Scientists at the ARS Produce Quality and Safety Laboratory in Beltsville, Maryland, are focusing on ways to keep packaged fresh-cut lettuce and leafy greens safe.

Arried health-conscious eaters love the convenience of purchasing fresh, bagged salads. Today, sales of fresh-cut lettuce and leafy greens have reached $3 billion annually, according to industry experts, and the demand is increasing.

But these increased demands also come with new food safety challenges, affirmed by the recent outbreaks of *E. coli* O157:H7-related illnesses traced to bagged fresh produce.

For example, cutting fresh produce during harvesting removes natural protective barriers, exposing cut surfaces to potential contaminants. Plant operators use cleaning treatments to wash surfaces and reduce cross-contamination from environmental pathogens that may be introduced before processing.

At the ARS Produce Quality and Safety Laboratory (PQSL) in Beltsville, Maryland, several researchers are focusing on ways to keep leafy greens safe at processing facilities and at home. Their studies range in focus from washing product before bagging to effective storage conditions after bagging.

**Sanitizing Before Bagging**

PQSL food technologist Yaguang Luo recently studied various wash waters and sanitizers that enhance the microbial safety of lettuce and leafy greens. Washing/sanitizing is a step taken by the produce industry. It is an important intervention used for reducing contamination on product surfaces to increase the safety and quality of precut produce before bagging.

Luo simulated washing techniques to learn more about how industry practices affect safety and quality of precut lettuce. “This study evaluated the effect of wash-water reuse on water quality and the subsequent effects on microbial growth and product quality,” says Luo.

For the study, romaine lettuce leaves were sliced and then rinsed in either fresh wash water or various types of reused wash waters. The washed leaves were then...
dried, placed into bags made from special oxygen-permeable films, and stored at 5°C (about 41˚F).

Microbial growth and product quality were monitored at 0, 4, 8, 11, and 14 days of storage. The results showed that at the end of storage time, unwashed control leaves and leaves washed with water that had been reused many times had higher bacterial populations than those washed with clean water.

Washing a small amount of lettuce thoroughly in a large amount of clean water resulted in the least off-odor development.

Luo also found that when water was reused during the washing process, organic matter rapidly accumulated within that wash water and then compromised the efficiency of any sanitizers that had been applied.

The study demonstrated the direct effect of wash-water quality on the final quality of the product.

“This new information gives produce packers new tools in designing wash systems and managing wash operations for enhancing food safety and quality,” she says. The study was published in HortScience.

**Which Treatments To Use**

Various new treatments are also being developed and tested to evaluate their effectiveness during industrial-scale washing of produce. Luo has been working with the industry to formulate a new sanitizer that works better than chlorine as a wash-solution ingredient. She and her collaborator at the University of Illinois specifically tested combining the use of several different sanitizers with ultrasound as a means to enhance removal of contaminants before bagging.

The study showed that combining the new wash-solution treatment with ultrasound treatment could reduce bacterial contamination by up to 4.5 logs—meaning the bacteria decreased from about 300,000 colony-forming units to less than 10. Therefore, “the combination of a selected sanitizer with ultrasound can potentially be used to enhance microbial safety of leafy green produce before the bagging process,” Luo says.

**Safety After Bagging**

While sealing fresh-cut lettuce and leafy greens in clear package wrappers, called “films,” provides some protection, research is needed to understand the environment inside the bags and how it affects bacterial growth and product quality.

An important part of maintaining safety, freshness, and shelf life of bagged lettuce and leafy greens is finding the right balance of gases inside the wrappers. This technology, called “modified atmosphere packaging,” or MAP, allows fresh-cut produce to respire slowly during storage.

Hundreds of manufactured films each have their own unique oxygen-transmission rate, which allows cut produce to continue “breathing” throughout storage and distribution periods. Luo has also been identifying package films that complement the characteristics of individual plant foods within.

In addition to the gas permeability of different film packages and the effect that those rates have on the quality of the produce within, many other factors contribute to the atmosphere inside bagged leafy greens.

New equipment called “GrowthCurves USA” allows researchers to monitor pathogen growth in individual wash-water samples—including reused wash water—in real time. Microbiologist Arvind Bhagwat, of the ARS Produce Quality and Safety Laboratory, and graduate student Liu Liu are using the equipment to help industry improve sanitizer efficiency.
**Bacterial Adaptability in the Bag**

PQSL microbiologist Arvind Bhagwat has been investigating whether there are differences in bacterial growth levels between cut lettuce bagged in certain types of sealed modified atmospheres and lettuce leaves bagged in regular air.

To keep plant tissue respiring at minimal levels—and thereby extend the time that bagged salad appears fresh—manufacturers have adopted a type of MAP that involves excessively low oxygen. To maintain functionality and a fresh appearance of the produce, other safe and acceptable gases are used in place of oxygen.

Bhagwat started with the premise that, on your everyday head of uncut lettuce, bacteria are starving for nutrients. In their desperation to survive and grow, these bacteria try to adapt to the changing environment. Bhagwat studied the survival skills of bacteria that had been applied to lettuce leaves and then sealed inside package films. He wanted to know whether the lack of oxygen would make a bacterium’s pursuit for survival in the human gut more difficult.

Surprisingly, he found that in response to the challenge of being air-starved, combined with the added nutrients provided by the cut leaves, the bacteria actually became harder, better surviving a challenge with synthetic gastric juice.

“Luckily, we found this phenomenon only took place when salad bags were left at what we call ‘abusive temperatures,’” says Bhagwat. “That is when bagged fresh-cut salad is left at room temperature or unrefrigerated for an extended period.”

**Finding the Right Temperature**

Since success with MAP is influenced by refrigeration, Bhagwat also tested different temperatures. In a study, bacteria were applied to fresh-cut lettuce and stored under different MAP conditions for 8 days.

Low-oxygen conditions were defined as between 0.5 percent and 1 percent oxygen in a sealed package. Regular-oxygen conditions were defined as 20 percent oxygen in a sealed package.

When stored under extremely low-oxygen conditions and at temperatures of 15°C (about 59°F) or above, bacteria became more resistant to synthetic gastric juice. In comparison, no resistance was induced among bacteria stored under extremely low-oxygen conditions and at temperatures of 10°C (about 50°F) or below.

In addition, regular levels of atmospheric oxygen did not induce resistance in the bacteria, even if the salad sample was left at abusive temperatures.