Robyn Miranda

Process and Agent-Based Modeling for Risk Assessment of Norovirus on Frozen Berries

During the last decade, an increased incidence of infections and outbreaks attributed to foodborne viruses, specifically norovirus, was observed worldwide. In 2012, a norovirus gastroenteritis outbreak in Germany affecting over 11,000 children was linked to contaminated frozen strawberries[4]. Most foodborne viruses cannot be cultured in the laboratory, which hinders studies of their stability in food. Cultivable surrogate viruses, genetically related to the human infecting strains, are used as a substitute. The purpose of this project is to perform survival and inactivation studies to contribute towards future risk assessment models for norovirus on frozen berries. Farm-to-fork risk assessment models will be developed to evaluate norovirus mitigation strategies in the frozen fruit supply chain. Past norovirus outbreaks will be simulated for validation of the models.

Freezing is a common practice to preserve berries, but survival characteristics of foodborne pathogens on frozen fruits is not well understood[1,2]. Frozen storage studies were conducted on raspberries and strawberries. The berries were inoculated with three different inoculum levels of bacteriophage MS2, a norovirus surrogate (~10 log PFU/ml, ~8 log PFU/ml, ~6 log PFU/ml) and stored in the freezer at -20°C. The berries will be removed at specific times over a two-year time period to quantify the viral load remaining on the fruit following frozen storage. While thermal destruction of pathogenic bacteria has been thoroughly studied in the food industry, heat inactivation of viruses in food has been poorly investigated. The objective of the heat inactivation study is to calculate heat resistance parameters (D and z values) using berry purees inoculated with bacteriophage MS2 at temperatures used in the industry[3]. Heat is known to affect the stability of viruses and thus be an effective means of virus inactivation.

Risk models will use input data collected from scientific literature, experimental studies and assumptions. The first model was built in Excel using the Monte Carlo modeling software @Risk add-in. This model assumed the initial contamination source was surface water used to apply pesticides to berries in the field. A sensitivity analysis was performed to identify the most relevant model inputs and to evaluate mitigation strategies. The @Risk model closely simulated the German outbreak when the highest concentration of 8 log genome copies (GC)/L for pesticide application was assumed. Variables that had the greatest impact on the outcome of illnesses include the concentration of norovirus in surface water and use of a heat step on the berries prior to consumption. The second model will be performed in AnyLogic, an agent-based modeling software, to represent ill field-workers contaminating the berries at harvest. Contamination of berries can occur pre- or post-harvest and they may be washed before freezing, but are not usually blanched or heat-treated unless they are used in preserves or other processed products.

Selected References

1. Baert, L., M. Uyttendaele, M. Vermeersch, E. Van Coillie, J. Debevere. 2008. Survival and Transfer of Murine Norovirus 1, a Surrogate for Human Noroviruses, during the

Production Process of Deep-Frozen Onions and Spinach. *Journal of Food Protection.* 71(8): 1590-1597.
2. Butot, S., T. Putallaz, G. Sanchez. 2008. Effect of sanitation, freezing and frozen storage on enteric viruses in berries and herbs. *International Journal of Food Microbiology.* 126: 30-35.
3. Deboosere, N., O. Legeay, Y. Caudrelier, M. Lange. 2004. Modelling effect of physical and chemical parameters on heat inactivation kinetics of hepatitis A virus in a fruit model system. *International Journal of Food Microbiology.* 93: 73-85.
4. Made, D., K. Trubner, E. Neubert, M. Hohne, R. Johne. 2013. Detection and Type of Norovirus from Frozen Strawberries Involved in a Large-Scale Gastroenteritis Outbreak in Germany. *Food and Environmental Virology.* 5:162-168.