

A side of *Salmonella* with your salad?

Salad leaves are an important part of a healthy diet but in recent years they have been associated with infection by food poisoning bacteria such as *Salmonella*. Research by **Giannis Koukkidis** in the Department of Infection, Immunity & Inflammation reported by the BBC, CBS, Reuters, The Guardian, Mirror, Daily Mail, NHS, and hundreds of internet news outlets and radio shows, has revealed that the juices which drip from the cut ends of leafy greens inside bagged salads can massively boost *Salmonella* growth even when refrigerated and increase its capacity to cause infection.

Not so innocent salad leaves

It is a common held misbelief that food poisoning can only originate from the fish or meat component of a meal. However, recent research highlights the contribution of your salad side dish in this issue. Surveys from the Food Standards Agency report that fresh produce such as salads and fruit are responsible for nearly 50,000 incidents of food poisoning in the UK every year. *Salmonella*, *E. coli*, *Campylobacter* and *Listeria* are the top bacterial pathogens associated with uncooked salads and other fresh produce. In the UK *Salmonella* is responsible for more than 2,500 hospital admissions every year.

“*Salmonella* can even grow in the fridge when it comes in contact with salad juices”

Healthy Salads?

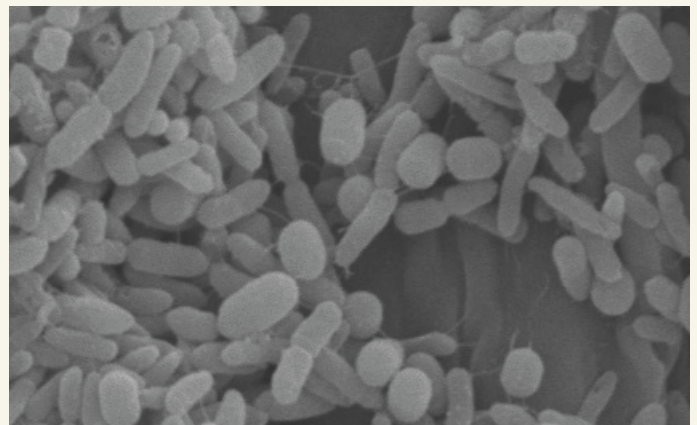
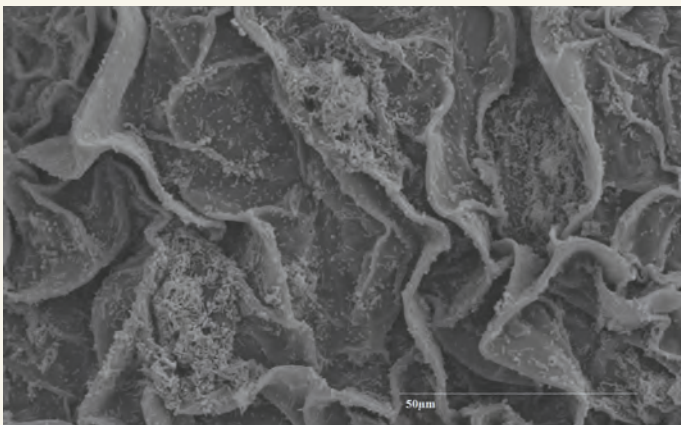
Because of their claimed health benefits, the consumption of salad leaves such as lettuce, spinach and rocket has increased

considerably in recent years, but along with this increase in consumption, increased cases of salad-associated food poisoning such as *Salmonella* and *E. coli*.

“Consumption of salad leaves such as lettuce, spinach and rocket have increased... so have cases of *Salmonella* and *E.coli*”

I was therefore curious about the behaviour of the bacteria if they got into the salad bag. To investigate, I extracted juices from salad leaves, and collected the juice-rich watery fluid that accumulates at the bottom of the plastic bag that holds ready cut salads, and looked at what it did to promote the risk of food poisoning by bacteria such as *Salmonella*.

The salad leaves used in our research were cos lettuce, baby green oak lettuce, red romaine lettuce, spinach and red chard.



Scanning electron micrograph (SEM) images of spinach juice-exposed *Salmonella* on a section of spinach leaf. *Salmonella* magnified (right).

Salad juices increase *Salmonella*... even in the fridge

Investigations began with *Salmonella*, as this is one of the most aggressive food-poisoning bacteria. It was found that even tiny traces of salad leaf fluids (as little as 1/500th of a teaspoon) or even the watery bag fluid massively stimulated the growth of *Salmonella*, even if the bacteria were incubated in the fridge. This was most surprising as it was believed that *Salmonella* should stop growing in any environment below 7 °C, yet it grows quite happily at 4 °C if provided with salad juices.

“Consumption of damaged leaves or salad juices may exacerbate a *Salmonella* infection carried by the salad”

In the fridge, I found that fewer than 100 *Salmonella* on day 1 (not enough to cause illness) became more than 100,000 by day 5 (more than enough to cause food poisoning), these 5 days represented the use by date of the salad, so many people would consider the salad still “safe” for consumption.



Fluid that accumulates in bagged salads (mixed-leaf varieties).

Behaviour of *Salmonella* in the salad bag

We also found that *Salmonella*'s ability to attach to the salad leaves was much greater when salad juice or fluid was present, and washing the leaves in water did not remove the bacteria.

When we used higher magnification electron microscopy we noticed that the *Salmonella* also made more biofilm, an aggregation of bacteria encased in a jelly like slime which they make to attach themselves to surfaces. This biofilm was extensive when salad juice



Rocket leaf and the resulting microbe print on Luria Agar Media plate¹

was present and extended as far as the stomata, the microscopic pores in the salad leaves. The *Salmonella* biofilm formation occurred despite the presence of many thousands of bacteria and other microbes that naturally colonise the salad leaves (known as the microflora).

“There are around 50,000 food poisoning cases per year in the UK which come from fresh produce”

What was also surprising, was that the salad juice helped the *Salmonella* to attach to the interior of the salad bag itself – previously, no one had considered the salad container was a bacterial attachment site.

In fact, the *Salmonella* treated with the salad juice displaced the leaf microflora that were naturally growing there! And, again, the biofilm formed with the salad juice was not washed away with water.

Implications of eating the infected salad?

Salad juices also increased *Salmonella* growth when added to a liquid designed to support the growth of bacteria and replicate the environmental conditions inside a human.

This was up to 2400-fold when compared to the non-juice supplemented controls. The salad juices stimulate growth by helping the bacteria steal iron from a normally non-accessible iron-

binding protein called transferrin, whose role is to prevent growth by sequestering all free iron and making bodily fluids bacteriostatic (inhibitory to bacterial growth).

Salad juices made *Salmonella* more aggressive, as we found increased expression of proteins involved in the direct invasion of human gut cells. This means that consumption of damaged leaves or salad juices may exacerbate a *Salmonella* infection carried by the salad.

“Salad juices made *Salmonella* more aggressive”



Top: Rocket leaf microbe print

Bottom: Variety of salad leaves used in our research (baby green oak lettuce, red romaine lettuce, spinach and red chard)



Rocket leaf and associated microbe print on Luria Agar Media plate (Above and Left)¹



Salad leaf microbe prints on Luria Agar Media plates (spinach, rocket, coriander, parsley and red chard)¹

Final thoughts - and how can we avoid food poisoning?!

Our project did not indicate that there was any increased risk to eating leafy salads, but it does provide a better understanding of the factors contributing to food poisoning risks from bagged salads.

Our research did not look for evidence of food poisoning bacteria in salad leaves, instead, it examined how *Salmonella* behaves on salad leaves when they are damaged inside the plastic bag environment.

The findings from this research compel us to provide the following advice when buying and storing bagged salads: when purchasing bagged salads choose those with the latest use by dates, avoid bags with mushy leaves, and bags or containers that look swollen.

Use the salad as quickly as possible after purchase to minimise the growth of any pathogens that might be present, and wash the leaves thoroughly before consumption – this will remove juices which could increase growth of food poisoning bacteria.

Our future work will investigate the use of novel techniques to prevent attachment of pathogenic bacteria to salad leaves and fresh produce.

Giannis Koukkidis began working in the field of food technology during his Masters this subsequently became his PhD project at the University of Leicester. He is currently studying the interaction of a range of food poisoning bacteria with bagged salad leaves with the aim of reducing infections from fresh produce.