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Assessment of the Microbiological Quality and Wash Treatments of Lettuce Produced in Hofuf City, Saudi Arabia

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ABSTRACT

Microbial contamination of vegetables, especially leafy ones, is a common problem during growth under field conditions and their consumption without washing can create human health hazards. Therefore, the main objective of this study was to assess the microbial quality and determine suitable treatments of lettuce produced in Hofuf, Saudi Arabia. A total of 150 lettuce samples were collected for the study. The samples were treated with vinegar (5% acetic acid) and with disinfection tablets containing sodium dichloroisocyanurate for microbial infection treatment. The contamination of different microbes was determined under different growth media and temperatures. Mean counts of mesophilic aerobic bacteria were 5.06-6.70, 3.38-4.80 and log 4.97-5.99 CFU g^{-1} in outer leaves, inner leaves and composite samples of whole head leaves, respectively. Mean counts of coliforms, yeasts and molds didn't reach log 3 CFU g^{-1}. The counts of mesophilic aerobic bacteria seem to increase during handling, counts of coliforms and yeasts remain constant while those of molds decrease. The *E. coli* 1 was detected in 5 out of 45 samples tested for this bacterium while no *Salmonella* or *S. aureus* were detected in these samples. Soaking in vinegar was effective against mesophilic aerobic bacteria, coliforms and yeasts at concentrations 25-100% and reduced contamination by up to 3 log cycles but it was less effective against molds. In conclusion, treatments such as washing with tap water and vinegar (5% acetic acid) reduced the microbial contamination level significantly for safe human consumption.

Key words: Lettuce, microbial contamination, washing, vinegar, water

INTRODUCTION

Generally, the lettuce (*Lactuca sativa*) is eaten raw as salad and its whole microbial content will be consumed with it. Washing before consumption is the only possible means for reducing this load. Microbial contamination of lettuce from the farm can reach up to 10^8 CFU g^{-1} (Priepke *et al*., 1976; Garg *et al*., 1990; Jayasekara, 1999; Delaquis *et al*., 1999; Amponsah-Doku *et al*., 2010; Moyne *et al*., 2011). The microbial contamination is mostly on the outer leaves. The main contaminants are bacteria and fungi (Nguyen-The and Carlin, 1994). In Spain, Garcia-Villanova *et al*. (1987) reported *Salmonella* contamination in 7.5% of vegetables sampled from the market. Reports indicate that washing with running tap water can reduce contamination by up to one log cycle while treatment with disinfectants like ozone, chlorine or vinegar reduced this load by 1-7 log cycles (Yuan, 2002; Rivera, 2002; Nacimento *et al*., 2003).

Presently, lettuce is being produced in agricultural farms in the suburbs of Hofuf City, Al-Ahsa, Eastern Province, Saudi Arabia under varying agricultural management practices. There is a possibility of its contamination with different microbes during growth. A little information is available on the microbial contamination of locally grown lettuce and can be a serious problem of human health upon its consumption without proper insect-pest management. Therefore, the aim of this study was to assess the microbial contamination of lettuce samples collected from a farm and from markets in Hofuf city and to investigate the effect of washing with tap water, vinegar and disinfection tablets on their microbial load.

MATERIALS AND METHODS

Collection of Samples: One hundred and fifty lettuce (*Lactuca sativa*) head samples were collected from a farm, whole sale market and shops in Hofuf City. The marketing chain of the crop involves many steps such as manual harvest, collection in plastic boxes, transportation to the whole sale market and from there to the shops without washing or further packaging treatments. The samples were collected on weekly basis over a period of 3 months. The investigators tracked and took samples from whole sale market and shops supplied with the products (lettuce) that originated from the same farm. First 45 samples were collected to investigate differences in the amount of contamination between outer and inner leaves of the head and to detect some potential pathogenic bacteria. Then 105 samples were taken to investigate the amount of contamination of composite outer and inner leaves of the whole head for washing and soaking treatments. The lettuce samples were taken to the laboratory and analyzed on the same day for levels of contamination.

Sample preparation: In order to determine the general contamination, the outer (about 3 layers) and the inner leaves (rest of the head) were analyzed separately or the leaves of the whole head were analyzed as a composite sample. All samples were minced in sterile mincer (Moulinex) before analysis.

Treatments of samples: The lettuce samples were washed in running tap water for 2 min or soaked in vinegar or water containing disinfection tablets for 15 min.

Vinegar: The vinegar was obtained from the local market containing 5% acetic acid.

Disinfection tablets: Disinfection tablets containing sodium dichloroisocyanurate, called as salad wash, were obtained from the local market. One tablet, when dissolved in 20 L water, was supposed to provide about 50 mg L^{-1} of available chlorine.

Microbiological analysis: Aerobic mesophilic bacteria were counted on Plate Count Agar dishes (PCA Oxoid, CM0325) by incubating at 30°C for 2-3 days. The coliforms were counted on Violet Red Bile Agar (VRBA Oxoid, CM0107) by incubating at 37°C for 24-48 h. The yeasts and molds were counted on Potato Dextrose Agar (PDA Oxoid, CM0139) by incubating at 30°C for 2-3 days. The *E. coli* were determined in Lauryl Tryptose Broth (LST, Oxoid, CM0451) by incubating at 35°C for 24-48 h. The EC broth (Oxoid, CM0853) were determined by incubating at 45.5°C for 24-48 h and in Levine's Eosin-Methylene Blue Agar (L-EMB Agar, Oxoid, CM0069) by incubating at 35°C for 18-24 h. For further identification of *E. coli,* 15 isolates were made from 5 positive samples (3 isolates from each) and tested using the api 20 E method and identified after the api 20 E analytical profile index (bioMérieux sa, France).

Salmonella was incubated in lactose broth (CM0137) for 24 h at 35°C. Then the samples were placed in Rappaport-Vassiliadis (RV, Oxoid CM0669) and tetrathionate (TT, Oxoid CM0671) broth for 24 h at 42 and 43°C, respectively. Next, the samples were placed on bismuth sulfite (BS, Oxoid CM0201) agar, xylose lysine desoxycholate (XLD, Oxoid CM0469) agar and Hektoen enteric (HE, Oxoid CM0419) agar for incubation at 35°C for 24 h. Finally the previously treated samples were placed in Triple sugar iron agar (TSI, Oxoid CM0277)) and Lysine iron agar (LIA, Oxoid CM0381) for incubation at 35°C for 24 h. The *Staphylococcus aureus* were determined on Baird-Parker agar (Oxoid, CM0961) by incubating at 35°C for 45-48 h.

Statistical analysis: Duncan multiple range test at 5% level of significance was used to compare between means. The analysis was carried out using the ANOVA procedure of Statistical Analysis System according to SAS Institute (2001).

RESULTS AND DISCUSSION

Forty five lettuce head samples (15 samples each from a farm, whole sale market and a shop) were analyzed for contamination of the outer and inner leaves with mesophilic aerobic bacteria, coliforms, yeasts, molds, *Escherichia coli*, *Salmonella* and *Staphylococcus aureus*. Mean total count of mesophilic aerobic bacteria in the outer leaves of farm samples was log 5.06 CFU g^{-1} with a range of log 3.00-6.38 CFU g^{-1}. In the whole sale market samples, mean count was log 6.07 CFU g^{-1} and ranged from log 5.08-7.11 CFU g^{-1} whereas, in the shop samples, mean count was log 6.70 CFU g^{-1} and ranged from log 5.53-8.18 CFU g^{-1} (Table 1). However, the count of this group of bacteria increased steadily from the farm to the shop with significant difference between the shop and the farm samples. This indicated that mesophilic aerobic bacteria grow on the outer leaves of the head during handling and hence can be regarded as potential spoilage agent. Johnston *et al.* (2005) reported increases in total bacterial count of cilantro, parsley and cantaloupe from harvest through packing. There were slight increases in the coliform contamination of outer leaves for samples from the farm, the market and the shop but with no significant differences. Means counts were log 1.51, 1.53 and 1.85 CFU g^{-1} and the ranges were from not detected to log 2.78, 2.74 and 3.18 CFU g^{-1} for the three locations, respectively (Table 1). This indicates that coliforms did not grow in lettuce during handling but remained persistent. In case of yeasts, the amount of contamination in the market and shop samples was higher than the farm samples. In the case of molds, it was higher in the farm samples than the market and shop samples, although the differences were not significant in both cases (Table 1). This means that yeasts can remain persistent while molds seem to die out during handling.

Table 1: Microbial contamination (log CFU g^{-1}) of outer leaves from 45 samples of lettuce heads collected from 3 sources (15 samples from each)

	MAB		Coliforms		Yeasts		Molds	
Source	Mean	Range	Mean	Range	Mean	Range	Mean	Range
Farm	5.06±1.46^{b}	3.00-6.38	1.51±1.12^{a}	n.d.-2.78	0.85±1.10^{a}	n.d.-2.69	2.71±0.39^{a}	2.40-3.30
WSM	6.07±0.92^{ab}	5.08-7.11	1.53±1.40^{a}	n.d.-2.74	2.30±1.29^{a}	n.d.-3.08	1.86±1.90^{a}	n.d.-4.48
Shop	6.70±1.14^{a}	5.53-8.18	1.85±1.18^{a}	n.d.-3.18	2.09±1.27^{a}	n.d.-3.18	0.90±1.23^{a}	n.d.-2.30

MAB: Mesophilic aerobic bacteria, WSM: Whole sale market, n.d.: Not detected. Mean±SD, Mean values in a column followed by the same letter are not significantly different at 5% level of confidence

The contamination of inner leaves with mesophilic aerobic bacteria was about 2 log cycles lower than that of outer leaves (Table 2). The mean values (log CFU g^{-1}) of mesophilic aerobic bacteria were 5.06, 6.07 and 6.70 for MAB, coliform, yeasts and molds under farm, whole sale market and shops, respectively. While for the coliform, the mean values (log CFU g^{-1}) were 3.00, 5.08, 5.53 for MAB, coliform, yeasts and molds under farm, whole sale market and shops, respectively. With respect to yeasts, the mean values (log CFU g^{-1}) were 0.85, 2.30 and 2.09 for MAB, coliform, yeasts and molds under farm, whole sale market and shops, respectively. For the molds, the mean values (log CFU g^{-1}) were 2.71, 1.86 and 0.90 for MAB, coliform, yeasts and molds under farm, whole sale market and shops, respectively. The load from this group of bacteria increased during handling from farm to market and shop, although the differences were not significant. The level of contamination of inner leaves with coliforms and yeasts was about the same as that of outer leaves, but no significant differences were observed in the contamination levels among the samples from farm, market and shop (Table 2). The contamination level with molds was generally lower in the inner than the outer leaves, but still no significant differences were observed in the level of contamination among the samples from three different locations (farm, market and shop). Similar views were expressed by Garcia-Villanova *et al.* (1987) for microbial contamination especially Salmonella on different vegetables sampled from the market.

The *E. coli* was detected in 5 out of 45 samples tested for this bacterium (about 11%). It was detected in the outer leaves only at 7.0, 39.0 and 93.0 MPN g^{-1} in 3 samples and at 1.5×10^2 and 4.6×10^2 MPN g^{-1} in 2 samples while *Salmonella* and *Staphylococcus aureus* were not detected in any sample. The Saudi Standard for fresh vegetables consumed without cooking (SASO, 1999) has *E. coli* limit of 10^2 CFU g^{-1}, hence only 2 samples were out of Saudi specifications. The 15 isolates made from the 5 lettuce samples were further identified as three strains of *E. coli* 1 according to api 20 E method (Table 3). Three isolates gave identical numerical profile of 5044572 and the quality of identification as *E. coli* 1 was very good with %id = 99.2 and T-value = 0.91. Six isolates gave identical numerical profile of 5144552 and the quality of identification as *E. coli* 1 was good with %id = 97.7 and T-value = 1.0. Six isolates gave identical numerical profile of 1044552 and the quality of identification as *E. coli* 1 was good with %id = 69.3 and T-value = 0.86.

An analysis of the composite whole lettuce head leaves of 105 samples from farm, market and shop (35 samples from each location) showed mean mesophilic aerobic bacteria counts of log 4.97, 5.56 and 5.99 CFU g^{-1} with ranges of log 3.52-5.48, 4.29-5.99 and 4.57-6.38 CFU g^{-1} for the three locations, respectively (Table 4). The load increased during handling from farm to market and shop with significant differences between farm samples and samples from market and shop. This confirms the observation made above that mesophilic aerobic bacteria grow in lettuce during handling. The mean counts of coliforms and yeasts were <log 2 CFU g^{-1} with no significant

Table 2: Microbial contamination (log CFU g^{-1}) of inner leaves from 45 samples of lettuce heads collected from 3 sources (15 samples from each)

	MAB		Coliforms		Yeasts		Molds	
Source	Mean	Range	Mean	Range	Mean	Range	Mean	Range
Farm	3.38±0.62[a]	2.30-3.89	1.08±1.08[a]	n.d.-2.43	0.86±1.30[a]	n.d.-2.92	0.36±0.81[a]	n.d.-1.81
WSM	4.00±0.91[a]	3.23-5.00	1.45±1.32[a]	n.d.-2.45	2.36±1.33[a]	n.d.-3.18	0.97±0.91[a]	n.d.-1.92
Shop	4.80±1.40[a]	3.26-5.98	1.59±0.97[a]	n.d.-2.40	2.08±1.35[a]	n.d.-3.66	0.62±0.84[a]	n.d.-1.60

MAB: Mesophilic aerobic bacteria, WSM: Whole sale market, n.d.: Not detected. Mean±SD, Mean values in a column followed by the same letter are not significantly different at 5% level of confidence

Table 3: Profiles of the api 20 E biochemical tests for 15 *E. coli* isolates obtained from 5 lettuce samples. Isolates 1-4, 5-10 and 11-15 have numerical profiles 5144552, 5044572 and 1044552, respectively, all identified as *E. coli* 1

	Isolates														
Test name	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
B-galactosidase	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Arginine dehydrogenase	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Lysine decarboxylase	-	-	-	+	+	+	+	+	+	+	+	-	+	+	+
Ornithine decarboxylase	-	-	-	+	+	+	+	+	+	-	-	-	-	-	-
Citrate	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
H_2S	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Urease	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Tryptophan deaminase	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Indole	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Voges proskauer	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Gelatin hydrolysis	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Glucose	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Mannitol	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Inositol	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Sorbitol	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Rhamnose	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Sucrose	-	-	-	-	-	-	-	-	-	+	+	-	+	+	+
Melibiose	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Amygdaline	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Arabinose	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Oxidase	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-

+: Present, -: Absent

Table 4: Microbial contamination (log CFU g^{-1}) of 105 composite samples (mixed leaves) from lettuce heads collected from 3 sources (35 samples from each)

	MAB		Coliforms		Yeasts		Molds	
Source	Mean	Range	Mean	Range	Mean	Range	Mean	Range
Farm	4.97±0.75^{b}	3.52-5.48	2.43±0.63^{a}	1.76-3.13	2.52±0.86^{a}	1.72-3.61	2.16±0.60^{a}	1.75-3.15
WSM	5.56±0.66^{a}	4.29-5.99	2.36±1.03^{a}	n.d.-3.52	2.11±1.01^{a}	n.d.-3.00	1.40±0.42^{ab}	n.d.-2.04
Shop	5.99±0.59^{a}	4.57-6.38	2.15±0.85^{a}	n.d.-2.97	2.71±0.73^{a}	1.99-3.75	1.17±0.74^{b}	n.d.-1.80

MAB: Mesophilic aerobic bacteria, WSM: Whole sale market, n.d.: Not detected. Mean±SD, Mean values in a column followed by the same letter are not significantly different at 5% level of confidence

differences among samples of farm, market and shop and the ranges were 1.76-3.13, n.d.-3.52 and n.d. -2.97 CFU g^{-1} for the farm, market and shop, respectively. With regard to molds, mean counts were 2.16, 1.40 and 1.17 with significant decrease during handling from farm to shop (Table 4) which confirms the observation made above that molds seem to die out during handling. Generally, the levels of contamination found in the samples tested were comparable to values reported in the literature (Iris *et al*., 2008; Soriano *et al*., 2000; Nguyen-The and Carlin, 2000; Ercolani, 1976).

Washing treatments with running tap water and soaking in different concentrations of vinegar or disinfection tablets gave varying degrees of effect on the microbial contamination of lettuce samples. The contamination of mesophilic aerobic bacteria was reduced by a mean of 0.95 log cycles by washing treatment (Table 5). Whereas, the mean reduction of this bacteria was 1.07 and

Table 5: Reduction (log cycles) in microbial contamination of lettuce samples after different washing treatments

Treatment	MAB	Coliforms	Yeasts	Molds
Water	0.95±0.05^{d}	1.14±0.11^{cd}	0.96±0.08^{c}	1.12±0.25^{b}
Tablets	1.07±0.03^{c}	0.90±0.05^{d}	1.15±0.06^{bc}	0.87±0.14^{bc}
10% vinegar	1.15±0.06^{c}	1.14±0.05^{cd}	1.05±0.11^{c}	0.36±0.19^{d}
25% vinegar	2.12±0.15^{b}	1.48±0.06^{bc}	1.33±0.04^{b}	0.59±0.28^{cd}
50% vinegar	2.23±0.06^{b}	1.72±0.30^{b}	1.37±0.34^{b}	0.89±0.08^{b}
100% vinegar	3.04±0.07^{a}	2.95±0.52^{a}	2.17±0.11^{a}	2.68±0.17^{a}

MAB: Mesophilic aerobic bacteria. Mean±SD, Mean values in a column followed by the same letter are not significantly different at 5% level of confidence

1.15 log cycles with soaking in disinfection tablets and 10% vinegar, respectively. The reduction was higher by treatment with disinfection tablets and the vinegar than simple washing treatment with tap water. However, there was no significant difference between these two treatments. The effectiveness of vinegar treatment increased significantly further to reach the mean values of 2.12 and 2.23 log cycle reductions when 25 and 50% vinegar was used, respectively, but no significant difference between the two treatments. The highest reduction in contamination with mesophilic aerobic bacteria reached when 100% vinegar was used, where a mean reduction of 3.04 log cycles was registered which was significantly higher than all other treatments. Since, contamination of lettuce with this group of bacteria is in the range log 4-6 CFU g^{-1}, soaking in vinegar in concentrations from 25-100% will reduce this contamination to levels that can increase shelf life of the produce considerably. In case of coliforms, the effects of washing with running tap water or soaking in disinfection tablets or 10% vinegar were not significantly different with mean reductions of 1.14, 0.90 and 1.14 log cycles, respectively. The effect increased with 25% vinegar to reach mean reduction of 1.48 log cycles, but it was not significantly different from the effects of washing with tap water or soaking in 10% vinegar (Table 5). The use of 50% vinegar reduced contamination of coliforms by a mean of 1.72 log cycles which was not significantly different from the effect of 25% vinegar. The highest effect was obtained by 100% vinegar to reach reduction with mean 2.95 log cycles which was significantly higher than all other treatments. Since, contamination of lettuce with coliforms is generally in the range of log 1-2, soaking in vinegar at 25-100% concentrations will reduce this contamination to generally safe levels. On the other hand, washing with running water and soaking in disinfection tablets and 10% vinegar reduced contamination with yeasts by means of 0.96, 1.15 and 1.05 log cycles, respectively, with no significant differences between these treatments (Table 5). Use of 25 and 50% vinegar increased the treatment effect to mean reductions of 1.33 and 1.37 log cycles, respectively which were not significantly different from each other and also not significantly different from the effect of the disinfection tablets (Table 5). The highest effect was obtained by using 100% vinegar with a mean reduction of 2.17 log cycles. Since, contamination of lettuce with yeasts is generally in the range of log 1-2, all of these washing treatments will reduce this contamination to safe levels. Molds seem to be the least group affected by treatment with vinegar. The 10 and 25% vinegar treatments caused mean reductions of 0.36 and 0.59 log cycles, respectively (Table 5). The effect of disinfection tablets was also low, with mean reduction of 0.87 log cycles which was not significantly different from the effect of 25% vinegar. The 50% vinegar brought mean reduction of 0.89 log cycles which was still lower than that of the water washing, but not significantly different from the effect of washing with running tap water of 1.12 log cycles. The highest effect was caused by using 100% vinegar with mean reduction of 2.68 log cycles which was significantly higher than all other treatments. Since, mold contamination

in lettuce seem to decrease during handling, hence they form no spoilage threat and the reductions reached by these treatments can be considered enough for their control. Nacimento *et al.* (2003) reported decimal reductions in contamination of lettuce with mesophilic aerobic bacteria, molds and yeasts, coliforms and *E. coli* of 0.78, 0.87, 0.82 and 0.14 log CFU g^{-1}, respectively, in water; these were in excess of 2.89, 3.41, 2.21 and 0.26 log CFU g^{-1}, respectively in 50% vinegar. While these were in excess of 2.42, 3.20, 1.99 and 0.26 log CFU g^{-1}, respectively in 25% vinegar treatment.

CONCLUSION

The study findings showed that mean total count of mesophilic aerobic bacteria in the outer leaves of farm samples was log 5.06 CFU g^{-1} with a range of log 3.00-6.38 CFU g^{-1}. In the whole sale market samples, mean count was log 6.07 CFU g^{-1} and ranged from log 5.08-7.11 CFU g^{-1} whereas, in the shop samples, mean count was log 6.70 CFU g^{-1} and ranged from log 5.53-8.18 CFU g^{-1}. Mean counts of coliforms, yeasts and molds didn't reach log 3 CFU g^{-1}. Also, mean counts of mesophilic aerobic bacteria seem to increase during handling, counts of coliforms and yeasts remain constant while those of molds decrease. The *E. coli* 1 was detected in 5 out of 45 samples tested for this bacterium while no *Salmonella* or *S. aureus* were detected in these samples. Soaking in vinegar was effective against mesophilic aerobic bacteria, coliforms and yeasts at concentrations 25-100% and reduced contamination by up to 3 log cycles but it was less effective against molds. The contamination of inner leaves with mesophilic aerobic bacteria was about 2 log cycles lower than that of outer leaves. An analysis of the composite whole lettuce head leaves of 105 samples from farm, market and shop (35 samples from each location) showed mean mesophilic aerobic bacteria counts of log 4.97, 5.56 and 5.99 CFU g^{-1} with ranges of log 3.52-5.48, 4.29-5.99 and 4.57-6.38 CFU g^{-1} for the three locations, respectively. The mean counts of coliforms and yeasts were <log 2 CFU g^{-1} with no significant differences among samples of farm, market and shop and the ranges were 1.76-3.13, n.d.-3.52 and n.d.-2.97 CFU g^{-1} for the farm, market and shop, respectively.

Overall study indicated that there is potential for reducing the microbial contamination of locally grown lettuce by treatment with vinegar, washing with tap water before consumption and packing it on scientific grounds before selling in the market.

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