

## **Investigating the efficacy of combined treatments of ultrasound and antimicrobial extract for the inactivation on food pathogens on ready-to-eat salad leaves**

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The consumption of ready-to-eat pre-washed salad leaves has been increasing due to health benefits and convenience. However, current processing methods, such as water washing, are not fully effective to eliminate pathogenic bacteria, as demonstrated by recent outbreaks linked to the consumption of such foods. It is therefore necessary to investigate other technologies to ensure the microbiological safety of these products.

Ultrasound is a novel non-thermal technology of interest. As it is a milder than traditional thermal technologies, ultrasound may be used to process heat-sensitive fresh produce i.e., salad leaves. However, its mild nature may instead represent a sublethal stress, leading to stress adaptation, post-treatment survival, and the potential for antimicrobial resistance development in pathogens. Previous studies indicate that combining ultrasound with other decontamination techniques as a hurdle technology can be effective to increase the antimicrobial efficacy; indeed, it could be combined easily with water washing of fresh produce. Furthermore, antimicrobial extracts from pomegranate peels have been shown to inhibit the growth of some pathogenic bacteria and utilise a by-product from food manufacturing. The combination of pomegranate extract and ultrasound has been investigated in fruit juice but has not yet been studied on salad leaves. As such, this work presents a systematic investigation into the efficacy of ultrasound in combination with pomegranate peel extract for microbial inactivation.

Ultrasound was applied at a range of frequencies (44 - 1000 kHz) either alone or in combination with pomegranate peel extract to salad leaves inoculated with *L. monocytogenes* or *E. coli*. The multi-frequency analysis was conducted in a reactor vessel with interchangeable transducers, allowing for consistency in experimental set-up and therefore a systematic approach to identify inactivation mechanisms. The efficacy and mechanisms of inactivation were found to depend on parameters including the applied frequency, applied power, the combination of treatments, and the microbial species. The effect of these inactivation treatments on the organoleptic properties and structure of salad leaves was also investigated.

This work indicates that water washing of salad leaves may be enhanced by the integration of pomegranate extract and ultrasound treatment, ensuring microbiological safety while maintaining structural properties.