APC / Pathogen Corellation Study of Food Safety Status of Reusable Packaging

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Project Proposal for Consideration by RPA's Food & Beverage Committee
Edited by Tim Debus, President, RPA
Draft Version 1.0, August 1, 2016

Abstract

Reusable Plastic Containers (RPCs) are a safer, more efficient and more environmentally sustainable alternative to traditional packaging most commonly used in the food sector. IFCO studies have shown that RPCs produce 82% less solid waste, require 49% less energy and consume 92% less water than other packaging. RPCs are washed, rinsed and sanitized between each use to ensure they present a sanitary transport medium from the origin of the food it carries to its ultimate destination, the consumer. RPC use has grown dramatically every year since their adoption in North America for these reasons and more.

However, the potential for contamination cannot be ignored if inadequate sanitation occurs. That risk is not just relegated to human health. Plant disease viruses are commonly transmitted by mechanical or incidental contact within greenhouses and growing operations. These plant pathogens have the potential to affect entire sections of crops under glass at significant monetary loss for the operator.

That said, it is incumbent upon RPC providers to properly explain to the market and RPC users at large what constitutes risk within the supply chain in an unbiased way. Opponents of RPC use have suggested that Aerobic Plate Count (APC) accurately represents a food safety risk. APC measurements provide a useful tool in understanding the possible performance of the sanitation cycle of RPCs. We believe APC values do not represent a reliable measure of food safety risk concerning reusable packaging. Only pathogen and/or documented indicator bacterial species can adequately predict food safety risk in the fresh food supply chain.

The proposed project will evaluate the correlation of Aerobic Plate Count (APC) values with the prevalence of human pathogens known to pose a risk within the North American food supply chain. The goal of this project is to determine the relationship, if any, to known risks to food safety (pathogens). A single variable design is proposed to explore this correlation. The collection of data will follow strict scientific methodology conducted by a reputable third party participant. The data and conclusions learned will be intended to help the fresh food supply chain conduct risk based analysis as well as help interested individuals, companies and organizations to determine any risk associated with RPCs in the fresh food supply chain in the most unbiased manner possible.

Logistic regression is a useful tool in predictive food microbiology to determine food safety in relation to food composition, process, and in this particular case, storage variables (Zhao etal., 2001; Belletti et al., 2007). Therefore, it is proposed to use a logit model to evaluate the possible relationship among storage variables (i.e. temperature, time, distance traveled) and the probability of transferring APC and/or pathogens from packaging materials to a sponge test in any recoverable volumes.

Study Objective

The objective of this project is to evaluate the correlation of Aerobic Plate Count (APC) values with the prevalence of human pathogens known to pose a food safety risk on Reusable Plastic Containers (RPCs) that are within the North American fresh food supply chain. The specific questions answered in the study will be:

1. Do any of the RPCs within the study sample have the following human pathogens in recoverable volumes?
    a. *Salmonella spc.*
    b. *E.coli 0157:H7*
    c. *Listeria monocytogenes*

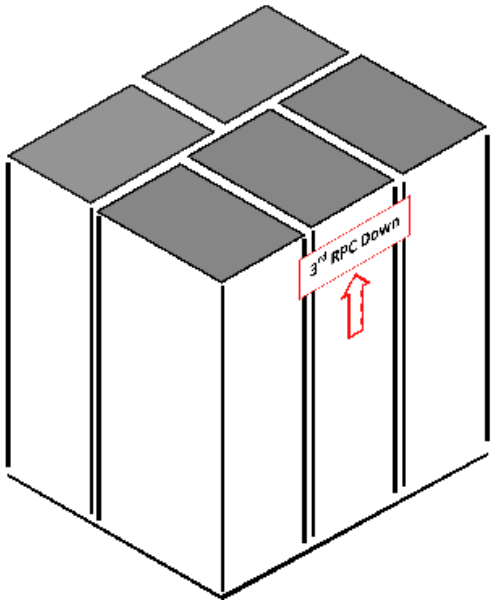
2. What are associated Aerobic Plate Count values of the RPC when an APC swab is taken directly adjacent to the previous pathogen swab on the same RPC?
    a. *Pepino Mosaic* Virus
    b. *Tomato Mosaic* Virus
    c. *Cucumber Mosaic* Virus

3. What, if any correlation value can be associated with the APC vs. Pathogen values of washed, rinsed and sanitized RPCs?

Study Methodology

Study Parameters

- The study will be conducted over 4 weeks of operation working with 10 separate RPC customers around North America.
- Each of the 10 customers will allow the contracted microbiologist or a trained food science technician designated by the Study Team to collect a maximum of 25 RPC surface samples per week.
- The 25 samples will be taken from incoming loads of RPCs from the manufacturers.
- The RPCs tested will be intact in their original form of packaging and be taken directly from their respective van trailer or shipping vehicle.
- Not more than 20 samples shall be taken from a specific load, and not more than 5 samples shall be taken from a specific pallet.
- The contracted microbiologist or a trained food science technician designated by the Study Team will adhere to the prescribed testing methodology found in the International Microbiology Laboratory Guideline
- Pallets of RPCs shall be chosen at random in an effort to avoid selection bias and maintain consistent results.
- In addition, the individual RPC to be sampled will be subject to a double blind selection bias protocol. Only the contracted microbiologist or a trained food science technician designated by the Study Team will know the exact RPC chosen.
    - For example: See illustration: RPC to be sampled might be the 3rd down from the top of the center stack on each pallet.



Contracted Microbiologist or their Trained Food Science Technician Designee

- Each sample will follow the "Swab Testing Procedures: EnviroSponge™ / HydraSponge™" below:

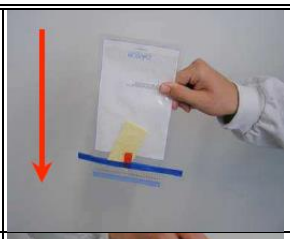
- Sample surface area of each swab will be the interior of the container on the base.
- A 30 x 30 cm^2 (11.8 x 11.8 in^2) area will be sampled.
- Once the sample swab has been taken correctly, the contracted microbiologist or a trained food science technician designated by the CHC RPC Task Force will place the sample on ice immediately after the procedure is complete.
- The entire set of samples for the day will be sent on ice, via overnight mail delivery to the following addresses:

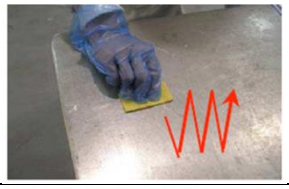
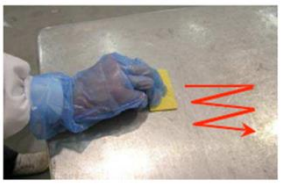
List Participating Labs

University of Guelph Laboratory

- The University of Guelph laboratory has bid on the project. The lab testing will be overseen by Todd Marrow – University of Guelph Agriculture & Food Laboratory Business Development / Diagnostics Manager and Amanda Hauck – University of Guelph Agriculture & Food Laboratory Services Division Supervisor.
- BAX method testing will be conducted on all microbiology tests. All testing results will be available 48 hours after testing.
- The University of Guelph laboratory will provide all the testing materials, swabs and lab analysis for all samples and all 6 pathogens scheduled.

Swab Method

Step 1	For convenience, move the sponge to the top of the sample pouch by shaking the pouch in a downward motion.	
Step 2	Tear open the pouch and push the sponge, from the outside of the bag, so that it just protrudes the opening in the pouch.	
Step 3	Open the sterile glove sachet and put it on with care.	
Step 4	Using the Sterile gloved hand, remove the sponge from the pouch. Do not touch the inside of the bag!	

Step 5	Swab the sampling surface (suggested 30 x 30 cm^3 area) with the sponge in a <u>vertical</u> (up and down) direction.	
Step 6	Turn the sponge over and swab the sample area again, this time in a <u>horizontal</u> (left to right) direction.	
Step 7	Return the sponge to the pouch with care.	
Step 8	Remove the glove and dispose.	
Step 9	Fold down the top of the pouch and use the wire tabs to secure the pouch.	

Literary Review

- Microbial cross-contamination refers to the transfer, direct or indirect, of microorganisms (bacteria, virus, parasites, or fungi) from a contaminated item to a non-contaminated one (Minnesota Department of Health, 2007). In food, cross contamination of foodborne pathogens is a major concern since it increases the health risk for humans due to the intake of contaminated food. Otherwise, cross-contamination of foodborne pathogens from inert surfaces to foods is well documented (Kusumaningrum et al., 2003; Lin et al., 2006; Wilks et al., 2006; De Candia et al., 2015; Erickson et al., 2015).

- The adhesion and persistence of microorganisms to the surfaces can spread pathogens and spoilage microorganisms to foods, influencing their shelf-life and safety (Barnes et al., 1999; Bae et al., 2012). Several studies have showed the ability of microorganisms to attach to all the surfaces commonly found in the food processing environment, such as stainless steel, polystyrene, rubber, glass, wood and so on (Czechowski, 1990; Mafu et al., 1990; Krysinski et al., 1992; Suárez et al., 1992; Barnes et al., 1999; Siroli et al., 2014). Additionally, if microorganisms remain on a given surface for a relatively long time, they can multiply and, eventually, form biofilms (Uhlich et al., 2006).

- On the other hand, fresh produce have been associated in several outbreaks caused by E. coli O157:H7, Salmonella spp. and L. monocytogenes (Alegre et al., 2010; Scallan et al., 2011; Oliveira et al., 2012; Siroli et al., 2014). According to EFSA (2013), these products are involved in more than 5% of food borne illness in Europe. Also the USA Centre for Disease Control and Prevention (CDC) clearly showed the fresh produce as a source of contamination leading to food borne illnesses. In fact, pathogens, eventually introduced during the production chain, may remain until the product consumption due to the lacking of treatments able to eradicate the microbial cells. The interruption of cold chain during distribution, sale and home storage determine rapid deterioration of these products due to the growth of spoilage microorganisms present on fruit and vegetable. To increase the limited shelf-life of fresh produce the tendency is to pack unripe fruit and vegetable characterized by lower sensory features compared to ripe fruits.

- The literature data on the contamination levels of packaging materials are few and fragmented. However, they demonstrated that packaging materials can be contaminated by spoilage and pathogenic microorganisms (Suominen et al., 1997). The cell loads normally detected for mesophylic aerobic bacteria ranged between 103 and 106 cfu/cm2 for packages of recycled materials and between 102 and 105 cfu/cm2 for products based on virgin fibers (Suominen et al., 1997).

- The few literature data show that spore-forming bacteria (belonging to the genera Bacillus, Geobacillus, Alicyclobacillus, and Clostridium) and molds (belonging mainly to the species Aspergillus niger, A. cinnamomeus, and Cladosporium herbarum) prevail on packaging microbiota. They are widespread microorganisms, resistant to adverse environmental conditions and endowed with high spoilage potential (Binderup et al., 2002; Turtoi and Nicolau, 2007). However, also yeast and other spoilage bacteria can be present on packaging materials. To avoid and/or minimize this issue, the use of appropriate packaging is essential, since it acts as a barrier that can protect fresh food from contamination (Campos et al., 2014).

- However, although the Regulation (EC) No 852/2004 on materials and articles intended to come into contact with food stipulates that “the packaging must not be a source of food contamination,” understanding the real contribution of the packaging material in product contamination is not very simple due to the impossibility to establish “a priori” the level of the naturally occurring fruit and packaging microflora. In addition, the microbial survival, growth or death on the packaging materials, and consequently their role in cross contamination of packed fruits, are affected by environmental conditions, including storage temperature, relative humidity and nutrient availability (Siroli et al., 2014; De Candia et al., 2015; Erickson et al., 2015). Also the growth potential of the microorganisms on fruit surface is affected by the intrinsic features of fruit species and variety (i.e., specific surface features, acidity, sugar content and so on), by the ripening and by the presence of wounds and exudates (Heaton and Jones, 2008).

- The attractiveness of fresh produces for consumers is determined also by organoleptic factors like appearance, firmness, taste and perceived health benefits as well as by safety and shelf-life of the product (Malmendal et al., 2011; Cuthbertson et al., 2012; Santucci et al., 2015).

- The fruit considered in this research (peach), being a living organism with high metabolic activity, is subjected to a rapid quality decreases after harvest due mainly to ethylene production. This causes several negative effects including senescence, accelerated quality loss, reduced nutrient composition, increased fruit pathogen susceptibility,

physiological disorders in fruit and vegetables, and consequently the growth potential of microorganisms present on fruit surfaces (Martínez-Romero et al., 2007; Liu et al., 2015).

- Microbiological Testing of Fresh Produce – United Fresh White Paper

- Average APC #'s – Bacteriological quality of some readhy to eat vegetables as retailed and consumed in Sabon-gari, Zaria, Nigeria (Abdullahi, I.O and Abdulkareem, S

- Shelf Life of Fresh-Cut Fruits and Vegetables
  - The shelf life (i.e., the length of time that corresponds to a tolerable loss in quality of a processed food and other perishable items) of fresh-cut fruits and vegetables ranges from 1 to 35 days depending on types of shelf life (such as marketing shelf life, food safety shelf life, sensory shelf life, or microbiological shelf life) (M. Barth et al, 2013)
  - The average shelf lives of fresh-cut fruits and vegetables are typically 10-14 days (Cantwell & Suslow, 2002)

- The microorganisms that exist on the surfaces of raw, whole produce appear to be the major source of microbial contamination and consequent spoilage of fresh-cut fruits and vegetables (Sapers, Miller, Jantschke, and Mattrazzo (2001) reported that, compared with good surface sanitation practices, no decontamination treatment or an ineffective antimicrobial treatment on whole cantaloupe resulted in premature microbiological spoilage of fresh cut cantaloupe.
  - Robbs et al. (1996) – determined that the most common bacteria on raw celery plants, including fluorescent *Pseudomonasi* spp. And *Aeromonas* spp., were also the most common bacteria on cut celery products.
  - Boyette, Ritchie, Carballo, Blankenship, and Sanders (1993) reported that the microbial decay of fresh-cut lettuce is largelyl due to the growth of microorganisms originating from preharvest environments.
  - Delaquis, Stewart, Toivonen, and Moyles (1999) determined that the types of microorganisms found on shredded lettuce were highly associated with the microorganisms detected on lettuce before shredding.
  - Several studies (Magnusson, King, & Torok, 1990; Geeson, Churey, & Splittstoesser, 1990; Torok & King, 1991) have revealed that yeast species identified on fresh-cut produce can also be isolated from raw materials prior to processing
  - Garg, Churey, and Splittstoesser (1990) concluded that peel is the major source of microbial contaminants on carrots sticks
  - Several outbreaks of salmonellosis that were associated with cut cantaloupe and watermelon have resulted from *Salmonella* present on the rind contaminated in the field or packinghouse (Harris et al., 2003)
  - Inoculation of *Listeria monocytogenes* and *Salmonella* on the surface of entire cantaloupes resulted in the contamination to fresh-cut pieces during cutting (Ukuku & Sapers, 2001, Ukuku & Fett, 2002)

- These results indicate that the bacteria on the surface of whole produce are the same as those on fresh-cut produce and can contaminate finished product through processing

- Raw materials can contribute to contamination of produce products during cultivation, harvesting, packaging, and shipping, and there are no definite decontamination steps during processing. It is no surprise that a variety of microbial populations are present.
  - Goepfert (1980) reported that mesophilic aerobic bacterial populations on vegetables sampled at processing plants ranged from 4.6 (carrots) to 7.5 (peas) $\log_{10}$ CFU/g fresh weight.
  - The mesophilic aerobic bacterial counts ranged from 4 to 6 $\log_{10}$ CFU/g fresh weight on finished cut vegetables and from 2 to 5 $\log_{10}$ CFU/g fresh weight on finished cut fruits, depending on the commodities, seasons of the year, and growing regions (Zhuang et al., 2003)
  - The mesophilic aerobic bacterial counts on bagged salads from the retail market ranged from 4.0 to 9.0 $\log_{10}$ CFU/g (Heard, 2000)

- Many types of microorganisms can be found on a cut fruit or vegetable, including Gram-negative bacteria, Gram-positive bacteria, fungi (yeasts and molds). The type of fresh-cut commodity and the pH of fresh-cut products are the two primary intrinsic factors that determine the microbiological spoilage profile of fresh-cut products.
  - Regulation (EC) No 852/2004 on materials and articles intended to come into contact with food stipulates that "the packaging must not be a source of food contamination,"
  - In fact France and Germany microbiological specifications for mesophilic aerobic bacterial populations or aerobic plate counts (APC) of salad vegetables at production (fresh) are $5x10^6$ CFU/g, for separating good quality from marginally acceptable quality. And at use by date are $5x10^7$ CFU/g (Francis, Thomas, & O'Beirne, 1999; Lund, 1993)

- Debevere (1996) proposed 10^8 CFU/g of aerobic psychrotrophic bacteria, 10^5 CFU/g of yeast, and 10^7 CFU/g for lactic acid bacteria as the limiting criteria for ready-to-eat vegetables.
- The Spanish legal limit (RD 3484/2000, 2001) for microbial populations on minimally fresh-processed fruit for safe consumption are 7, 5, and 3 $\log_{10}$ CFU/g for aerobic bacteria, yeasts, and molds, respectively.

- Regardless of raw material quality, GMPs, processing conditions, antimicrobial treatments, types of antibacterial packaging, temperature abuse shortens the shelf life of fresh-cut produce.(M. Barth et. Al 2013)
  - Temperature is one of the most impactful factors affecting the quality and microbiological characteristics of produce (M. Barth et. Al 2013

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