**Article title:** Removal of grit from baby leafy salad vegetables by combinations of sanitiser and surfactant

**Article type:** Original research paper

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**Abstract**

Grit composed of dirt, sand and small stones adheres to baby leafy salad vegetables during the growing period and can sometimes be difficult to remove with sanitiser only or tap water. For the first time, the effect of a surfactant, sodium dodecyl sulphate (SDS), alone (0.025, 0.05, 0.1 % SDS) and in combination (0.05 % SDS) with peroxyacetic acid (40 mg L⁻¹, PAA), on grit removal, quality, shelf-life and taste of baby spinach was investigated. Increasing SDS from 0.025 to 0.1 % resulted in a 21-50 % increase in grit removal on spinach and coral lettuce. Overall, SDS treatments had no effect on microbial growth, colour and electrolyte leakage during shelf-life. An increase in bruising, sliming and yellowing scores was also observed regardless of the treatment, reaching an unacceptable score (<3) by day-12 for all samples, however yellowing scores were still within an acceptable range (>3) on d-14. There were no differences in sensorial attributes namely, flavour, aroma and texture, between baby spinach samples treated with PAA alone or in combination with SDS. These results demonstrate that SDS treatment can be used to increase grit removal on baby leafy salad vegetables without compromising quality.
Key words:

baby spinach; coral lettuce; grit; peroxyacetic acid (sanitiser); sodium dodecyl sulphate (surfactant), shelf-life, taste.
1.0 INTRODUCTION

Baby leafy salad vegetables are minimally processed, which includes washing with a sanitiser to minimise microbial cross-contamination and to reduce microbial load, pesticide residues, soil and grit [1]. Therefore, sanitising improves customer satisfaction, convenience and visual appeal [2, 3]. Grit can attach to leafy vegetables grown in the open field, due to wind or splashing from rain and irrigation, or through mechanical harvesting and can contaminate produce [4]. Grit increases the hydrophobic properties of the leaf surface and thus, hinders direct contact between the leaf surface and sanitiser wash water reducing decontamination efficacy [5, 6]. Furthermore, grit can habour microorganisms and therefore facilitate their attachment to produce surfaces [5]. Ingestion of improperly washed leafy vegetables with grit and soil can have a negative impact on health, if the soil has pathogenic microorganisms, heavy metals, pesticides or fertilisers [7]. Surfactants have been suggested to facilitate removal of bound contaminants from fresh produce surfaces [8].

Surfactants are surface-active amphiphilic molecules that reduce interfacial/ surface tension of solutions [8-10]. They consist of a non-polar group attached to a polar group that can either be cationic, anionic, zwitterionic or non-ionic [11]. Surfactants may enhance contact between sanitiser and microorganisms, thus improving microbial inactivation [5, 12, 13], and can enable sanitisers to gain access to crevices and cracks in the lettuce structure [14]. Raiden [15] states that detergents can successfully clean produce without compromising their structural integrity. SDS is a food grade anionic surfactant that has previously been used with leafy salad vegetables [5, 16, 17]. Huang and Nitin [5] observed that sodium dodecyl sulphate (SDS), Tween 20 and lauric arginate at 0.1 % lowered the surface tension of water from 71.17 mN m\(^{-1}\) to 46.6, 36 and 36 mN m\(^{-1}\) respectively. In the same study, soil particles reduced the ability of the surfactants SDS, lauric arginate and Tween 20 to remove _Escherichia coli_ 0157:H7-lux and _Listeria innocua_ from romaine lettuce leaf surface by 0.2-0.5 and 0.7-0.8 log CFU cm\(^{-2}\), respectively, compared to control lettuce leaves without soil.
Xiao [10] demonstrated the importance of using surfactants at concentrations exceeding the critical micelle concentration in order to realise its benefits.

The efficacy of a wide range of surfactants to inactivate bacteria and viruses, alone and in combination with sanitisers on leafy salad vegetables has been examined with varying results. Baby spinach leaves (Spinacea oleracea) inoculated with E. coli 0157:H7 showed a 3.1 log CFU leaf-1 reduction following treatment with 1 % thiamine dilauryl sulphate (TDS) in comparison to a simple water wash, and a further 1.4 CFU leaf-1 reduction during 7-d of shelf-life [18]. In contrast, 0.1 % SDS and 0.1 % Tween 80 did not increase the removal of Salmonella sp. and Shigella sp. on green-leaf lettuce surfaces compared to tap water [15].

The combination of surfactants and sanitisers has not always been beneficial. For example, Zhao [16] observed 4.2-4.5 log CFU g-1 reduction in S. enteritis, S. typhimurium and E. coli 0157:H7 on inoculated romaine lettuce after treatment with 0.3 and 0.5 % levulinic acid in combination with 0.05 % SDS for 1 min at 21 °C. However, Keskinen [12] observed 0.85-1 log CFU g-1 reduction of E. coli 0157:H7 on inoculated romaine lettuce after treatment with chlorine-based sanitisers, and their efficacy was not improved with addition of either 0.2 % dodecylbenzenesulfonic acid or sodium 2-ethyl hexyl sulphate surfactants for 2 min at 22 °C.

Sanitisers for fresh produce include; chlorine dioxide, hydrogen peroxide, PAA, ozone, electrolysed oxidizing water and organic acids [3, 12, 19]. PAA is a non-foaming strong oxidant composed of hydrogen peroxide and acetic acid in an equilibrium mixture and decomposes into benign products that include: water, acetic acid, carbon dioxide and oxygen [20, 21]. PAA sanitiser is preferred over chlorine, as chlorine reacts with organic matter to form trihalomethanes which are potentially harmful to human health [22].
Despite the presence of grit affecting consumer acceptability, no other studies have considered and quantified the efficacy of SDS alone, and in combination with the sanitiser, peroxycetic acid (PAA), on the removal of grit from vegetables and fruit in general including leafy salad vegetables. Most of the studies cited above focused on the effect of surfactants on microbial safety, very few of these studies assessed shelf-life and sensory quality [14, 17] and none involved tasting.

The main objective of this study was to evaluate the effect of SDS treatment alone and in combination with PAA (15.2 %) on grit removal, microbial quality, sensorial attributes and shelf-life of baby leaf salad vegetables. Two leaf varieties were selected based on their difference in morphology: baby spinach representing flat leaves varieties and coral lettuce (Lactuca sativa var. crispa) represented curly leaves. The investigation was divided into two stages, involving initial work to identify effective concentrations of SDS namely: 0.025 % 0.05 % and 0.1 % on baby spinach and coral lettuce. A subsequent experiment involved a shelf-life study of baby spinach treated with tap water as control, PAA alone and 0.05 % SDS + PAA including organoleptic evaluation.

2.0 MATERIALS AND METHODS

2.1 Plant material

Fresh baby spinach and coral lettuce were harvested manually from a commercial farm in Tasmania, Australia (Richmond Latitude: 42° 44′ 2.40″ S, Longitude: 147° 26′ 24.00″ E) at a maturity stage of 40-100 mm length. Given the nature of the study, plant material with a high load of grit was selected. Samples were transported to the laboratory in an ice box taking no longer than 40 min. Upon arrival, bruised leaves were manually removed. The baby leaves were stored at 4 °C for a maximum of 16 h before use in experiments 1 and 2.
2.2 Preparation of treatment solutions
Wash solutions were prepared using potable tap water, Tsunami 100 (active compound, peroxycetic acid, ‘PAA’, at 15 %; Ecolab, Minnesota, USA) and sodium dodecyl sulphate (SDS; Sigma-Aldrich, St Louis, MO, USA) (Table 1). In both experiments, potable tap water was used as the control and the concentration of PAA used was 40 mg L\(^{-1}\).

<table>
<thead>
<tr>
<th>Experiment number</th>
<th>Baby leafy vegetable</th>
<th>Treatment solutions</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>spinach and coral lettuce</td>
<td>✓ 0.025, 0.05, 0.1</td>
</tr>
<tr>
<td>2</td>
<td>spinach</td>
<td>✓ ✓ ✓</td>
</tr>
</tbody>
</table>

Table 1: Details on variety of leafy salad vegetable, concentrations of surfactant and sanitiser solutions used for experiments 1 and 2

Treatment solutions were stored overnight at 4 °C. The pH, oxidation-reduction potential (ORP) and turbidity of the solutions was measured by a pH meter (Orion 250A, USA), ORP meter (Milwaukee MW 500, Romania) and turbidity meter (Hach 2100P, USA), respectively.

2.3 Sanitising treatment of baby spinach and lettuce
All batches of samples were immersed for 45 s in processing wash water containing sanitizing solution with or without SDS in a ratio of 1:30 (produce:water w/v) containing sanitising solution with or without SDS. In experiment 1, each batch involved washing 30 g of baby spinach and lettuce separately in 900 mL of solution, whereas in experiment 2, 100 g of baby spinach were washed in 3 L wash water. Excess wash water was removed manually with a manual salad spinner and spun three times (8 revolutions/ spin on average). The wash water was collected to allow measurement of total grit removed. Out of the three SDS
concentrations tested in experiment 1, 0.1 % SDS produced the most foam therefore, 0.05 % SDS was selected for experiment 2.

Total grit removed was quantified by filtering the wash water through (Whatman filter paper no 1, 18.5cm) by gravity; these filter papers were oven-dried until constant weight at 80 °C. Wash solutions from experiment 2 were double-filtered, using fluted fast flowing VWR filter paper 415 (38.5 cm) first, and then medium-fast flowing fluted Whatman filter paper no. 1 (24 cm) to capture smaller particles. The amount of grit removed was expressed as g per g of fresh leaf biomass

\[
\text{Grit removed} = \frac{N_{\text{max}}}{(1 + \left( \frac{N_{\text{max}}}{N_{\text{min}}} - 1 \right) \cdot e^{(-\text{rate} \cdot %\text{SDS})}}
\]

(1).

\( N_{\text{max}} \) = maximum grit that can be removed 0.0106, 0.0141, \( N_{\text{min}} \) = minimum grit that can be removed 0.00679, 0.00977, rate = 33.9 and 38.5 for spinach and coral lettuce respectively.

For experiment 2, 40 g of processed baby spinach were packaged manually in oriented polypropylene (OPP) film (Apex films, Victoria, Australia) bags (28 x 16 cm). Bags were stored at 4 °C for subsequent quality assessment during a 14-d shelf-life trial.

On days 0, 4, 7, 10 and 14, three bags per treatment were analysed for microbial load, whereas five bags per treatment were assessed for electrolyte leakage and colour measurements. Prior to washing, samples were also analysed for microbial load on the day of processing. The organoleptic properties of the samples were evaluated during shelf life as described below.
2.4 Microbial analysis
Samples of 10-g from each package were transferred aseptically to sterile filter bags (190 x 300 mm), diluted 1:10 (wt/wt) in 0.1 % sterile buffered peptone water (Oxoid LP0037, UK) and homogenised for 120 s using a stomacher (Colworth Stomacher 400, Seward, London, UK). Subsequently, serial decimal dilutions in peptone were performed and appropriate dilutions were surface-plated on tryptone soya agar (TSA) (Oxoid CM0129, Basingstoke, Hampshire, England) and Pseudomonas agar (Oxoid CM0559, Basingstoke, Hants, UK) for enumeration of total aerobic plate count (TPC) (72 h at 25 °C) and Pseudomonas spp. (48 h at 25 °C), respectively. Microbial populations were expressed as log CFU g⁻¹ of spinach.

2.5 Colour measurements
Colour changes of baby spinach, \( L^* \) for lightness (ranging from 0 for black to 100 for white), \( a^* \) (degree of redness a+ or greenness a-), \( b^* \) (degree of yellowness b+ or blueness b-) were assessed during shelf-life. Measurements were taken at two different points on the upper surface of 15 different leaves per treatment using a colourimeter (Konica Minolta chroma meter CR400, Washington, USA) with an 8 mm diameter viewing aperture.

2.6 Electrolyte leakage
Following a modified method of Lopez-Galvez, [20], electrolyte leakage was measured using a Conductivity-TDS-pH-temperature instrument (WP-81 version 6, TPS, Brisbane, Australia). Samples (2-g) were cut approximately into 1 cm² squares and immersed in 40 mL of distilled water at room temperature for 1 h to obtain the initial electrical conductivity of each solution (C1) and of distilled water (C0). Samples were then frozen at -18 °C for 24 h and the total conductivity (C2) measured after thawing in water at room temperature for 3 h. Tissue electrolyte leakage was calculated using the formula:

\[
E = \left( \frac{C_1 - C_0}{C_2} \right) \times 100
\]
2.7 Organoleptic evaluation

For experiment 2, visual quality assessment of nine samples (3 replicates per treatment) was conducted by a panel of up to seven trained members on 0, 3, 7, 10 and 14-d of the shelf-life experiment. Quality deterioration parameters (bruising, sliming and yellowing) were evaluated on a scale of 1-5, with 5 being the highest quality (no defects, no yellowing), 1 the lowest quality and 3 commercially acceptable.

Panel tasting was performed for samples treated with 40 mgL⁻¹ PAA (considered as the control treatment) and 40 mg L⁻¹ PAA + 0.05 % SDS. Due to food safety reasons, samples washed with portable water only were not included for tasting. Samples were stored at 4 °C for 48-64 h and removed from the fridge before serving. 48-64 h is the shortest time it takes for the packaged product to reach the consumer after processing. During the evaluation, two samples treated with PAA and the other treated with PAA + SDS were served at the same time to 34 panelists. Coded samples were rated on flavour, aroma, texture, and overall liking on a 9-point hedonic scale of 1-9 (dislike extremely - like extremely). Panelists were also asked to indicate their purchase intent on a scale of 1-5 (definitely would buy - definitely would not buy). This study was approved by the University of Tasmania Social Sciences Human Research Ethics Committee – ethics reference number H0016331. Written consent to participate was sort from the panelists, specifying that only the sensory evaluation data will be published without identifying individuals involved.

Statistical analysis

Data were analysed using JMP statistical software (version 11, SAS Institute Inc, USA). The relationship between grit removed and % SDS from experiment 1 was evaluated using regression analysis. For experiment 2, two-way analysis of variance (ANOVA) was used to analyse TPC, Pseudomonas count, electrolyte leakage and colour parameters shelf-life data.
with day and treatment as the independent variables. Grit data was analysed using one-way
ANOVA followed by Tukey’s honestly significant difference (HSD) test. To understand
whether treatment had an effect on taste attributes, data were analysed using the chi-square
test in JMP. ANOVA for sensory evaluation data (visual quality assessment) was calculated
using “proc mixed” in SAS (version 9.3, USA), a random effect was included for the panelist.
A repeated measures approach was assumed with a spatial correlation structure, where the
sample code was used as the repeated experimental unit. Assumptions for homogeneity of
variance and normality were checked before each analysis. Significance was calculated at p
= 0.05.

3.0 RESULTS AND DISCUSSION

3.1 Optimising SDS concentration for grit removal from baby spinach and
coral lettuce
There was a significant positive correlation between the amount of grit removed and %SDS
(Fig. 1), $R^2$ was higher for coral lettuce than spinach. ($R^2_{\text{coral}} = 0.734$, $p<0.0001$; $R^2_{\text{spinach}} = 0.372$, $p=0.0043$).
Figure 1: Relationship between grit removed per g of coral lettuce and spinach and % SDS concentration. (SDS = sodium dodecyl sulphate).

Increasing SDS concentration also resulted in increased foaming. Ho [23] also observed excessive foaming in wash tanks containing 250 ppm SDS in combination with peroxyacetic acid + lactic acid.

3.2 The effect of PAA + SDS treatment on grit removal, microbial load, shelf-life and taste of baby spinach

3.21 Wash water characteristics

Addition of SDS to PAA did not influence pH and ORP values (table 2) which suggests that SDS does not influence antimicrobial properties of the sanitiser. Zhao [16] observed a pH of 6 for 0.05% SDS, 3.0 for levulinic acid (LA) and 3.1 for LA combined with SDS. Guan [17] also observed pH of 3.04 for 0.5% LA + 0.05% SDS.

Table 2: pH and ORP values for wash water solutions used in experiment 2
Wash solution | pH  | ORP |
---|---|---|
Tap water | 6.82 | 363 |
PAA (40 mg L\(^{-1}\)) | 4.25 | 587 |
PAA (40 mg L\(^{-1}\)) + 0.05 % SDS | 4.24 | 557 |

Although turbidity values of PAA + SDS solution after washing were high (195-228 NTU) compared to the control (79 NTU) and PAA solutions (76 NTU) due to the presence of grit, PAA+SDS solution also had high turbidity values (90-114 NTU) even before washing (supplementary table S1).

3.22 Grit removed

In experiment 2, the combination of SDS (0.05 %) and PAA resulted in a significant increase (p = 0.0012) in the amount of grit removed as compared to tap water and PAA alone by 19 and 21 %, respectively (Fig. 2). Grit removed by tap water and PAA was comparable (Fig. 2; p >0.05). Preliminary trials also proved that SDS alone washed more grit as compared to tap water (similar results to Fig. 1) and PAA+SDS washed off more grit compared to PAA alone (data not shown).
Figure 2: Grit removed gram /gram of baby spinach using washing solution treatments (control = tap water, PAA 40 ppm, SDS = 0.05 % sodium dodecyl sulphate). Error bars represent standard error of the mean (n=5). Different letters show significant differences at p < 0.05.

3.23 Microbiological analysis
The initial TPC of baby spinach was 6.6 ± 0.1 log CFU g⁻¹ (Fig. 3) with significant reductions of 0.85, 1.28 and 1.50 log CFU g⁻¹ observed after washing with tap water, PAA and PAA + SDS, respectively (Fig 3; p < 0.001). A progressive increase in TPC from 5.1-5.8 log CFU g⁻¹ was observed during storage across all treatments, reaching similar levels of 7.9-8.3 log CFU g⁻¹ on day-10. Samples washed with tap water alone had 0.4 log CFU g⁻¹ higher counts (p = 0.0002) during the first few days of shelf-life in comparison to PAA and PAA + SDS treated samples during storage (Fig. 3). However, no significant difference (p >0.05) in TPC were observed between PAA and PAA + SDS treated spinach throughout the storage period.

Initial *Pseudomonas* count was 5.0-5.5 log CFU g⁻¹ (Fig. 4) with an increase of 2.5-2.9 log CFU g⁻¹ observed during shelf life for all treatments. However, there was no significant treatment effect (p >0.05) during storage (Fig. 4). The growth trend of *Pseudomonas* spp. was similar to that of TPC (Fig. 3).
Figure 3: Total aerobic plate count of baby spinach leaves treated with tap water (control), peroxyacetic acid (PAA), or peroxyacetic acid + sodium dodecyl sulphate (PAA + SDS), before wash (UN) and after wash during storage at 4 °C for 14 d. Error bars represent the standard error of the mean (n=3). Different letters show significant differences at p < 0.05.
PAA + SDS treatment did not produce higher initial TPC log reductions or reduce microbial growth during shelf-life in comparison to PAA treatment, and thus, SDS had no effect on microbial quality. Similar results were obtained by Ho [23] whereby 0.02-0.025 % SDS did not improve the efficacy of PAA (70 mg L⁻¹) and lactic acid (4500 mg L⁻¹) treatment against E. coli K-12 and L. innocua on inoculated romaine lettuce and spinach. Salgado [14] studied the effect of treating lettuce with 1 g L⁻¹ SDS + 80 ml L⁻¹ Tsunami 100 + ultrasonication on quality aspects. Treatment of inoculated iceberg lettuce with 0.25 % sodium acid sulphate + 0.5 % SDS resulted in 0.87 log CFU g⁻¹ decrease in E. coli 0157:H7, similar to 0.94 log CFU g⁻¹ observed after treatment with 100 ppm chlorine solution [17]. In the same study, 0.41 log CFU g⁻¹ was observed after treatment with 0.5 % LA + 0.05 % SDS for 5 min. Using 0.1 % SDS improved the removal of L. innocua from inoculated romaine lettuce by 0.95 log CFU m⁻² in comparison to deionised water, therefore yielding a total reduction of 1.79 log CFU m⁻² [5]. In contrast, Zhao [16] observed 4.2-4.5 log CFU g⁻¹ reduction of Salmonella spp. and E. coli 0157:H7 on inoculated romaine lettuce after treatment with 0.3 and 0.5 % levulinic acid in combination with 0.05 % SDS for 1 min at 21 °C. Therefore, in literature there is varying evidence on the effect of surfactants on leafy salad vegetables.

3.2.4 Colour and electrolyte leakage

No changes in colour L* a* and b* parameters were observed during shelf-life across all treatments (p > 0.05) (supplementary table S2). Huang and Nitin [5] only observed marginal colour changes after washing romaine lettuce with 0.1 % SDS in comparison to water wash. On each sampling day, there was no significant difference in electrolyte leakage (p>0.05) between treatments (supplementary table S3). Electrolyte leakage of romaine lettuce
washed with 0.1 % SDS alone was not significantly different from the control leaves washed with tap water [5].

3.25 Sensory evaluation

Scores for bruising, sliming and yellowing of baby spinach were similar across treatments during shelf-life (Fig. 5; p > 0.05). Regardless of the treatment, an increase in bruising and sliming was observed on baby spinach leaves during storage, reaching unacceptable levels (< 3) by day-12. Yellowing scores were still within acceptable range (≥ 3) at the end of shelf-life (Fig. 5).

Similarly, Gómez-López [24] observed a decrease in overall quality of baby spinach treated with PAA (80 mgL⁻¹), during shelf-life from day-4. In contrast, lettuce treated with water and sodium hypochlorite maintained better visual quality compared to lettuce treated with 0.5 % - 3% levulinic acid + 0.05 % SDS and 0.25 – 0.75 % sodium acid sulphate + 0.05 % SDS during 14-day storage period at 4 °C [17].
Figure 5: Changes in sensorial attributes, bruising, sliming, and yellowing scores for baby spinach samples treated with tap water, peroxyacetic acid (PAA), and peroxyacetic acid + sodium dodecyl sulphate (PAA+SDS), stored at 4 °C for 14-d. Error bars represent the standard error of the mean (n=7 assessors). Effect of the treatment was not significant. Panelists did not identify any significant differences in taste attribute scores nor overall liking between the spinach samples treated with PAA and PAA (40 mgL⁻¹) + 0.05 % SDS + SDS Fig. 6; p > 0.05).
Figure 6: Panel test scores for baby spinach treated with peroxycetic acid (PAA), and peroxycetic acid + sodium dodecyl sulphate (PAA + SDS), stored at 4 °C for 48-64 h. Error bars represent the standard error of the mean (n=30-34 panelists). Effect of the treatment was not significant.

Similar results were obtained by Zhou [25] where panelists did not observe differences in flavour, appearance and texture between strawberries washed with 0.5 % levulinic acid + 0.5 % SDS and 50 mL⁻¹ chlorine solution for 2 min. Though no studies have examined the effect of PAA + surfactant treatment on the taste of leafy vegetables, Ho [23] observed no differences in appearance, colour, aroma, taste texture and overall liking of leaf mix containing spinach, chopped iceberg and romaine lettuce treated with PAA + lactic acid compared to samples treated with chlorinated water.

Seventy percent of the consumers reported that they would be willing to purchase baby spinach treated with PAA + SDS based on sensorial quality, 21% were unsure and only 9% were unwilling.

Conclusions
The use of SDS (0.05, 0.1 %) significantly improved grit removal from baby spinach and coral lettuce in comparison to tap water wash or sanitiser alone. 0.05 % SDS + PAA (40 mgL⁻¹) treatment aids in grit removal without affecting microbial quality, electrolyte leakage, colour L*, a*, b*, shelf-life, sensorial and organoleptic properties of baby spinach. Future research in this area should consider scaling up to pilot plant with the aim of using low concentrations of SDS to reduce potential foaming issues to assess the feasibility of using SDS in a commercial processing facility.

Data availability

The data table and figures used to support the findings of this study are included within this article and the supplementary material.

Declaration of conflicting interests: “The Authors declare that there is no conflict of interest”.

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Data tables S1 - S3 are included within the supplementary information file

REFERENCES


