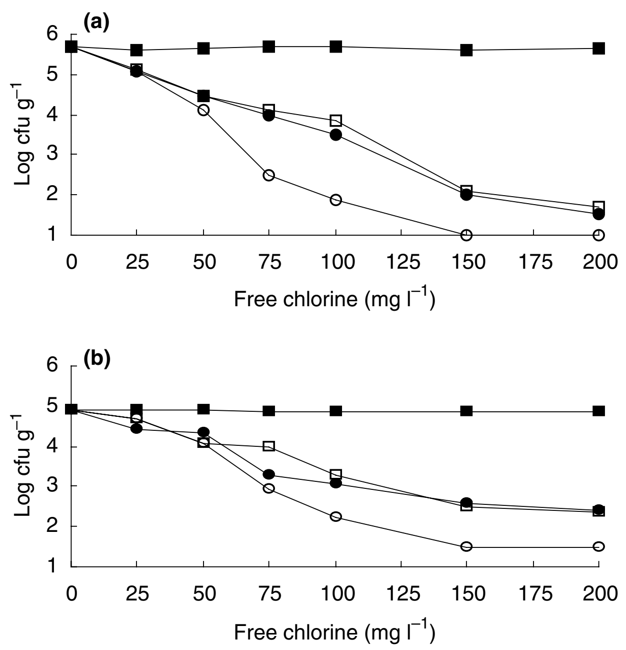

Fig. 4 Mesophilic counts after adding different chlorine concentrations to bacterial extracts obtained from (a) artichoke and (b) from borage. ●, Without pH control; ■, pH 4·5 with citric acid; ○ pH 4·5 with acetic acid; and □, pH 7·0

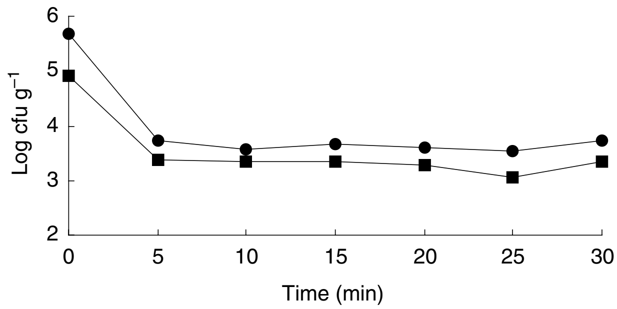

Fig. 5 Mesophilic counts after different contact times with washing solutions. ●, Artichoke with 50 p.p.m. free chlorine; and ■, borage with 100 p.p.m. free chlorine pH 7·0

The washing conditions tested were: 50 mg l^{-1} of free chlorine for artichoke and 100 mg l^{-1} pH 7·0 for borage.

Figure 5 shows the microbial counts obtained. After 5 min of washing, the microbial counts were 3·7 log cfu g^{-1} for artichoke and 3·4 log cfu g^{-1} for borage.

Extending washing times did not result in further reductions in the total counts. However, the appearance of both the artichoke and the borage was negatively affected by the extended contact times and the sensorial scores were lower for longer washing times (Fig. 6).

DISCUSSION

The microbial results obtained for raw and peeled vegetables were in line with those reported by other authors for similar vegetables and treatments (Adams *et al.* 1989; Garg *et al.* 1990). The limited effect on the microbial counts after washing the artichokes and the borage with tap water has also been observed by other authors with salad leaves (Von Jöckel and Otto 1990) and other vegetables (Garg *et al.* 1990).

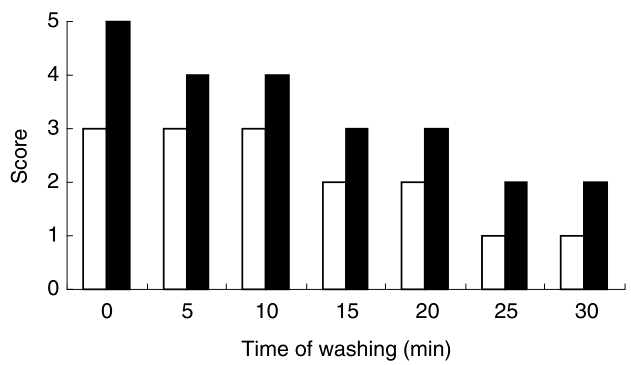

Fig. 6 Scores obtaining in sensorial evaluation after different contact times with washing solutions. □, Artichoke with 50 p.p.m. free chlorine; and ■, borage with 100 p.p.m. free chlorine pH 7·0

The reductions obtained after washing artichoke and borage with different chlorine solutions were also in line with those reported by other authors (Brackett 1987, 1992; Mazollier 1988; Beuchat 1992, 1999; Beuchat *et al.* 1998; Pao and Brown 1998; Taormina and Beuchat 1999; Li *et al.* 2001; Tomasoni *et al.* 2001). These authors suggested that the low effectiveness of chlorine may be due to the attachment of bacteria to the vegetal surface and/or to the protection provided by some special sites of attachment.

The higher effectiveness of chlorine on artichoke than on borage could be due to structural differences, or to the microflora composition. Different authors (Giese 1991; Mossel *et al.* 1995) found that the bacterial spores show a high resistance to the chemical agents and that the agents which contain chlorine only show a moderate activity against the spores. However, counts of spore-forming bacteria were significantly higher for artichoke than for borage (4·2 log cfu g^{-1} for artichoke before washing and of 2·7 log cfu g^{-1} for borage), and thus, the presence of this kind of resistance does not seem to account for the different results obtained in the disinfection of the two vegetables tested. Therefore, these differences can be mainly attributed to structural (morphological) differences.

The presence of higher amounts of organic material in the borage washing solution could be the reason for the lower effectiveness of chlorine on borage than on artichoke. The cut fleshy borage stems showed higher liquid exudative losses than the artichokes. Organic solubles react rapidly with the chlorine and decrease its disinfection effectiveness (Garg *et al.* 1990). The presence of an additional organic load under the conditions tested could be expected to reduce the effectiveness of the chlorine solutions that are neutralized by the contact with organic material.

The loss of chlorine effectiveness with high pH values found in our study had been also reported by other authors (Adams *et al.* 1989). In line with this, Mazollier (1988) studied the effect of the concentration of chlorine solutions on the total counts on green salad leaves during washing. The total microbial counts were reduced when the concentration of free chlorine was increased to 50 mg l^{-1}, but higher concentrations did not reduce the counts any further.

An antagonistic effect between citric acid and chlorine at high concentrations was also observed. Annous *et al.* (2001) found that 200 mg l^{-1} chlorine (pH 6·4) was ineffective in reducing populations of *Escherichia coli* in apples. The use of citric acid to adjust the pH would be another cause, in addition to those reported by these authors, of the low effectiveness of chlorine under these conditions.

The most important cause in spoilage of artichoke was the enzymatic browning. This explains the highest scores obtained for artichoke washed with solutions whose pH was expected to inhibit the polyphenoloxidase activity (Whitaker 1995) (i.e. 25, 50, 75 and 100 mg l^{-1} of free chlorine without pH adjustment), when the pH values in the solution were 8·1, 8·6, 8·9 and 9·2, respectively (Table 2). For borage, a relationship between spoilage and enzymatic browning was not so clear. Thus, borage washed with pH 7 solutions obtained high scores in sensory evaluation.

The fact that the effectiveness of chlorine does not improve with the increase of the washing times had already been reported by others authors (Mazollier 1988; Adams *et al*. 1989; Beuchat *et al*. 2001; Tomasoni *et al*. 2001). The adverse effect on the appearance of both vegetables of the extended contact time shows the advisability of limiting the disinfection time to 5–10 min.

The ineffectiveness of hypochlorite might be due to different factors, as suggested by Adams *et al*. (1989): an aqueous hypochlorite solution may not wet the hydrophobic surface of the waxy cuticle of the vegetables and the formation of a biofilm could protect the microorganisms against the lethal effects of the hypochlorite. Lund (1983) suggested that the contact with a host tissue may inactivate hypochlorite. Attachment to various inert supports increased the resistance of *Listeria* to sanitizers (Mustafa and Liewen 1989; Frank and Koffi 1990; Lee and Frank 1991). Recently, Han *et al*. (2000) and Takeuchi and Frank (2001) demonstrated the importance of the structures of the pepper surface and the lettuce leaf, respectively, in the protection of *Escherichia coli* O157:H7 cells against chlorine inactivation.

For artichoke, the effectiveness of chlorine was conditioned by the complexity of its morphology. Thus, the relative structural simplicity of the inner bracts and stems could explain the higher effectiveness of the chlorine disinfection. The lower reduction rate in the outer and medium areas suggested that the disinfection effectiveness on whole artichokes was improved by the removal of the external bracts before washing.

The lack of a bacterial protection in some particular sites of attachment over the complex surface of the vegetables explains the greater effect of chlorine on bacterial suspensions than on whole vegetables.

When homogenized vegetables were examined, the differences between the effects of the different chlorine solutions were similar to those found when washing whole vegetables. Thus, the count reductions were related to the chlorine concentration and no significant differences were found at 4·5–7·0 pH. Since in the washing solutions where the pH was not adjusted this value remained near to neutrality due to the buffer effect of the peptone solution used on the bacteria extract, the reduction obtained was similar to that obtained with solutions adjusted at pH 7·0. An antagonistic effect between citric acid and chlorine was also observed in the bacterial suspensions: the high citric acid addition (5000 mg l^{-1}) necessary to obtain a value of pH 4·5 in the stock chlorine solution caused the complete loss of the disinfectant effect of chlorine.

In conclusion, the characteristics of each vegetable must be taken into account to design a disinfection procedure. In fact, different disinfection conditions have been found for artichoke and for borage in order to decrease their microbial counts without damaging the appearance. An antagonistic effect between chlorine and citric acid was also detected in this work.

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REFERENCES

Ackers, M., Mahon, B., Leahy, E., Damrow, T., Hutwagner, L., Barrett, T., Bibb, W., Hayes, P., Griffin, P. and Slutsker, L. (1996) An outbreak of *Escherichia coli* O157:H7 infections associated with leaf lettuce consumption, Western Montana. *36th Interscience Conference on Antimicrobiological Agents of Chemotherapy*. Abstract K43, p. 258. Washington, D.C: American Society for Microbiology.

Adams, M.R., Hartley, A.D. and Cox, L.J. (1989) Factors affecting the effectiveness of washing procedures used in the production of prepared salads. *Food Microbiology* **6**, 69–77.

Annous, B.A., Sapers, G.M., Mattrazzo, A.M. and Riordan, D.C.R. (2001) Effectiveness of washing with a commercial flatbed brush washer, using conventional and experimental washing agents, in reducing populations of *Escherichia coli* on artificially inoculated apples. *Journal of Food Protection* **64** (2), 159–163.

Bari, M.L., Kusunoki, H., Furukawa, H., Ikeda, H., Isshiki, K. and Uemura, T. (1999) Inhibition of growth of *Escherichia coli* O157:H7 in fresh radish (*Raphanus sativus* L.) sprout production by calcinated calcium. *Journal of Food Protection* **62**, 128–132.

Barriga, M.L., Trachy, G., Willemot, C. and Simard, R.E. (1991) Microbial changes in shredded iceberg lettuce stored under controlled atmospheres. *Journal of Food Science* **56**, 1586–1588, 1599.

Beuchat, L.R. (1992) Surface disinfection of raw produce. *Dairy Food and Environmental Sanitation* **12**, 6–9.

Beuchat, L.R. (1996) Pathogenic microorganisms associated with fresh produce. *Journal of Food Protection* **59**, 204–216.

Beuchat, L.R. (1998) Surface decontamination of fruit and vegetables eaten raw: a review. WHO/FSF/FOS 98 2. Rome, Italy: Food Safety Unit, World Health Organization.

Beuchat, L.R. (1999) Survival or enterohemorrhagic *Escherichia coli* O157:H7 in bovine feces applied to lettuce and the effectiveness of chlorinated water as a disinfectant. *Journal of Food Protection* **62**, 845–849.

Beuchat, L.R., Nail, B.V., Adler, B.B. and Clavero, M.R.S. (1998) Efficacy of spray application of chlorinated water in killing pathogenic bacteria on raw apples, tomatoes and lettuce. *Journal of Food Protection* **61**, 1305–1311.

Beuchat, L.R., Ward, T.E. and Pettigrew, C.A. (2001) Comparison of chlorine and prototype produce wash product for effectiveness in killing *Salmonella* and *Escherichia coli* O157:H7 on alfalfa seeds. *Journal of Food Protection* **64** (2), 152–158.

Bolin, H.R. and Huxsoll, C.C. (1991) Effect of preparation procedures and storage parameters on quality retention of salad-cut lettuce. *Journal of Food Science* **56**, 60–62, 67.

Brackett, R.E. (1987) Antimicrobial effect of chlorine on *Listeria monocytogenes*. *Journal of Food Protection* **50**, 999–1003.

Brackett, R.E. (1992) Shelf stability and safety of fresh produce as influenced by sanitation and disinfection. *Journal of Food Protection* **55**, 808–814.

Brackett, R.E. (1994) Microbiological spoilage and pathogens in minimally processed refrigerated fruits and vegetables. In *Minimally Processed Refrigerated Fruits and Vegetables* ed. Wiley, R.C. pp. 269–312. New York: Chapman & Hall.

Brecht, J.K. (1995) Physiology of lightly processed fruit and vegetables. *Hortscience* **30**, 18–22.

Center for Disease Control and Prevention (CDC). (1997) Update: Outbreaks of cyclosporiasis. *Morbid Mortality Weekly Report* **46**, 521–523.

Frank, J.F. and Koffi, R.A. (1990) Surface-adherent growth of *Listeria monocytogenes* is associated with increased resistance to surface to surfactant sanitizers and heat. *Journal of Food Protection* **53**, 550–562.

Garg, N., Churey, J.J. and Splittstoesser, D.F. (1990) Effect of processing conditions on the microflora of fresh-cut vegetables. *Journal of Food Protection* **53**, 701–703.

Giese, J.H. (1991) Sanitation: the key to food safety and public health. *Food Technology* **45** (12), 74–83.

Han, Y., Sherman, D.M., Linton, R.H., Nielsen, S.S. and Nelson, P.E. (2000) The effects of washing and chlorine dioxide gas on survival and attachment of *Escherichia coli* O157:H7 to green pepper surfaces. *Food Microbiology* **17**, 521–533.

ICMSF (1978) Microorganisms in foods. 1. Their significance and methods of enumeration. *International Commission on Microbiological Specifications for Foods.* 2nd edn. Toronto: University of Toronto Press.

Izumi, H. (1999) Electrolyzed water as a disinfectant for fresh-cut vegetables. *Journal of Food Science* **64**, 536–539.

Karapinar, M. and Gönül, S.A. (1992) Removal of *Yersinia enterocolitica* from fresh parsley by washing with acetic acid or vinegar. *International Journal of Food Microbiology* **16**, 261–270.

Lee, S.H. and Frank, J.F. (1991) Inactivation of surface adherent *Listeria monocytogenes* hypochlorite and heat. *Journal of Food Protection* **54**, 4–12.

Li, Y., Brackett, R.E., Shewfelt, R.L. and Beuchat, L.R. (2001) Changes in appearance and natural microflora of iceberg lettuce treated in warm, chlorinated water and then stored at refrigeration temperature. *Food Microbiology* **18**, 299–308.

Lund, B.M. (1983) Bacterial spoilage. In *Post-Harvest Pathology of Fruits and Vegetables* ed. Dennis, C. pp. 219. London: Academic Press.

Magnuson, J.A., King, A.D. Jr and Török, T. (1990) Microflora of partially processed lettuce. *Applied and Environmental Microbiology* **56**, 3851–3855.

Mazollier, J. (1988) IVème gamme. Lavage-désinfection des salades. *Infos-Ctifl* **41**, 20–23.

Mossel, D.A.A., Corry, J.E.L., Struijk, C.B. and Baird, R.M. (1995). *Essentials of Microbiology of Foods.* Chichester, UK: John Wiley.

Mustafa, A. and Liewen, M.B. (1989) Destruction of *Listeria monocytogenes* by sodium hypochlorite and quaternary ammonium sanitizers. *Journal of Food Protection* **52**, 306–312.

Nguyen-the, C. and Carlin, F. (1994) The microbiology of minimally processed fresh fruits and vegetables. *Critical Review of Food Science and Nutrition* **34**, 371–401.

Odumeru, J.A., Mitchell, S.J., Alves, D.M., Lynch, J.A., Yee, A.J., Wang, S.L., Styliadis, S. and Farber, J.M. (1997) Assessment of the microbiological quality of ready-to-use vegetables for health-care food services. *Journal of Food Protection* **60**, 954–960.

Pao, S. and Brown, G.E. (1998) Reduction of microorganisms on citrus fruits surfaces during packinghouse processing. *Journal of Food Protection* **61**, 903–906.

Saltveit, M.E. (1997) Physical and physiological changes in minimally processed fruits and vegetables. In *Phytochemistry of Fruit and Vegetables* ed. Tomás-Barberán, F.A. pp. 205–220. Oxford: Oxford University Press.

Shewfelt, R.L. (1987) Quality of minimally processed fruits and vegetables. *Journal of Food Quality* **10**, 143–156.

Simons, L.K. and Sanguansri, P. (1997) Dangerous residues after washing of vegetables. *Food Australia* **49**, 75–84.

Takeuchi, K. and Frank, J. (2001) Quantitative determination of the role of lettuce leaf structures in protecting *Escherichia coli* O157:H7 from chlorine disinfection. *Journal of Food Protection* **64** (2), 147–151.

Taormina, P.J. and Beuchat, L.R. (1999) Comparison of chemical treatments to eliminate enterohemorrhagic *Escherichia coli* O157:H7 on alfalfa seeds. *Journal of Food Protection* **62**, 318–324.

Tauxe, R., Kruse, H., Hedberg, C., Potter, M., Maddes, J. and Wachsmuth, K. (1997) Microbiological hazards and emerging issues associated with produce; a preliminary report to the National Advisory Committee on microbiologic criteria for foods. *Journal of Food Protection* **60**, 1400–1408.

Tomasoni, L., Previdi, M.P., Sansebastiano, G. and Chiuri, A. (2001) Lavaggio e sanitizzazione di verdure destinate alla IV gamma. *Industria Conserve* **76**, 21–31.

Von Jöckel, J. and Otto, W. (1990) Technologische und hygienische Aspekte bei der Herstellung und Distribution von vorgeschnittenen Salaten. *Archiv für Lebensmittelhygiene* **42**, 129–135.

Whitaker, J.R. (1995) Polyphenol oxidase. In *Food Enzymes. Structure and Mechanism* ed. Wong, D.W.S. pp. 271–307. New York: Chapman & Hall.

Wiley, R.C. (1994) Introduction to minimally processed refrigerated fruits and vegetables. In *Minimally Processed Refrigerated Fruits and Vegetables* ed. Wiley, R.C. New York: Chapman & Hall.