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Pre-wash chopping and storage conditions of parsley limit the effectiveness of washing methods in SMEs against *S.*Typhimurium

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Abstract

Initial chopping of parsley before washing for subsequent processing into ready-to-eat foods later in the day is common in some restaurants. The aim was to evaluate the influence of pre-wash chopping on the *S.* Typhimurium decontamination by common washing and disinfection methods. including the use vinegar (4%, v/v, acetic acid), 0.25g/l sodium dichloroisocyanurate (NaDCC), and water combined with manual agitation. This study demonstrated limited efficiency of applied methods and that holding pre-wash chopped leaves at 30ºC reduced the effectiveness of all washing solutions. SEM imaging indicated initiation of biofilm formation after 24 h at 5ºC with noticeable adhesion of cells to inaccessible folds of the vein on the leaf surface. NaDCC was shown to be the most effective solution achieving log reductions of 1.92 to 3.12. Its effect was reduced by 0.73 and 1.3 log cfu/g on chopped leaves at 5ºC and on both intact and chopped leaves at 30ºC, respectively. This type of decontamination could apply well to other leafy greens as an affordable and convenient sanitation method in small restaurants. In conclusion, strict temperature control and avoiding pre-wash chopping are highly recommended during handling of parsley for the optimal elimination of pathogenic microorganisms.

1. Introduction

Fresh leafy greens continue to pose health risks due to its exposure to microbiological contamination though usage of untreated irrigation water (Pachepsky et al., 2011) , inappropriate organic fertilizers and untreated manure, presence of wildlife or malpractices that can take place during harvesting, handling, transportation, processing and packaging (Olaimat & Holley, 2012). Hence, it is widely recognized that fresh produce is among foods that necessitate safe handling practices to prevent foodborne disease (McCabe-Sellers & Beattie, 2004). However, these are not easily achieved and despite the numerous studies and efforts to develop mitigation strategies, several outbreaks of human infections linked to consumption of fresh vegetables persisted and have been increasingly documented (Buck et al., 2003; Sodha et al., 2011). These have been linked to norovirus, *E. coli* O157:H7, *Campylobacter*, *Listeria monocytogenes,* and *Salmonella* with the last being the most frequently encountered in outbreaks, especially linked to leafy greens (Barton et al., 2011; IFSAC, 2015; Patel & Sharma, 2010). *Salmonella* spp. are usually transmitted to humans by eating food contaminated with animal faeces (e.g., birds, domestic and wild animals grazing on crop fields); Some habitats, such as ponds and drainage ditches are also potential avenues for fresh produce contamination, besides the unhygienic hand contacts during the post-harvest practices though the food chain (Beuchat & Ryu, 1997; Buck et al., 2003).

Besides *E.coli* O157, *Campylobacter* spp., and *Listeria monocytogenes, Salmonella* is considered as the one of those most common severe pathogens associated with outbreaks linked to fresh produce (Barton et al., 2011; IFSAC, 2015), in addition to leafy greens being recognized as the leading source of food poisoning illnesses(Patel & Sharma, 2010). Within the environment, *Salmonella* spp. are usually transmitted to humans by eating food contaminated with animal faeces (e.g., birds, domestic and wild animals grazing on crop fields), as well as water from ponds and drainage ditches in addition to unhygienic hand

contacts during harvesting and post-harvesting practices though the food chain ((Beuchat & Ryu, 1997; Buck et al., 2003).

Inadequate post-harvest cleaning procedures allow the bacteria remaining on surfaces of contaminated fresh vegetables to initiate growth when subjected to optimum conditions during handling and storage (Koseki & Isobe, 2005).. There is a body of evidence that pathogenic microorganisms attached on the surfaces of vegetables particularly on cut surfaces are able to colonize in biofilms (Beuchat, 2002; Ells & Truelstrup Hansen, 2006; Fett, 2000; Tang, 2012) which could actually limit and interfere with the disinfecting efficacy of various sanitizers (Koseki et al., 2001b; H. Ölmez & Temur, 2010). Consequently, the survival of attached pathogens on fresh produce not subjected to subsequent heat treatment pose health risks to consumers.

Further down the produce chain, mishandling in food service operations has been linked to several reported food poisoning outbreaks involving fresh vegetables (CDC, 1999, 2007; De Jong et al., 2007; MacDonald et al., 2011). Investigations of outbreaks of foodborne disease in England and Wales (1992-2006) that were associated with ready-to-eat salads indicated that the majority of the outbreaks occurred in the foodservice and catering sectors and were linked to infected food handlers, cross contamination and poor storage (Little & Gillespie, 2008). The most common pathogen involved was *Salmonella* followed by norovirus (Todd and Greig (2015). Figures for the developing countries such as those in the Middle East are relatively scarce on foodborne illnesses associated with the consumption of raw vegetables, although leafy vegetables such as parsley are often consumed raw in various traditional salad meals, or mezze garnishes. However, one recent study pointed out at several risk factors that may contribute to microbial contamination of leafy greens from farms destined to wholesale and retail markets in Beirut (Lebanon) by Faour-Klingbeil et al. (2016). One challenge is to find effective washing and sanitization procedures for fresh vegetables, as critical steps to ensure appropriate safety without adversely affecting the sensory, and

nutritional characteristics of fruits and vegetables (Martínez-Sánchez et al., 2006). To this end, the use of sanitizing agents such as chlorine-based compounds, ozone, peroxyacetic acid, electrolyzed water, and organic acids in various postharvest operations is widespread (Kilonzo-Nthenge et al., 2006; Hülya Ölmez & Akbas, 2009; H. Ölmez & Temur, 2010; Rahman et al., 2010; Ramos et al., 2014; Vandekinderen et al., 2009). However, chemical compounds based sanitizers such as the inorganic chlorine compounds have been reported to produce hazardous by-products (FDA, 1998; Kim et al., 2012) and alteration of the quality at doses permissible to eliminate pathogens (Beuchat & Ryu, 1997). Hence, Sodium dichloroisocyanurate (NaDCC) known also as Troclosene Sodium, has been advocated as an alternative to chlorine to treat water, with the advantage of leaving no odour or taste and prolonged effectiveness (Clasen & Edmondson, 2006).

NaDCC is a di-chlorinated isocyanuric acid derivative (Arbor, 2008) that upon dissolving in water releases a variety of chlorinated and non-chlorinated isocyanurates and free available chlorine in the form of hypochlorous acid, recognized for its oxidation property and as a microbicidal agent (Clasen & Edmondson, 2006). Furthermore, it has been reported to be effective to sanitize fresh vegetables against *Salmonella* spp.(Nascimento et al., 2003). As efforts are concerted towards seeking new interventions and bio sanitizers, organic acids such as acetic and citric acids have been tested for removal of pathogens from fresh fruits and vegetables (Rhee et al., 2003; Wu et al., 2000, Karapinar et al., 1992). These have been shown to be effective, convenient and economic to reduce microbial populations at the foodservice and household levels, with the additional advantage of a cleaner image. These sanitizers have to be effective enough to eliminate really low levels as few as 10 to 100 cells of *Salmonella* on parsley leaves which still constitute a potential health risk (Kisluk et al., 2012) and improper storage of cut produce can allow rapid growth of bacteria, as reported in an outbreak of salmonellosis in Germany that was traced to paprika with an estimated infective dose as low as 4 to 45 *salmonella* and stated by Kisluk et al. (2012).

Unfortunately, in many small and medium sized foodservice facilities (SMEs) in the Middle East, these sanitizing agents are rarely used. For instance, in an assessment of food handlers knowledge and practices in 50 restaurants in Beirut (Faour-Klingbeil et al., 2015), it was observed that washing fresh vegetables with tap water was the most common method, followed with the use of a locally available commercial sanitizer, and vinegar. Parsley was often chopped before washing and in some cases kept on hold in warm ambient temperatures of 30-33ºC, in suitable conditions for pathogenic bacterial growth. (Faour-Klingbeil et al., 2015).

There has been no attempt so far, at least in the Middle East and North Africa region (MENA) to address the efficacy and safety of washing methods typically applied in foodservice establishments on intact and cut parsley leaves *in situ* conditions. SMEs serving raw parsley in ready-to-eat salads or sandwiches are popular, not only in Lebanon, but also for Syrian and Turkish food outlets. The aim of this study was to examine the effect of the pre-wash chopped parsley in different time-temperature conditions on the decontamination effect of simple and practical washing methods, , with the view of supporting recommendations for safe handling practices of fresh leafy greens in SMEs.

2. Materials and methods

2.1 Preparation of parsley

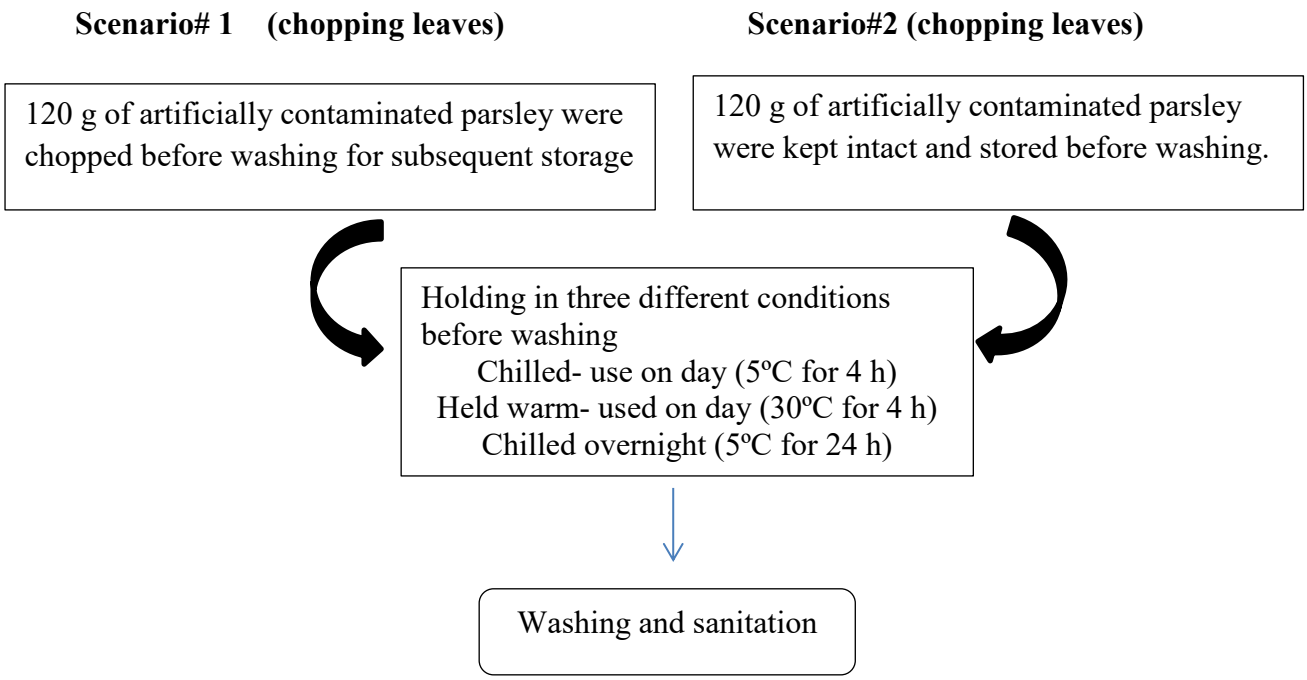
Bundles of fresh parsley (*Petroselinum crispum*. var. *neapolitanum)* were purchased from a local retailer and used on the same day. Bruised and yellow leaves were discarded and the remaining intact green leaves were washed with running tap water to remove soils and dirt (H. Ölmez & Temur, 2010; Sengun & Karapinar, 2004) for approximately 1 minute. Leaves were taken off the stems while keeping 2-3cm of the petioles, as prepared locally.

2.2 Rationale for the applied scenarios

The scenarios used in this study were based on observations derived from an assessment survey of food handlers practices in 50 restaurants in Beirut (Faour-Klingbeil et al., 2015) and on observed practices of handling salad vegetables. It was noted that parsley is often chopped early mornings before washing, to preserve the leaves texture by avoiding sogginess if chopped wet. It is held in uncontrolled environments, either in refrigerators or on shelves for variable periods until subsequent washing procedures, prior to serving at lunch or dinner services. . In some cases, the chopped parsley was kept in a refrigerator until next day (when not served).

Washing was done by immersing parsley leaves in water for 15 minutes followed by a rigorous manual agitation in the sink, then rinsing two or three times was observed in some small establishments, whereas others used NaDCC. A few outlets applied white vinegar in water for 15 minutes, however in unspecified and variable amounts. The experimental design resembled the same washing methods while maintaining the exposure time constant (15 min) for all solutions to observe of the effects of either chopping or not before washing, 3 different holding time-temperatures, and 5 different washing solutions on S.Typhimurium decontamination.

The scenarios were as follows:



2.3 Preparation and application of washing solutions

Commercial white vinegar containing 5% acetic acid was purchased from a local supermarket and diluted with sterile water to prepare a solution of 4% acetic acid (pH 2.9). This concentration has been previously reported by Sengun and. Karapinar (2005) and Ramos et al. (2014). A solution of 1000 ppm available chlorine (Chlor-Clean®, pH 5.94) and 0.25g/l Sodium dichloroisocyanurate (NaDCC) (Presept®, pH 6.14) were prepared. The 1000 ppm chlorine solution was included for reference (a concentration greater than 200 ppm of total chlorine is sufficient to achieve the desired sanitizing effect (FDA, 1998). Deionized water (Milli-Q plus) was used for rinsing twice, with manula agitation (2-3 seconds in 3 successions).. The pH of all treatment solutions was measured before and after 15 minutes exposure.

The inoculated parsley (20 g) was immersed into 200 ml of each washing solution in sterile bags to cover all the leaves for 15 min at about22ºC. After decanting (Lang et al., 2004),

sterile bags were held upright in biosafety cabinet for 2-3 minutes, with additional light shaking to remove remaining drops of solutions on the leaves.

All experiments were replicated at least 3 times and carried out in duplicate.

2.4 Preparation of *S.* Typhimurium culture and cell suspension

Freeze-dried *Salmonella enterica* serovar Typhimurium (*S.* Typhimurium) strain LT2 was obtained from the School of Biological Sciences (Plymouth University). A. Cultures were grown from a stock kept at -80ºC, in brain heart infusion (BHI) broth overnight at 37ºC, streaked on blood agar plates and incubated overnight at 37ºC.

Then 1-2 colonies were cultured for 18 h at 37 °C in tryptone soya broth (TSB,Oxoid, Basingstoke, Hampshire, England) to provide an initial inoculum of approximately 10^9cells/ml grown to stationary phase, as confirmed by plating, because bacterial cells are generally more tolerant than are logarithmic growth phase cells to environmental stresses (Miller et al., 2009) and *Salmonella* cells showed 1000-fold more acid resistance than logarithmic phase cells exposed to pH 3 for 1 hour (Lee et al., 1994).

Simultaneously, 1-2 colonies of the *S.* Typhimurium stock cultures adapted gradually to nalidixic acid (50µg/ml) were cultured by transferring to 10 ml tryptone soya broth (TSB,Oxoid, Basingstoke, Hampshire, England) supplemented with nalidixic acid (50µg/ml) (TSBN) followed by incubation for 18 h at 37ºC.

Inoculum cell suspension of *S.* Typhimurium was prepared by transferring 1 ml of the overnight suspension containing 10^9cfu/ml into 1 l of 0.1% peptone water (PW) to achieve the targeted concentration of approximately 10^6CFU/ml. To obtain the low inoculum level (10^3 cfu/ml), 1 ml of 10^6 dilutions prepared from overnight culture was transferred into 1 l of PW.

2.5 Inoculation of parsley

Washed parsley was left to drain on sterile paper towels in a biosafety cabinet for approximately 1 h prior to dipping on in inoculum suspensions (120g in 1l), containing targeted levels of bacteria, for 60 min with occasional manual agitation (Lapidot et al., 2006). After draining, the samples were dried on sterile towels in the biosafety cabinet for 1 h at ambient temperature (22±1ºC). The target inoculation level was higher than the typically expected cross contamination levels, to allow an effective observation of bacterial reductions and the elimination effect of each factor. Considering the unlikelihood of high concentration of *Salmonella*, we examined the factors effects on parsley with low inoculum levels in two selected variables for additional validation of the trend in log reduction.

Control samples of unwashed inoculated parsley were taken before and after the washing procedures.

2.6 Detection and enumeration of *S.*Typhimurium

To determine the initial presence of *Salmonella* on parsley, homogenates of 25g in 225ml buffered peptone water (Biorad, UK) were incubated at 37ºC for 24 h, then 0.5ml was transferred to 9.5ml selective enrichment in Rappaport Vassalidis (Biorad, UK) and incubated at 45ºC for 24 h. Later, a loopful of enriched solution was streaked on Rapid'*Salmonella* agar (Biorad, UK) for detection purposes.

*S.*Typhimurium enumeration was performed before and after the washing procedures. A 10 g sample of parsley was aseptically suspended into 90 ml of TSB in a stomacher bag and homogenized for 2 minutes at 230 rpm. To determine the levels of *Salmonella* on the washed parsley, homogenates were serially diluted and 0.1 ml aliquots was plated in duplicate, in addition to 1 ml aliquots pipetted over 4 plates of PCA supplemented with 50µg/ml nalidixic acid. The background flora still overgrew the test pathogen in a number of replicates, which has been also reported by Gündüz et al. (2010). In preliminary trials on non-selective media (unreadable plates), the population size difference was 0.5 log cfu/g compared to selective

agar and 1 Log cfu/g for chlorine-treated samples. It is worth noting that the difference on the recovery of *Salmonella* cells between selective and non-selective media was found to be insignificant by Gündüz et al. (2010). Therefore, to determine the survival of *Salmonella* enumerations were performed on a selective agar (Karapinar & Sengun, 2007; Patel & Sharma, 2010) where typical pink colonies were counted after incubation at 37ºC for 24-48 h. Initial trials with nalidixic acid supplemented selective media to increase the selectivity resulted in smaller *Salmonella* colonies and occasional loss; hence we omitted this step as this medium was already highly selective.

To determine the viable uncultured cells on samples with inoculum of low levels (10^3), non-selective pre-enrichment, followed by selective enrichment according to ISO 16140 N° BRD 07/11-12/05 was performed.

Mean values of bacterial counts (CFU/g) from duplicate plate samples were log10 converted..

2.7 Scanning Electron Microscopy (SEM) of parsley leaves

The SEM imaging was performed to examine attachment of the test pathogen on the surface of parsley leaf to determine sites that featured preferential attachment and tor understand potential reasons for washing efficiency. The procedure of sample preparation for SEM examination was based on the protocol of Pathan et al. (2008) and on that described by Ells and Truelstrup Hansen (2006) and Ölmez and Temur (2010). Parsley leaves were removed from the bacterial suspensions after 24 h at 5ºC. Some leaves were treated for 15 min. by immersion in vinegar (4%) and in NaDCC (0.25g/l). Afterwards, leaves were rinsed twice in 0.1% PW and portions were immediately cut with sterile scalpel and sterilized cork-borer to the size of the stub diameter, and fixed for 2 h at 4 °C in 0.1 M cacodylate buffer, pH 7.2, containing 2.5% glutaraldehyde. Samples were rinsed three times with 0.1M Cacodylate, and then dehydrated by ethanol gradient series of 10, 30, 50, 70, 90, and 100. The exposure in each step was 15-20 min with the final concentration being repeated three times for 30 min. Samples were then critical point dried with carbon dioxide (EMS Qourom 850) and mounted

on specimen stub for coating with gold. Different locations of the samples were viewed using scanning electron microscope (Tescan Mira, Czech Republic).

3. Statistical analysis

A two-way ANOVA was used to assess the interaction of pre-wash parsley processing (chopping) and types of the washing methods on the reduction of attached S. Typhimurium using SPSS statistics version 21. The level of significance for all tests was 0.05. When no interaction effect existed, simple main effects of each factor for the chopping process, for the different variables categories were examined by one-way ANOVA. The inspection Q-Q plots, tests for normality, examining standardized skewness and the Shapiro-Wilk tests were performed to check assumptions. As the analysis of variance is robust to violations of the homogeneity of variances, provided that the ratio of the largest group is not more than 3 times the smallest group, data were interpreted by Welch robust test and Games-Howell post hoc testing (Howell, 2007).

Independent t-tests were also performed for differences in mean values between both groups, chopped and unchopped, for each washing method treatment.

The treatment effects on microbial loads were assessed by calculating the reduction of microbial content in relation to untreated samples, expressed as log-cycles, i.e. log (N/N_0) (Ramos et al., 2014), where N_0 is the sample initial microbial load and N is the microbial load after treatment.

4. Results

The results indicated no significant interaction (combination effect) between pre-wash processing of parley (chopping) and washing methods on reducing the number of *S.*Typhimurium counts in all tested conditions ($p>0.05$), i.e., the pattern of change in *Salmonella* counts was fairly consistent across each type of washing solution on chopped and unchopped leaves.

On the other hand, F-test results indicated that the main effects of the pre-wash chopping and types of washing methods were significant ($p< 0.05$). One-way ANOVA analysis showed that all types of washing solutions resulted in a significant reduction in mean values of *S.*Typhimurium on pre-wash unchopped parsley held at 5ºC for 4 h compared to control group ($p<0.05$), which was also observed on unchopped parsley at 30ºC for 4 h and at 5ºC for 24 h ($P<0.001$) (Table 1). On the contrary, both vinegar and water did not result in a statistically significant reduction in contamination levels on pre-wash chopped parsley compared to control group, with both inoculum levels and under all conditions (Tables 1 and 2).

Overall, the difference among the mean values of all washing solutions was significant at low temperatures; Chlorine followed by NaDCC was the most effective in reducing contamination levels compared to vinegar and water (Table 2). This was also notable on unchopped and chopped leaves with low inoculum levels at 5ºC for 24 h ($p<0.001$). However, NaDCC did not differ significantly from vinegar on unchopped leaves held at 30ºC for 4 h, and from chlorine on chopped parsley at 5ºC for 24h ($p>0.05$).

Interestingly, the reduction in pathogen levels was not statistically different when comparing water and vinegar under all conditions ($p>0.05$).

4.1 The effect of the pre-wash chopping process

T-test results indicated that unchopped parsley washed by soaking for 15 minutes in water followed by manual agitation after holding at 5ºC for 4 h and 24 h had a statistically significant lower contamination level compared to chopped leaves with a mean difference of 0.76 (95% CI, 0.17-1.34) log and of 0.898 (95%CI, 0.47-1.32) log, respectively.

Similarly, vinegar was more effective on unchopped than on chopped parsley held at 5ºC for 4 h with a mean difference of 0.898 (CI95%, 0.12-1.67) log as illustrated in Figure 1. A. On the contrary, it was found that application of vinegar did not result in a significantly different contamination level between both groups when parsley were held at 30ºC for 4 h and 5ºC for 24 h, ($p>0.05$) (Figure1. B-C).

The effect of NaDCC and chlorine also differed significantly in both groups at 5ºC for 4 h; NaDCC (approached significance) with a mean difference 0.91 (CI95 %,-0.02-1.84) log and chlorine with a mean difference 1.22 (CI 95%, 0.24-2.2) log. But when inoculated parsley was held for 20 h more at 5ºC, chlorine did not result in any further significant difference in reduction levels between both groups.

In general, when unwashed parsley leaves were kept at 30ºC for more than 2-3 h, the mean values in both groups did not differ significantly for all washing solutions. The maximum reduction was mainly achieved on unchopped parsley, particularly with NaDCC and chlorine. This trend was also observed on samples with low inoculum levels (Table 2). NaDCC was capable of reducing the initial inoculum levels to an undetectable level in one sample of unchopped leaves (Table 2). Also, water and vinegar did not have a significant decontamination effect on chopped parsley compared to control.

4.2 The main effect of temperature/time conditions

The two-way ANOVA tests showed a statistically significant interaction effect of temperature and chopping process before washing, ($p<0.001$).

Pairwise comparisons showed that *S.* Typhimurium counts were significantly reduced ($p<0.001$). after washing unchopped parsley held at 5ºC for 4h and 24 h compared to 30ºC for 4 h, with a mean difference of -1.647and -1.528 respectively

Further analysis revealed that the mean values in chopped and unchopped parsley held at 5ºC for 4 h and for 24 h were significantly lower ($p<0.05$) than those held at 30ºC for 4 h for all washing solutions groups indicating the pivotal role of temperature in altering the washing solutions efficiency relatively to other assessed individual factors (Table 3).

4.3 Scanning Electron Microscopy

The SEM imaging of *S.*Typhimurium on parsley samples incubated for 24 h at 5ºC demonstrated of what appears to be clusters of cells agglomerated in the netting and crevices of small veins of the parsley leaf (Figure 2). There has been no clear indication of a preferential adhesion of colonies at the cut edges of the leaf as we have not noticed any substantial and clear indication for a differential attachment around the scar.

There was apparently an initiation of formation of polysaccharide matrix and strands after 24 h incubation at 5ºC that held cells together and to the plant tissue (Figure 3). The observed clusters were apparently embedded within the folds and capable of evading most commonly used washing solutions (Figure 4), having being adhered to inaccessible sites on the leaf surface.

5. Discussion

According to the present results, the current washing methods applied in the SMEs using water and vinegar were only capable of ≤ 90% reduction of the contamination level on intact and chopped leaves which is not considered sufficient to ensure microbiological safety given the very low infectious dose of *Salmonella* as well as the practice of uncontrolled dilution of vinegar.

Water wash achieved negligible log reduction with a range of 0.59-0.93 and 0.84-1.5 for pre-wash chopped and unchopped leaves, respectively, which is in agreement with several authors. Sengun and Karapinar (2005) reported a 0.5–1 log reduction for wash with sterile water. Similarly, Neal et al. (2012) found only 0.7 log reduction in *Salmonella* with water wash of spinach, whereas a lower reduction was reported elsewhere (Tan et al., 2015). The higher numbers observed in our unchopped samples might be due dislodging more cells by the rinsing in conjunction with successive rigorous agitation. The exertion of additional physical cleansing such as scrubbing in water was shown to increase reduction in log CFU/g compared to soaking (Parnell et al., 2005).

The limited studies on the decontamination effect of vinegar on produce, particularly on parsley, are very limited (M. a. G. Karapinar, S.A. 1992, 1992; Sengun & Karapinar, 2004; Wu et al., 2000); and gave varying results. In our study, log reduction with vinegar achieved a maximum reduction of 0.54 and 1.08, for chopped and unchopped leaves, respectively. With similar concentration and exposure time, Sengun and Karapinar (2004) showed a maximum reduction of 1.87 log cfu/g and 2.45 log cfu/g with low inoculum levels of *S.* Typhimurium, in contrast to higher reduction levels on rocket leaves obtained in their other study . We assume that the lower values obtained in this study were attributable to attachment time of inoculum and to topography of parsley surface characterized by folds and niches that shield bacteria from treatment accessibility.

As *Salmonella* can survive and grow in a wide range of pHs (4-9), besides that the pH value of vinegar was constant in all tested conditions, it is postulated that properties other than acidity of vinegar (hydrogen ion effect) underlie its effect on reducing the cell counts, such as the antimicrobial properties of phenolic compounds naturally existing in grape juice (Oliveira et al., 2013; Rhodes et al., 2006). Overall, this study has confirmed the equivalent efficacy of water and vinegar (4%) and the unlikelihood to reduce the numbers of bacteria by more than 1-2 log (Nastou et al., 2012). Although the US Environmental Protection Agency (EPA) Scientific Advisory panel proposed that at least a 2 log microbial reduction is considered as significant (São J. et al., 2015), the food safety laws require strict sanitation measures to achieve a reduction of 99.99683% (Fallik, 2004) which remains a challenge for SMEs in view of limited practical washing methods.

Our study showed that NaDCC was the most effective method against *S.*Typhimurium with a log reduction range of 1.92-3.12. Its affordable price and convenience offer SME's with limited resources a practical alternative for fresh produce sanitation. There are few documented reports on the use of sodium dichloroisocyanurate in fresh produce (Nicholl & Prendergast, 1998; São J. et al., 2015; Tan et al., 2015), particularly in eliminating *Salmonella* on parsley. A log reduction of 99-99.99% was readily achieved in this study, which was consistent with a recent work on *S.*Typhimurium on turnip by Tan (2015). The effectiveness of NaDCC (200 mg/L) on other species was also demonstrated but in varying levell indicating that the sanitization effect vary depending on the produce type, contamination and attachment levels and, bacterial species.

In general, all washing methods, with exception to chlorine, failed to eliminate *S.* Typhimurium, with high and low inoculum levels, with exception to few cases where NaDCC and chlorine reduced the pathogen to below the detection limit. We think that the inaccessibility of washing solutions to crevices and folds on parsley surface, hydrophobic pockets where bacteria hide and attach (Adams et al., 1989) in addition to the strength of

attachment undoubtedly contributed to reducing the efficacy of sanitizing treatments as previously suggested by Ölmez and Temur (2010).

Interestingly, the effectiveness of washing solutions significantly dropped on samples subjected to pre-wash chopping and notably as storage temperature increased to 30ºC. The decrease in the initial inoculum levels was generally more significant on intact parsley leaves than on chopped samples with all washing methods. These results are in line with Patel who observed higher numbers of *Salmonella* attached preferentially to produce with a damaged surface, perhaps due to stronger binding properties on cut leaves. There are hypothetically a number of possible reasons for this. It is increasingly evident that *Salmonella* are capable of instant adherence to fresh produce surface (Ells & Truelstrup Hansen, 2006; Patel & Sharma, 2010). Additionally, the tissue damage and release of exudates by slicing, peeling or shedding of plant tissues produce abundance of nutrients to enteric bacteria enabling the cells growth on the produce (Sapers, 2002; Sela & Fallik, 2009). It is also substantiated that cutting plant surfaces resulted in larger surface area that support higher attachment levels (Ells & Truelstrup Hansen, 2006). It is however noteworthy to mention that other authors stated that *S.*Typhimurium did not differ in attachment strength to cut and intact lettuce at 4ºC for 8h (Kroupitski et al., 2009; Takeuchi et al., 2000). In this context, several citations shed the light on the attachment properties varying with produce types, exposure time to contamination and strains (Patel & Sharma, 2010; Reina et al., 2002).

Images obtained by SEM indicated the adhesion of *S.* Typhimurium and clusters of cells within inner folding of the veins on the surface of inoculated parsley held for 24 hs at 5ºC (Figure 2 A) which is likely due to the adhesion of bacteria. We have not observed preferential attachment or clusters of cells anchored at the cut edges as we expected. Our observation corroborates with Takeuchi et al. (2000) who demonstrated by means of confocal scanning laser microscopy (CSLM) that different species of microorganisms attach differently to lettuce structures and *P.fluorencens* attached preferentially to intact surface than

to cut edges. Apparently, there was a constant trend, although not significant, of a diminishing decontamination effects on parsley held at 5ºC for 24 h than for 4 h. It is well established that that longer attachment time allowed more cells to attach ,which is thought to be due the development of cell aggregates and biofilm formation that confer *Salmonella* its resistance to disinfectants and conventional household methods of washing (Burnett & Beuchat, 2000; Koseki & Itoh, 2001a; Lapidot et al., 2006; Takeuchi et al., 2000).

The higher reductions observed on samples at 5ºC 24h treated with vinegar were negligible and might be due to rough and folds of the parsley surface that led to minor variations among replicated experiments. It is conceivable that the declining pattern as validated with high chlorine concentration resulted from formation of extracellular polymers and increase in attachment with time (H. Ölmez & Temur, 2010; Reina et al., 2002). The SEM micrographs Figure 3 show cell clusters possibly on the initial formation stages of exopolysaccharide matrix (biofilm) and strands connecting cells together and to the plant tissue. Embedded cells inside the matrix on parsley surface were most probably able to escape effective contact with washing solutions, hence complete elimination by sanitizing agents was not observed in this study (Figure 4).

The decontamination effect of all solutions was the least effective at higher temperature (30ºC) perhaps because of a lower permeability of treatment in view of increased cells attachment. It is generally agreed that low storage temperature (4 °C) suppresses the microbial growth (Dinu & Bach, 2011; Tan et al., 2015). Nevertheless, review of literature reflected the complexity of the attachment process as affected by temperature conditions and the disparities in several suggested underlying mechanisms. Ells and Truelstrup Hansen (2006) indicated that at 37ºC, cells exhibited significantly lower attachment strengths during the first 4 h given the lack of production of flagella at this temperature. Earlier, Herald and Zottola (1988) reported on the effect of flagella on attachment. Findings showed an increase production of flagella with the decrease in temperature hence the decreased numbers of

bacterial attachment at low temperature. Whereas, Reina (2002) proposed that binding strength increases with contact time, but a temperature dependent response was mainly noted in the early stages of exposing the produce surface to inoculum. Recently, Patel and Sharma (2010) pointed out that low temperatures and short periods of contact with the produce surface will reduce the potential for bacterial adhesion; at the same time, the increase level of attachment of *Salmonella* at higher temperature was proven (McAuley et al., 2015). It is believed that this effect is due to a decrease in the bacteria surface polymer at lower temperatures as well as to reduced surface area (Garrett, Bhakoo, et al., 2008). On the other hand, Stepanović et al. (2003) stated that optimum temperature results in rapid bacterial growth and biofilm formation of bacteria in association with an increase in nutrients due to increase in the bacterial enzymatic reactions which control the development of many physiological and biochemical properties of bacteria (Garrett, Manmohan, et al., 2008). It is perhaps not easy to ascertain the precise mechanism of the study results as several factors could have been possibly involved, nevertheless, the alteration in efficiency of washing methods by temperature and chopping practice was verified.

6. Conclusion

Our findings highlighted the importance for temperature control over time during handling of parsley for the optimal elimination of pathogenic microorganisms. We demonstrated that chopping parsley leaves before washing and sanitization, and storing them at inappropriate temperature would reduce the effectiveness of washing procedures typically applied in the SME's by 0.5-1.9 log compared to cold storage temperature. Since *S.* Typhimurium has the ability to persist in soils contaminated by manure or irrigation water and contaminate parsley (Kisluk et al., 2012) and is not eliminated by inappropriate post-harvest washing and employee mishandling (Faour-Klingbeil et al., 2016), it is critical that the most effective sanitizers are used during parsley in the food service operations. Chlorine compounds are the

most effective and economic to use but are avoided by many facilities because of their undesirable sensory characteristics. Our results showed that NaDCC is an acceptable substitute to chlorine and other tested solutions that should be used to intact leaves stored under controlled temperature and storage conditions. Its use could be as well advocated by local authorities as an alternative sanitizer for reducing risks of foodborne illnesses associated with consuming raw parsley and other leafy greens in food service establishments. This is the first study to evaluate the effectiveness of common washing methods against *S.* Typhimurium in scenarios that represent SMEs practices in the Middle East/MENA region for processing raw parsley.

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8.References

Adams, M. R., Hartley, A. D., & Cox, L. J. (1989). Factors affecting the efficacy of washing procedures used in the production of prepared salads. Food Microbiol, 6(2), 69-77. doi: 10.1016/s0740-0020(89)80039-5

Barton, B. C., Mody, R. K., Jungk, J., Gaul, L., Redd, J. T., Chen, S.,Salmonella Saintpaul Outbreak Investigation, T. (2011). 2008 outbreak of Salmonella Saintpaul infections associated with raw produce. *N Engl J Med, 364*(10), 918-927. doi: 10.1056/NEJMoa1005741

Beuchat, L. R. (2002). Ecological factors influencing survival and growth of human pathogens on raw fruits and vegetables. *Microbes infect, 4*(4), 413-423. doi: 10.1016/s1286-4579(02)01555-1

Beuchat, L. R., & Ryu, J. H. (1997). Produce handling and processing practices. *Emerg Infect Dis, 3*(4), 459-465.

Buck, J. W., Walcott, R. R., & Beuchat, L. R. (2003). Recent trends in microbiological safety of fruits and vegetables *Plant Health Progress*.

Burnett, S. L., & Beuchat, L. R. (2000). Human pathogens associated with raw produce and unpasteurized juices, and difficulties in decontamination. *J Ind Microbiol Biotechnol, 25*(6), 281-287. doi: 10.1038/sj/jim/7000106

CDC. (1999). Outbreaks of Shigella sonnei infection associated with eating fresh parsley--United States and Canada, July-August 1998. *MMWR Morb Mortal Wkly Rep, 48*(14), 285-289.

CDC. (2007). Multistate outbreaks of Salmonella infections associated with raw tomatoes eaten in restaurants--United States, 2005-2006. *MMWR Morb Mortal Wkly Rep, 56*(35), 909-911.

Clasen, T., & Edmondson, P. (2006). Sodium dichloroisocyanurate (NaDCC) tablets as an alternative to sodium hypochlorite for the routine treatment of drinking water at the household level.. *Int J Hyg Environ Health, 209*(2), 173-181. doi: 10.1016/j.ijheh.2005.11.004

De Jong, B., Oberg, J., & Svenungsson, B. (2007). Outbreak of salmonellosis in a restaurant in Stockholm, Sweden, September - October 2006. *Euro Surveill, 12*(11), E13-14.

Dinu, L. D., & Bach, S. (2011). Induction of viable but nonculturable Escherichia coli O157:H7 in the phyllosphere of lettuce: a food safety risk factor. *Appl Environ Microbiol, 77*(23), 8295-8302. doi: 10.1128/AEM.05020-11

Ells, T. C., & Truelstrup Hansen, L. (2006). Strain and growth temperature influence Listeria spp. attachment to intact and cut cabbage. *Int J Food Microbiol, 111*(1), 34-42. doi: http://dx.doi.org/10.1016/j.ijfoodmicro.2006.04.033

Fallik, E. (2004). Prestorage hot water treatments (immersion, rinsing, brushing). *Postharvest Biol Tec, 32.*, 125-134.

Faour-Klingbeil, D., Kuri, V., & Todd, E. C. D. (2015). Investigating a link of two different types of food business management to the food safety knowledge, attitudes and practices of food handlers in Beirut, Lebanon. *Food Control, 55*, 166-175.

Faour-Klingbeil, D., Murtada, M., Kuri, V., & Todd, E. C. D. (2016). Understanding the routes of contamination of ready-to-eat vegetables in the Middle East. *Food Control, 62*, 125-133. doi: http://dx.doi.org/10.1016/j.foodcont.2015.10.024

FDA. (1998). *Guide to minimize microbial food safety hazards for fresh fruits and vegetables*. U.S. Food and Drug Administration Retrieved from http://edocket.access.gpo.gov/2008/pdf/E8-20187.pdf.

Fett, W. F. (2000). Naturally occurring biofilms on alfalfa and other types of sprouts. *J Food Prot, 63*(5), 625-632.

Garrett, T. R., Manmohan, B., & Zhang, Z. (2008). Bacterial adhesion and biofilms on surfaces. *Prog Nat Sci, 18*(9), 1049-1056. doi: http://dx.doi.org/10.1016/j.pnsc.2008.04.001

Gündüz, G. T., Gönül, Ş. A., & Karapınar, M. (2010). Efficacy of oregano oil in the inactivation of Salmonella typhimurium on lettuce. *Food Control, 21*(4), 513-517. doi: http://dx.doi.org/10.1016/j.foodcont.2009.07.016

Herald, P., & Zottola, E. (1988). Attachment of Listeria monocytogenes to stainless steel surfaces at various temperatures and pH values. *J Food Sci, 53*, 1549–1552.

Howell, D. C. (2007).Statistical methods for Psychology (6th ed.). Wadsworth.

IFSAC. (2015). Foodborne Illness Source Attribution Estimates for Salmonella, Escherichia coli O157 , Listeria monocytogenes , and Campylobacter using Outbreak Surveillance Data. Interagency Food Safety Analytics Collaboration (IFSAC) Project. http://www.cdc.gov/foodsafety/pdfs/ifsac-project-report-508c.pdf.

Karapinar, M., & Sengun, I. Y. (2007). Antimicrobial effect of koruk (unripe grape—Vitis vinifera) juice against Salmonella typhimurium on salad vegetables. *Food Control, 18*(6), 702-706. doi: http://dx.doi.org/10.1016/j.foodcont.2006.03.004

Karapinar, M. a. G., S.A. 1992. (1992). Removal of Yersinia enterocolitica from fresh parsley by washing with acetic acid or vinegar. *Int J Food Microbiol, 16*, 261-264.

Kilonzo-Nthenge, A., Chen, F. C., & Godwin, S. L. (2006). Efficacy of home washing methods in controlling surface microbial contamination on fresh produce. *J Food Prot, 69*(2), 330-334.

Kim, S. W., Baek, S. B., Ha, J. H., Lee, M. H., Choi, C., & Ha, S. D. (2012). Chlorine treatment to inactivate norovirus on food contact surfaces. *J Food Prot, 75*(1), 184-188. doi: 10.4315/0362-028X.JFP-11-243

Kisluk, G., Hoover, D. G., Kneil, K. E., & Yaron, S. (2012). Quantification of low and high levels of Salmonella enterica serovar Typhimurium on leaves. *LWT - Food Sci Technol, 45*(1), 36-42. doi: http://dx.doi.org/10.1016/j.lwt.2011.07.029

Koseki, S., & Isobe, S. (2005). Prediction of pathogen growth on iceberg lettuce under real temperature history during distribution from farm to table. *Int J Food Microbiol, 104*(3), 239-248. doi: 10.1016/j.ijfoodmicro.2005.02.012

Koseki, S., & Itoh, K. (2001a). Prediction of microbial growth in fresh-cut vegetables treated with acidic electrolyzed water during storage under various temperature conditions. *J Food Prot, 64*(12), 1935-1942.

Koseki, S., Yoshida, K., Isobe, S., & Itoh, K. (2001b). Decontamination of lettuce using acidic electrolyzed water. *J Food Prot, 64*(5), 652-658.

Kroupitski, Y., Pinto, R., Brandl, M. T., Belausov, E., & Sela, S. (2009). Interactions of Salmonella enterica with lettuce leaves. *J Appl Microbiol, 106*(6), 1876-1885. doi: 10.1111/j.1365-2672.2009.04152.x

Lang, M. M., Harris, L. J., & Beuchat, L. R. (2004). Survival and recovery of Escherichia coli O157:H7, Salmonella, and Listeria monocytogenes on lettuce and parsley as affected by method of inoculation, time between inoculation and analysis, and treatment with chlorinated water. *J Food Prot, 67*(6), 1092-1103.

Lapidot, A., Romling, U., & Yaron, S. (2006). Biofilm formation and the survival of Salmonella Typhimurium on parsley. *Int J Food Microbiol, 109*(3), 229-233. doi: 10.1016/j.ijfoodmicro.2006.01.012

Lee, I. S., Slonczewski, J. L., & Foster, J. W. (1994). A low-pH-inducible, stationary-phase acid tolerance response in Salmonella typhimurium. *J Bacteriol, 176*(5), 1422-1426.

Little, C. L., & Gillespie, I. A. (2008). Prepared salads and public health. *J Appl Microbiol, 105*, 1729-1743.

MacDonald, E., Heier, B., Stalheim, T., Cudjoe, K., Skjerdal, T., Wester, A., Vold, L. (2011). Yersinia enterocolitica O:9 infections associated with bagged salad mix in Norway, February to April 2011. *Euro Surveill, 16*(19).

Martínez-Sánchez, A., Allende, A., Bennett, R. N., Ferreres, F., & Gil, M. I. (2006). Microbial, nutritional and sensory quality of rocket leaves as affected by different sanitizers. *Postharvest Biol Tec, 42*(1), 86-97. doi: http://dx.doi.org/10.1016/j.postharvbio.2006.05.010

McAuley, C. M., Duffy, L., Subasinghe, N., Hogg, G., Coventry, J., & Fegan, N. (2015). Salmonella Typhimurium and Salmonella Sofia: Growth in and Persistence on Eggs under Production and Retail Conditions. *BioMed Res Int, 2015*, 8. doi: 10.1155/2015/914987

McCabe-Sellers, B. J., & Beattie, S. E. (2004). Food safety: emerging trends in foodborne illness surveillance and prevention. *J Am Diet Assoc, 104*(11), 1708-1717. doi: 10.1016/j.jada.2004.08.028

Miller, F. A., Ramos, B., Gil, M. M., Brandao, T. R., Teixeira, P., & Silva, C. L. (2009). Influence of pH, type of acid and recovery media on the thermal inactivation of Listeria innocua. *Int J Food Microbiol, 133*(1-2), 121-128. doi: 10.1016/j.ijfoodmicro.2009.05.007

Nascimento, M.S., Silva, N., Catanozi, M.P., & Silva, K.C. (2003). Effects of different disinfection treatments on the natural microbiota of lettuce. *J Food Prot, 66*(9), 1697-1700.

Nastou, A., Rhoades, J., Smirniotis, P., Makri, I., Kontominas, M., & Likotrafiti, E. (2012). Efficacy of household washing treatments for the control of Listeria monocytogenes on salad vegetables. *Int J Food Microbiol, 159*(3), 247-253. doi: http://dx.doi.org/10.1016/j.ijfoodmicro.2012.09.003

Neal, J. A., Marquez-Gonzalez, M., Cabrera-Diaz, E., Lucia, L. M., O'Bryan, C. A., Crandall, P. G., Castillo, A. (2012). Comparison of multiple chemical sanitizers for reducing Salmonella and Escherichia coli O157:H7 on spinach (Spinacia oleracea) leaves. *Food Res Int, 45*(2), 1123-1128. doi: http://dx.doi.org/10.1016/j.foodres.2011.04.011

Nicholl, P., & Prendergast, M. (1998). Disinfection of shredded salad ingredients with sodium dichloroisocyanurate. *J Food Process Pres, 22*, 67-79. doi: DOI: 10.1111/j.1745-4549.1998.tb00805.

Olaimat, A. N., & Holley, R. A. (2012). Factors influencing the microbial safety of fresh produce: A review. *Food Microbiol, 32*(1), 1-19. doi: http://dx.doi.org/10.1016/j.fm.2012.04.016

Oliveira, D. A., Salvador, A. A., Smania, A., Jr., Smania, E. F., Maraschin, M., & Ferreira, S. R. (2013). Antimicrobial activity and composition profile of grape (Vitis vinifera) pomace extracts obtained by supercritical fluids. *J Biotechnol, 164*(3), 423-432. doi: 10.1016/j.jbiotec.2012.09.014

Ölmez, H., & Akbas, M. Y. (2009). Optimization of ozone treatment of fresh-cut green leaf lettuce. *Journal of Food Engineering, 90*(4), 487-494. doi: http://dx.doi.org/10.1016/j.jfoodeng.2008.07.026

Ölmez, H., & Temur, S. D. (2010). Effects of different sanitizing treatments on biofilms and attachment of Escherichia coli and Listeria monocytogenes on green leaf lettuce. *LWT - Food Sci Technol, 43*(6), 964-970. doi: 10.1016/j.lwt.2010.02.005

Pachepsky, Y., Shelton, D. R., McLain, J. E. T., Patel, J., & Mandrell, R. E. (2011). Irrigation Waters as a Source of Pathogenic Microorganisms in Produce- Chapter two: A Review. In L. S. Donald (Ed.), *Adv Agron* (Vol. 113, pp. 75-141) Burlington: Academic Press.

Parnell, T. L., Harris, L. J., & Suslow, T. V. (2005). Reducing Salmonella on cantaloupes and honeydew melons using wash practices applicable to postharvest handling, foodservice, and consumer preparation. *Int J Food Microbiol, 99*(1), 59-70. doi: http://dx.doi.org/10.1016/j.ijfoodmicro.2004.07.014

Patel, J., & Sharma, M. (2010). Differences in attachment of Salmonella enterica serovars to cabbage and lettuce leaves. *Int J Food Microbiol, 139*(1–2), 41-47. doi: http://dx.doi.org/10.1016/j.ijfoodmicro.2010.02.005

Pathan, A. K., Bond, J., & Gaskin, R. E. (2008). Sample preparation for scanning electron microscopy of plant surfaces—Horses for courses. *Micron, 39*(8), 1049-1061. doi: http://dx.doi.org/10.1016/j.micron.2008.05.006

Rahman, S. M. E., Ding, T., & Oh, D.-H. (2010). Inactivation effect of newly developed low concentration electrolyzed water and other sanitizers against microorganisms on spinach. *Food Control, 21*(10), 1383-1387. doi: http://dx.doi.org/10.1016/j.foodcont.2010.03.011

Ramos, B., Brandão, T. R. S., Teixeira, P., & Silva, C. L. M. (2014). Balsamic vinegar from Modena: An easy and effective approach to reduce Listeria monocytogenes from lettuce. *Food Control, 42*(0), 38-42. doi: http://dx.doi.org/10.1016/j.foodcont.2014.01.029

Reina, L. D., Fleming, H. P., & Breidt, F., Jr. (2002). Bacterial contamination of cucumber fruit through adhesion. *J Food Prot, 65*(12), 1881-1887.

Rhodes, P. L., Mitchell, J. W., Wilson, M. W., & Melton, L. D. (2006). Antilisterial activity of grape juice and grape extracts derived from Vitis vinifera variety Ribier. *Int J Food Microbiol, 107*(3), 281-286. doi: http://dx.doi.org/10.1016/j.ijfoodmicro.2005.10.022

São J., Jackline, F., & Maria, C. (2015). Application of ultrasound and chemical sanitizers to watercress, parsley and strawberry: Microbiological and physicochemical quality. *LWT - Food Sci Technol, 63*(2), 946-952. doi: http://dx.doi.org/10.1016/j.lwt.2015.04.029

Sapers, G. M., (2002). *Washing and sanitizing raw materials for minimally processed fruit and vegetable products*: CRC Press (2002).

Sela, S., & Fallik, E. (2009). Microbial Quality and Safety of Fresh Produce. Chapter 13 - In F. R. Edited by Wojciech J., Shewfelt B., Stanley E. (Ed.), *Postharvest Handling (Second Edition)* (pp. 351-398). San Diego-USA: Academic Press.

Sengun, I. Y., & Karapinar, M. (2004). Effectiveness of lemon juice, vinegar and their mixture in the elimination of Salmonella typhimurium on carrots (Daucus carota L.). *Int J Food Microbiol, 96*(3), 301-305. doi: http://dx.doi.org/10.1016/j.ijfoodmicro.2004.04.010

Sengun, I. Y., & Karapinar, M. (2005). Elimination of Yersinia enterocolitica on carrots (Daucus carota L.) by using household sanitisers. *Food Control, 16*(10), 845-850. doi: http://dx.doi.org/10.1016/j.foodcont.2004.07.006

Sengun, Y. I., & Karapinar, M. (2005). Effectiveness of household natural sanitizers in the elimination of Salmonella typhimurium on rocket (Eruca sativa Miller) and spring onion (Allium cepa L.). *Int J Food Microbiol, 98*(3), 319-323. doi: 10.1016/j.ijfoodmicro.2004.07.011

Sodha, S. V., Lynch, M., Wannemuehler, K., Leeper, M., Malavet, M., Schaffzin, J., Braden, C. (2011). Multistate outbreak of Escherichia coli O157:H7 infections associated with a national fast-food chain, 2006: a study incorporating epidemiological and food source traceback results. *Epidemiol Infect, 139*(2), 309-316. doi: 10.1017/S0950268810000920

Stepanović, S., Ćirković, I., Mijač, V., & Švabić-Vlahović, M. (2003). Influence of the incubation temperature, atmosphere and dynamic conditions on biofilm formation by Salmonella spp. *Food Microbiol, 20*(3), 339-343. doi: http://dx.doi.org/10.1016/S0740-0020(02)00123-5

Takeuchi, K., Matute, C. M., Hassan, A. N., & Frank, J. F. (2000). Comparison of the attachment of Escherichia coli O157:H7, Listeria monocytogenes, Salmonella typhimurium, and Pseudomonas fluorescens to lettuce leaves. *J Food Prot, 63*(10), 1433-1437.

Tan, S., Mikš-Krajnik, M., Neo, S., Tan, A., Khoo, G., & Yuk, H. (2015). Effectiveness of various sanitizer treatments for inactivating natural microflora and Salmonella spp. on turnip (Pachyrhizus erosus). *Food Control, 54*, 216-224. doi: http://dx.doi.org/10.1016/j.foodcont.2015.02.012

Tang, P. L., Pui, C. F., Wong, W. C., Noorlis, A. and Son, R. (2012). Biofilm forming ability and time course study of growth of Salmonella Typhi on fresh produce surfaces. *Int Food Res J, 19*(1), 71-76.

Todd, E. C. D., & Greig, J. (2015). Viruses of foodborne origin: a review. *Virus Adaptation and Treatment 7*, 25–45.

Vandekinderen, I., Devlieghere, F., De Meulenaer, B., Ragaert, P., & Van Camp, J. (2009). Optimization and evaluation of a decontamination step with peroxyacetic acid for fresh-cut produce. *Food Microbiol, 26*(8), 882-888. doi: 10.1016/j.fm.2009.06.004

Wu, F. M., Doyle, M. P., Beuchat, L. R., Wells, J. G., Mintz, E. D., & Swaminathan, B. (2000). Fate of Shigella sonnei on parsley and methods of disinfection. *J Food Prot, 63*(5), 568-572.

Table1 *S.* Typhimurium mean levels (Log_{10} CFU/g) on chopped and unchopped parsley leaves hold on different time-temperature conditions and washed applying different solutions

Pre-wash leaves preparation	Wash treatment		Pre-wash storage conditions (Temperature/Time)		
			5ºC/4 h	5ºC/24 h	30ºC/ 4 h
	Solutions	pH[a]	Mean±SD	Mean±SD	Mean±SD
Chopped	Control[1]		6.38 ±0.54	5.91 ±0.10	7.14 ± 0.44
	Water	6.3	5.44 ±0.36	5.64 ±0.27	6.55 ± 0.48
	Vinegar	3.6	6.02 ±0.55	5.32 ±0.47	6.60 ± 0.25
	NaDCC	6.0	3.99* ±0.79	3.62* ±0.12	5.21* ± 0.19
	Chlorine	6.1	2.51* ±0.28	2.64* ±0.45	4.19* ± 0.11
Un-chopped	Control[1]		6.20 ±0.53	5.84 ±0.09	6.98 ± 0.45
	Water	6.4	4.69* ±0.38	4.74* ±0.13	6.16* ±0.22
	Vinegar	3.4	5.12* ±0.51	4.66* ±0.12	6.11 ± 0.61
	NaDCC	6.1	3.08* ±0.45	3.18* ±0.27	5.09* ± 0.20
	Chlorine	6.0	1.28†* ±0.80	2.55* ±0.63	4.26* ± 0.21

[1] Mean value of attached cells after holding under tested conditions and the initial inoculation with 10^6 CFU/g

* The mean value is significantly lower than the control group at $p<0.05$ (significant difference from control) for each tested variable.

† For 2 out of 5 replicate experiments, no growth of *Salmonella* was noted after enrichment (no-detection limit < 0.7 log cfu/g).

[a] Mean pH of washing solutions decanted after the 15 minutes. Values consistent for all settings and over time.

Table 2. . Log reduction (Log N/N_0) of *S.*Typhimurium‡ on pre-wash chopped and unchopped parsley inoculated with low inoculum levels† under selected temperature/time conditions

	5ºC/24 h	5ºC/ 24 h
Washing method	Unchopped Log (N/N_0)	Chopped Log (N/N_0)
Water	-0.98	-0.48
Vinegar	-1.25	-1.14
NaDCC	-1.85[b]	-1.71*
Chlorine	-2.27*[c]	-2.62*[d]

†Mean values for control for whole leaves parsley at 5C/24h and for chopped leaves at5C for 4 h were 4.08 and 3.85 Log cfu/g, respectively The star on mean values indicates significantly lower mean compared to control group ($p<0.05$).

‡Minimum detection limit was set to 0.7 log cfu/g to avoid under or overestimation in statistical analysis

[b,c] In 1 out of 4 replicated experiments showed undetectable levels ($\leq$ 0.7 log cfu/g for low inoculum). Detection test was positive after enrichment.

[d] 2 out of 4 replicates showed undetectable levels ($\leq$ 0.7 log cfu/g for low inoculum). Detection test was positive after enrichment

Table3. The difference in log reduction (Log N/N_0) of *S.*Typhimurium on parsley among the different temperature/time conditions of each group (pre-wash chopped and unchopped)

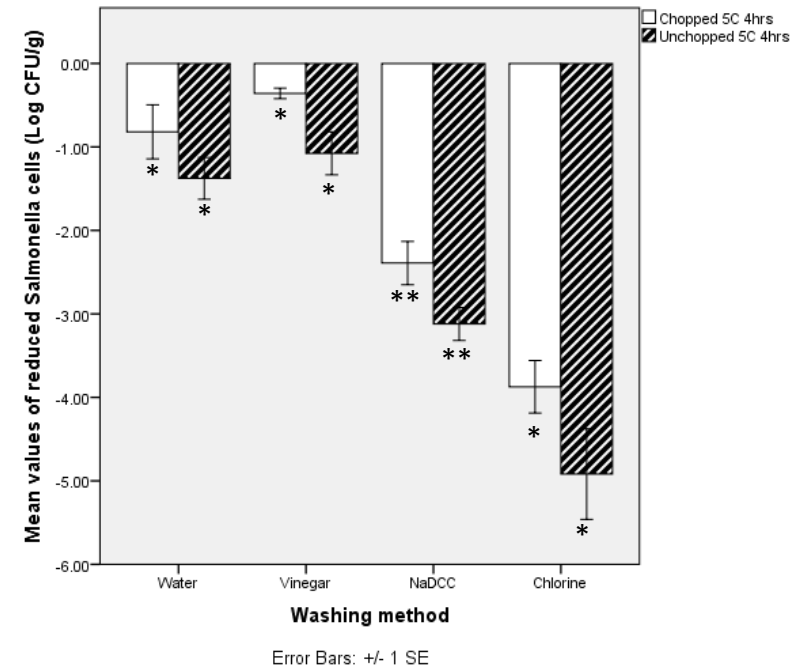
	Pre-wash storage conditions (Temperature/Time)					
	Chopped			Unchopped		
	5ºC/4h	5ºC/24 h	30ºC/4 h	5ºC/4 h	5ºC/24 h	30ºC/4 h
Washing solutions	Log (N/N_0)	Log (N/N_0)	Log (N/N_0)	Log (N/N_0)	Log (N/N_0)	Log (N/N_0)
Water	-0.93[a]	-0.27	-0.59[a]	-1.51[a]	-1.098[b]	-0.84[ab]
Vinegar	-0.36	-0.59[a]	-0.54[a]	-1.08[a]	-1.18[b]	-0.90[ab]
NaDCC	-2.39[a]	-2.19[b]	-1.93[ab]	-3.12[a]	-2.65[b]	-1.92[ab]
Chlorine[*]	-3.87[a]	-3.11[b]	-2.96[ab]	-4.92[a‡]	-3.29[b]	-2.74[ab]

Similar superscript letters in each row of each group(chopped and unchopped) indicate significant differences in mean values at $p<0.05$

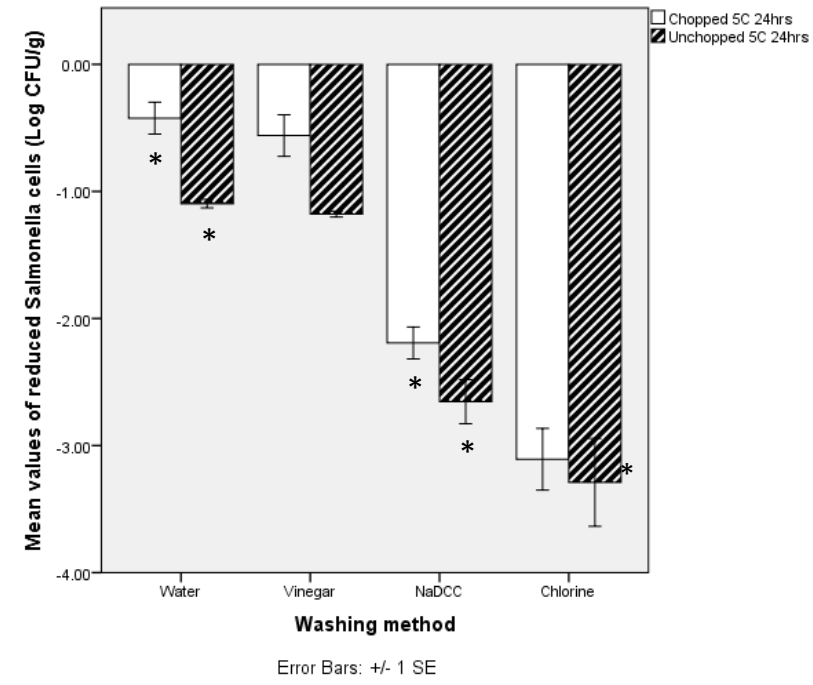
[*] Games-Howells post hoc test performed assuming non variances. For remaining variables, Tuckey post-hoc test was run.

‡ 2 out of 5 replicated experiments showed no visual growth of Salmonella (undetectable levels $\leq$ 0.7 log cfu/g) after selective and non-selective enrichment. Mean value of log reduction would be equal to -3.7 if zero values were given in the event of undetected cells.

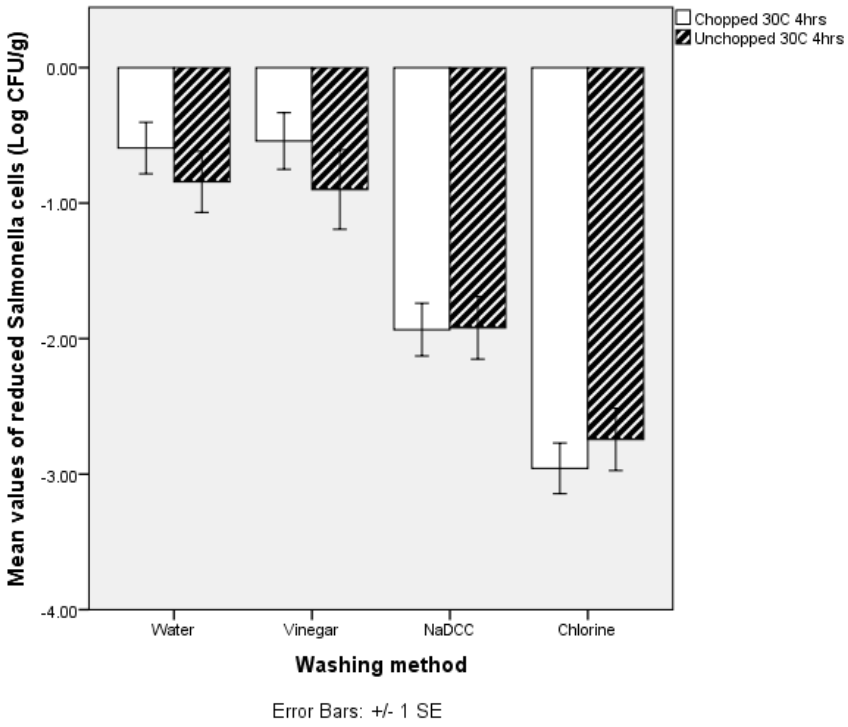
A


Chopped 5C 4hrs
Unchopped 5C 4hrs
Mean values of reduced Salmonella cells (Log CFU/g)
0.00
-1.00
-2.00
-3.00
-4.00
-5.00
-6.00
Water
Vinegar
NaDCC
Chlorine
Washing method
Error Bars: +/- 1 SE

B


Chopped 5C 24hrs
Unchopped 5C 24hrs
Mean values of reduced Salmonella cells (Log CFU/g)
0.00
-1.00
-2.00
-3.00
-4.00
Water
Vinegar
NaDCC
Chlorine
Washing method
Error Bars: +/- 1 SE

C



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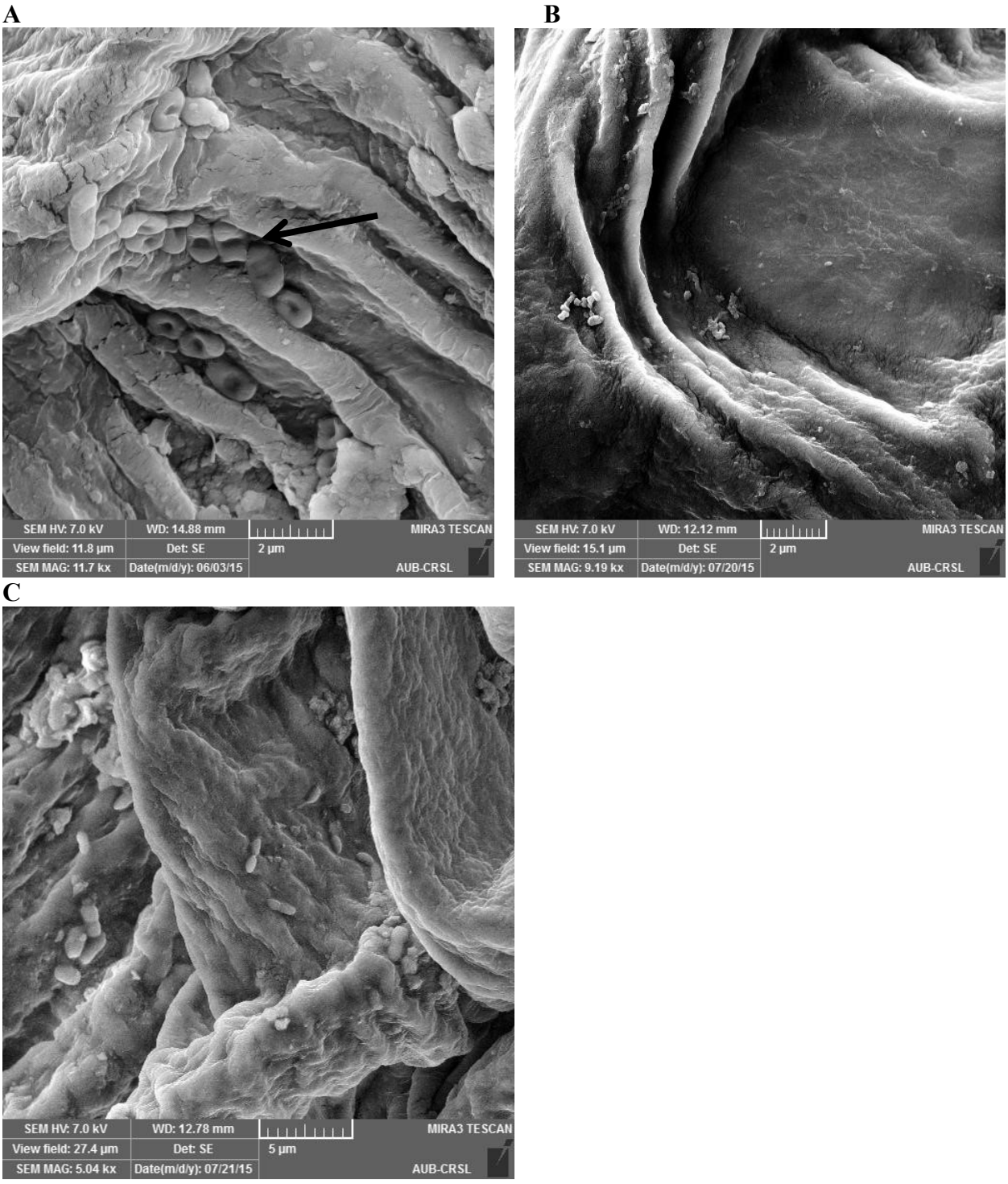

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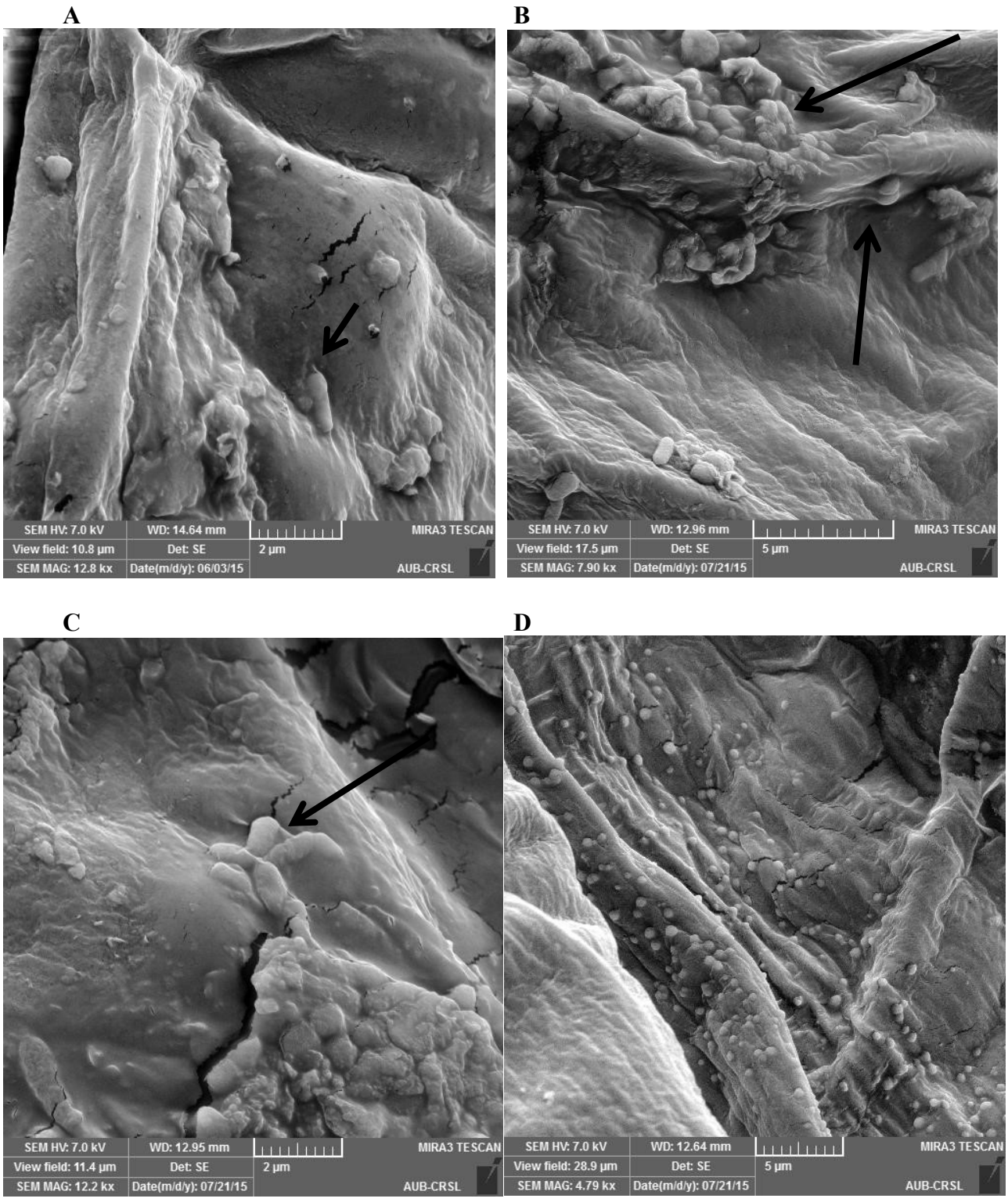

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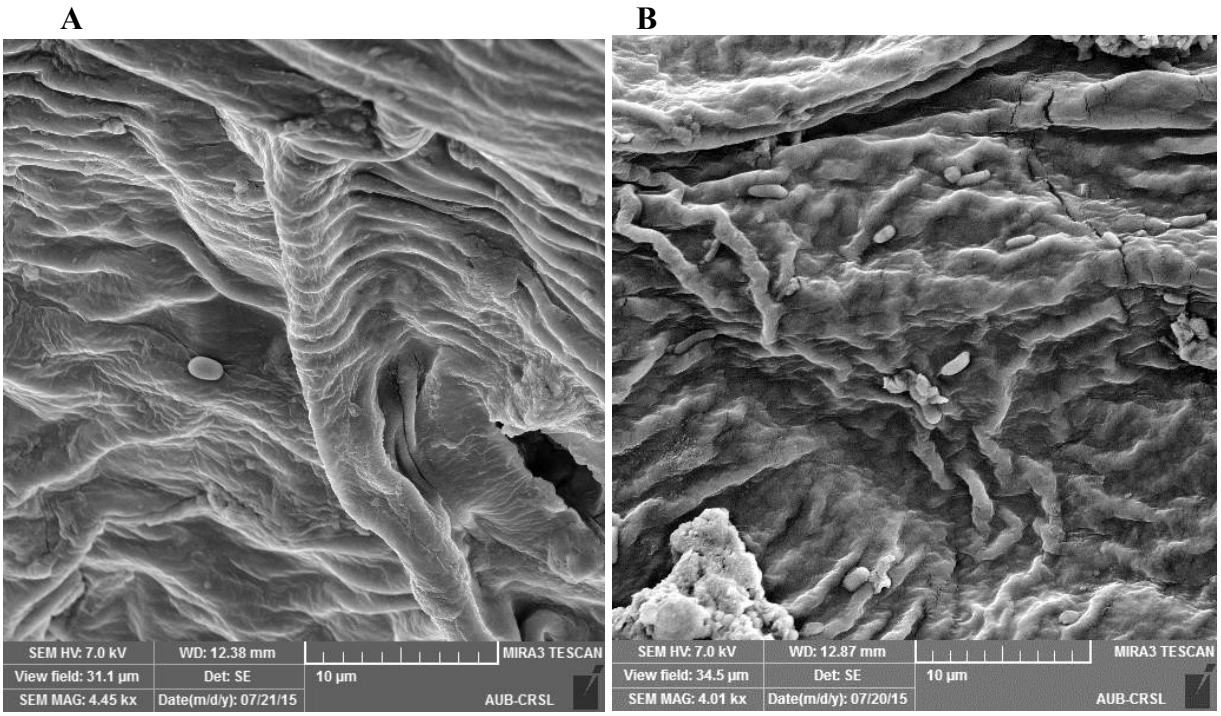

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Table1 *S.* Typhimurium mean levels (Log_{10} CFU/g) on chopped and unchopped parsley leaves hold on different time-temperature conditions and washed applying different solutions

Pre-wash leaves preparation	Wash treatment		Pre-wash storage conditions (Temperature/Time)		
			5ºC/4 h	5ºC/24 h	30ºC/ 4 h
	Solutions	pH[a]	Mean±SD	Mean±SD	Mean±SD
Chopped	Control[1]		6.38 ±0.54	5.91 ±0.10	7.14 ± 0.44
	Water	6.3	5.44 ±0.36	5.64 ±0.27	6.55 ± 0.48
	Vinegar	3.6	6.02 ±0.55	5.32 ±0.47	6.60 ± 0.25
	NaDCC	6.0	3.99* ±0.79	3.62* ±0.12	5.21* ± 0.19
	Chlorine	6.1	2.51* ±0.28	2.64* ±0.45	4.19* ± 0.11
Un-chopped	Control[1]		6.20 ±0.53	5.84 ±0.09	6.98 ± 0.45
	Water	6.4	4.69* ±0.38	4.74* ±0.13	6.16* ±0.22
	Vinegar	3.4	5.12* ±0.51	4.66* ±0.12	6.11 ± 0.61
	NaDCC	6.1	3.08* ±0.45	3.18* ±0.27	5.09* ± 0.20
	Chlorine	6.0	1.28†* ±0.80	2.55* ±0.63	4.26* ± 0.21

[1] Mean value of attached cells after holding under tested conditions and the initial inoculation with 10^6 CFU/g

* The mean value is significantly lower than the control group at $p<0.05$ (significant difference from control) for each tested variable.

† For 2 out of 5 replicate experiments, no growth of *Salmonella* was noted after enrichment (no-detection limit < 0.7 log cfu/g).

[a] Mean pH of washing solutions decanted after the 15 minutes. Values consistent for all settings and over time.

Table 2. . Log reduction (Log N/N_0) of *S.*Typhimurium‡ on pre-wash chopped and unchopped parsley inoculated with low inoculum levels† under selected temperature/time conditions

	5ºC/24 h	5ºC/ 24 h
Washing method	Unchopped Log (N/N_0)	Chopped Log (N/N_0)
Water	-0.98	-0.48
Vinegar	-1.25	-1.14
NaDCC	-1.85[b]	-1.71*
Chlorine	-2.27*[c]	-2.62*[d]

†Mean values for control for whole leaves parsley at 5C/24h and for chopped leaves at5C for 4 h were 4.08 and 3.85 Log cfu/g, respectively The star on mean values indicates significantly lower mean compared to control group ($p<0.05$).

‡Minimum detection limit was set to 0.7 log cfu/g to avoid under or overestimation in statistical analysis

[b,c] In 1 out of 4 replicated experiments showed undetectable levels ($\leq$ 0.7 log cfu/g for low inoculum). Detection test was positive after enrichment.

[d] 2 out of 4 replicates showed undetectable levels ($\leq$ 0.7 log cfu/g for low inoculum). Detection test was positive after enrichment

Table3. The difference in log reduction (Log N/N_0) of *S.*Typhimurium on parsley among the different temperature/time conditions of each group (pre-wash chopped and unchopped)

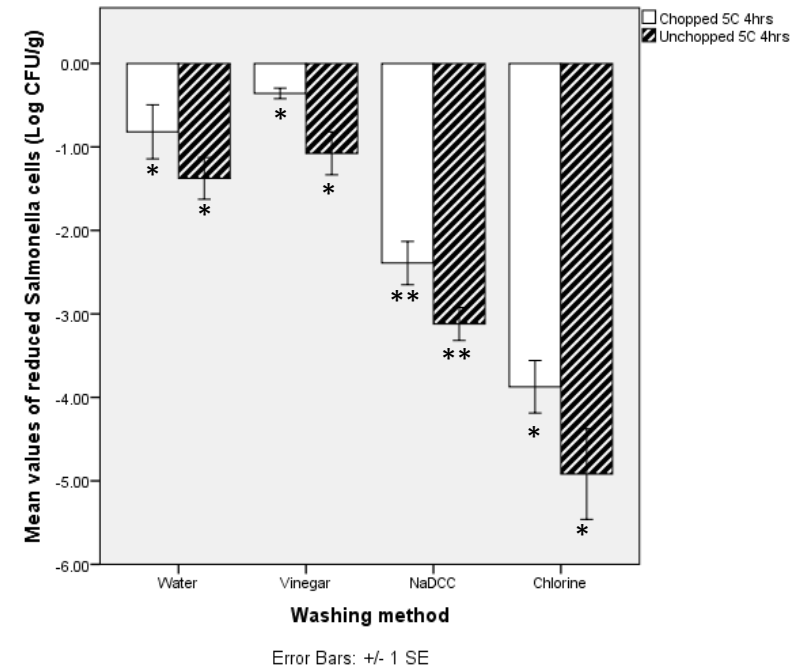
	Pre-wash storage conditions (Temperature/Time)					
	Chopped			Unchopped		
	5ºC/4h	5ºC/24 h	30ºC/4 h	5ºC/4 h	5ºC/24 h	30ºC/4 h
Washing solutions	Log (N/N_0)	Log (N/N_0)	Log (N/N_0)	Log (N/N_0)	Log (N/N_0)	Log (N/N_0)
Water	-0.93[a]	-0.27	-0.59[a]	-1.51[a]	-1.098[b]	-0.84[ab]
Vinegar	-0.36	-0.59[a]	-0.54[a]	-1.08[a]	-1.18[b]	-0.90[ab]
NaDCC	-2.39[a]	-2.19[b]	-1.93[ab]	-3.12[a]	-2.65[b]	-1.92[ab]
Chlorine[*]	-3.87[a]	-3.11[b]	-2.96[ab]	-4.92[a‡]	-3.29[b]	-2.74[ab]

Similar superscript letters in each row of each group(chopped and unchopped) indicate significant differences in mean values at $p<0.05$

[*] Games-Howells post hoc test performed assuming non variances. For remaining variables, Tuckey post-hoc test was run.

‡ 2 out of 5 replicated experiments showed no visual growth of Salmonella (undetectable levels $\leq$ 0.7 log cfu/g) after selective and non-selective enrichment. Mean value of log reduction would be equal to -3.7 if zero values were given in the event of undetected cells.

A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Unchopped 5C 4hrs
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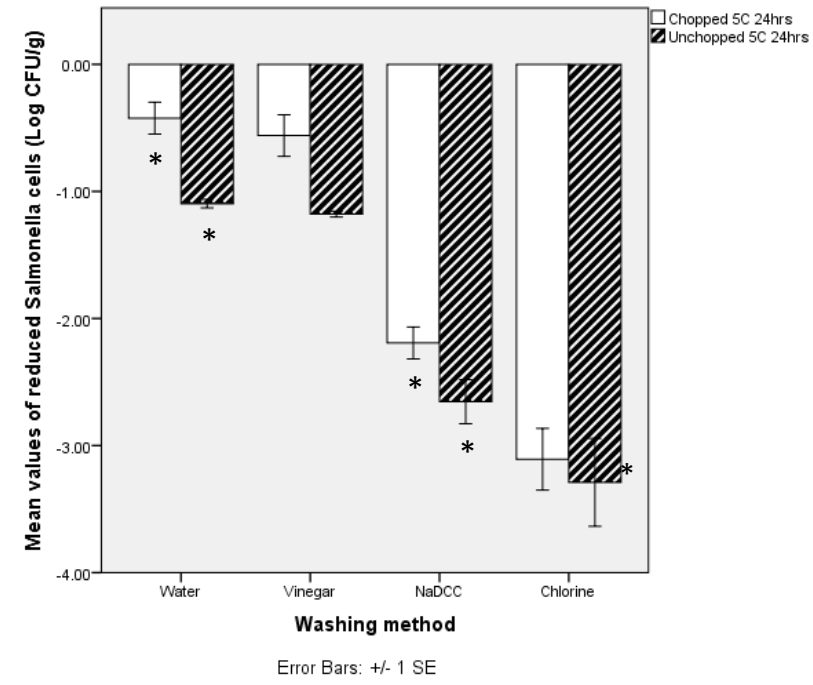
-2.00
-3.00
-4.00
-5.00
-6.00
Water
Vinegar
NaDCC
Chlorine
Washing method
Error Bars: +/- 1 SE

B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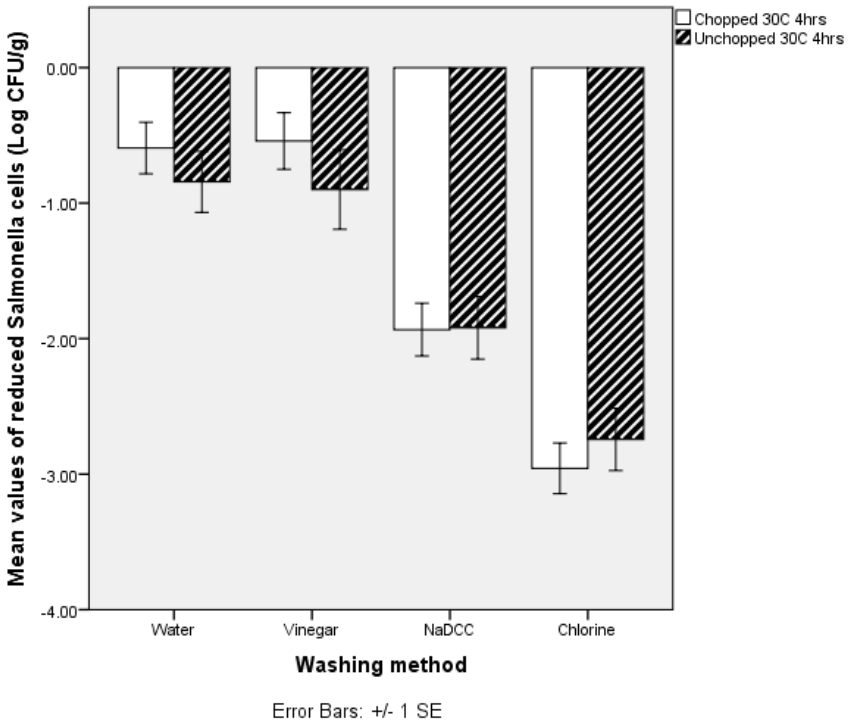

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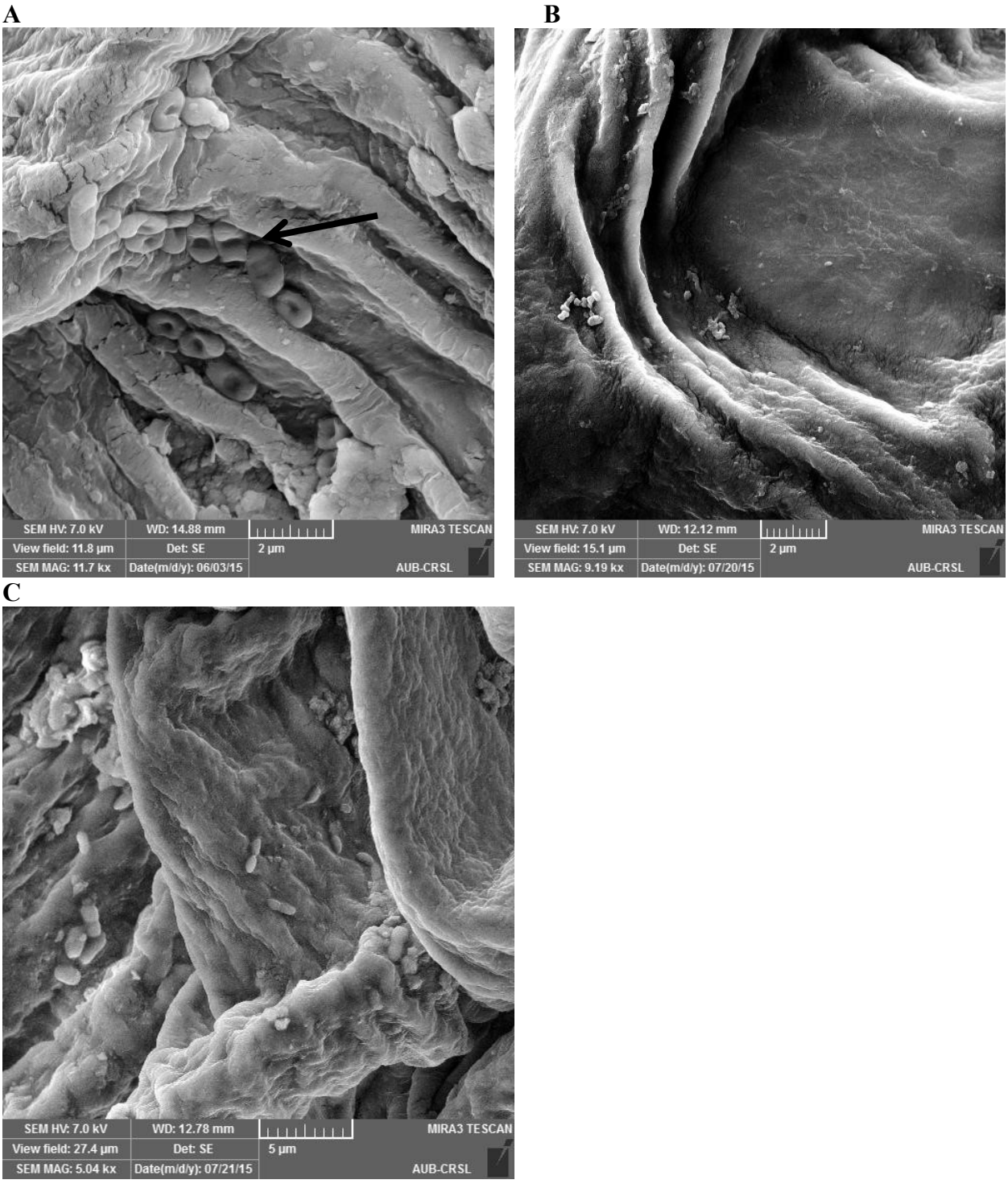

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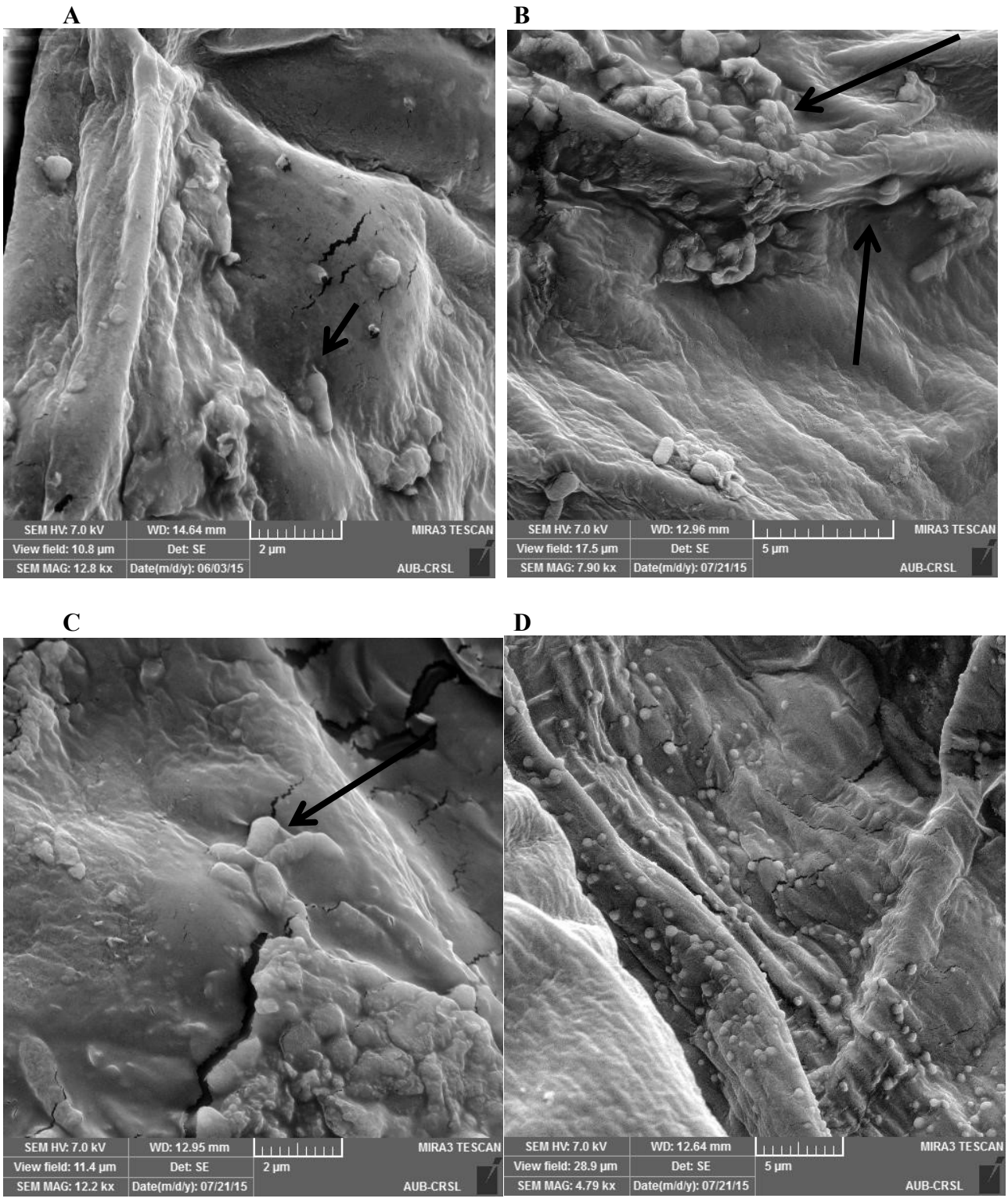

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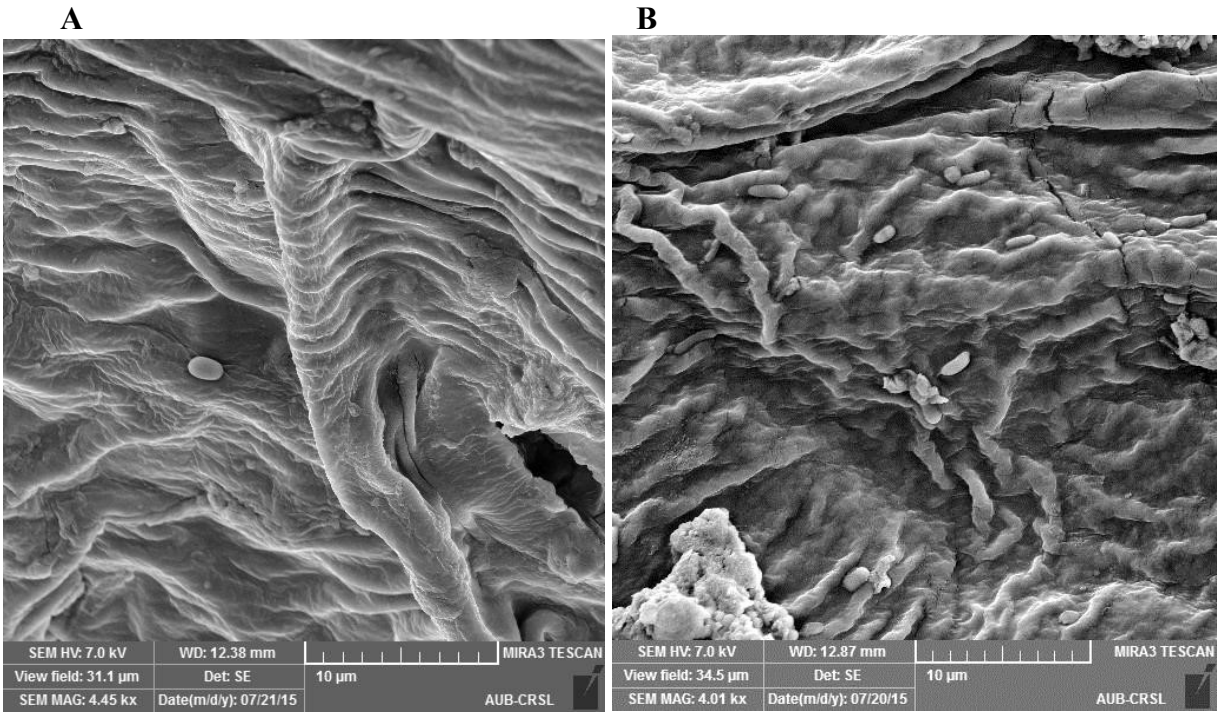

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