UNIVERSITY of GUELPH
ONTARIO AGRICULTURAL COLLEGE
Department of Food Science

Microbiological Standards for Reusable Plastic Containers within Produce Grower Facilities within Ontario and Quebec

Aim

To assess the microbiological standard of reusable plastic containers (RPC's) used in different fresh produce packing stations.

Summary

The sanitary status of reusable plastic containers (RPC) has been evaluated using visual inspection, ATP readings, prevalence of food safety indicators (Enterobacteriaceae, coliforms, E coli and Listeria spp) and spoilage indicators (Total Aerobic Count, Yeast and Moulds). Different batches of RPC's were sampled over a 7 week period at different geographical locations (Hamilton, Leamington and Quebec). On each sampling visit 10 unused RPC units were randomly selected for sampling and had been newly delivered to the packing plant. From the results it was found that a proportion of trays (ca. 10%) had visible dust or organic residues. Approximately, 30% had labels from previous users some of which had signage "Product of Mexico". The criteria used to define a sanitary RPC was applied as in previous studies (ATP <3 log RLU, TAC <4 log cfu/tray, enterobacteriaceae <3 log cfu/tray, coliforms <3 log cfu/tray, Yeast & moulds <3log cfu/tray and absence of *E coli* and *Listeria*). It was found that 43% of RPC's failed due to high ATP readings, 73% exceeded the TAC criteris with 51% and 35% failed in terms of enterobacteriacece and coliform levels respectively. Of more concern was the recovery of *E. coli* on 13% of the RPC's tested although none were positive for *Listeria* spp. In terms of Yeast & Moulds, 15% exceeded the 3 log cfu/tray limit which can be considered to be an acceptable proportion of defects. In comparison to the 2013 study, TAC, enterobacteriaceae, coliforms and E coli prevalence had increased in 2014. In conclusion, the results from the study have confirmed the unsanitary status of RPC with no improvements being observed compared to the 2013 study.

Methods

Five fresh produce packing operations located in Hamilton, Leamington and Montreal were visited in the course of the study between July and September 2014.
On each visit, location 10 randomly selected RPC's were sampled from different lots of trays that had been delivered on pallets wrapped in plastic film. A visual inspection was performed to note visible soil or organic debris and labels attached from previous users. After visual inspection samples were collected to determine ATP and microbial counts. ATP swabs were taken from approximately 10cm^2 areas of the container base and one side. Sponge samples were taken from the entire inside surface of the tray as outlined in SOP (Annex A). The sponge samples were returned to the laboratory and submerged in 30 ml saline then stomached for 30s. A dilution series was prepared in saline then plated on Total Aerobic Count, E. coli/Coliform and

Enterobacteriaceae petri films and Yeast & Mould Petri Films. The TAC was incubated at 34°C for 48h with the other petri films being incubated at 37°C for 24h. Yeast & mould petri films were incubated at 25°C for 5 days. The colonies were enumerated and converted into log values. The remaining homogenate from the sponge was added to 225 ml One Step Listeria enrichment broth and screened for *Listeria* spp using the 3M Microbial Detection system.

There is no specific criteria set for the microbiological standards for RPC's and as a consequence those based on food contact surfaces were used to designate pass or fails. Specifically, for ATP testing a fail was designated at >3 log RLU, for TAC the limit was 4 log cfu/tray, Enterobacteriaceae or coliforms >3 log cfu and presence of *E coli* or Listeria. The limit for Yeast & mould counts limit was set at 3 log cfu.

Results

Table 1: ATP (RLU) readings taken from unused Reusable Plastic Trays sampled at different produce packing stations located in Hamilton, Leamington or Quebec.

		ATP (log RLU/10cm^2)				
Farm #	**Total RPC Tested**	**Min**	**Max**	**Median**	**Pass**	**Fail (%)**
Farm 1	40	1.15	3.71	2.34	31	9 (22.5)
Farm 2	90	0.95	5.09	2.75	41	49 (54.4)
Farm 3	30	0.85	4.24	1.95	20	10 (33.3)

RPC returning an RLU reading > 3 log. Ten RPC were sampled on each visit and taken from a different lot of trays on each occasion.

Table 2: Microbial counts recovered from unused Reusable Plastic Trays sampled at different produce packing stations located in Hamilton (A), Quebec (B) and Leamington (C).

A		**Log cfu/Tray**				
Farm 1	**Total RPC Tested**	**Min**	**Max**	**Median**	**Pass**	**Fail (%)**
TAC	40	0.78	4.92	2.69	30	10 (25)
Enterobacteriaceae	40	0	2.580	0.65	40	0 (0)
Coliforms	40	0	1.71	0	40	0 (0)
E. coli	40	0	0	0	40	0 (0)
Listeria	40	0	0	0	40	0 (0)
Yeast and Moulds	40	0.30	2.72	1.11	40	0 (0)

UNIVERSITY of GUELPH
ONTARIO AGRICULTURAL COLLEGE
Department of Food Science

B		Log cfu/Tray				
Farm 2	**Total RPC Tested**	**Min**	**Max**	**Median**	**Pass**	**Fail (%)**
TAC	89	2.28	8.51	6.54	15	74 (83)
Enterobacteriaceae	89	0	5.17	3.23	38	51 (57)
Coliforms	89	0	5.11	2.50	34	55 (62)
E. coli	89	0	5.08	0	68	21 (24)
Listeria	89	0	0	0	89	0
Yeast and Moulds	89	0	4.82	2.23	67	22 (25)

C		Log cfu/Tray				
Farm 3	**Total RPC Tested**	**Min**	**Max**	**Median**	**Pass**	**Fail (%)**
TAC	30	4.94	7.18	5.26	0	30 (100%)
Enterobacteriaceae	30	0	1.20	0.74	0	30 (100%)
Coliforms	30	0	0	0	30	0 (0)
E. coli	30	0	0	0	30	0 (0)
Listeria	30	0	0	0	30	0 (0)
Yeast and Moulds	30	0	3.49	0.99	28	2 (7)

Table 3: Pooled data for ATP readings and microbial counts recovered from RPC sampled at different produce packers located within Ontario and Quebec.

		Log cfu/Tray				
Farm #	**Total RPC Tested**	**Min**	**Max**	**Median**	**Pass**	**Fail (%)**
ATP	160	0.85	5.09	2.50	92	68 (42.5)
TAC	159	0.84	8.51	5.26	45	114 (72)
Enterobacteriaceae	159	0	5.17	2.28	78	61 (51)
Coliforms	159	0	5.10	1.28	104	55 (34.6)
E. coli	159	0	5.08	0	138	21 (13.2)
Listeria	159	0	0	0	159	0 (0)
Yeast and Moulds	159	0	4.82	1.52	135	24 (15)

UNIVERSITY of GUELPH
ONTARIO AGRICULTURAL COLLEGE
Department of Food Science

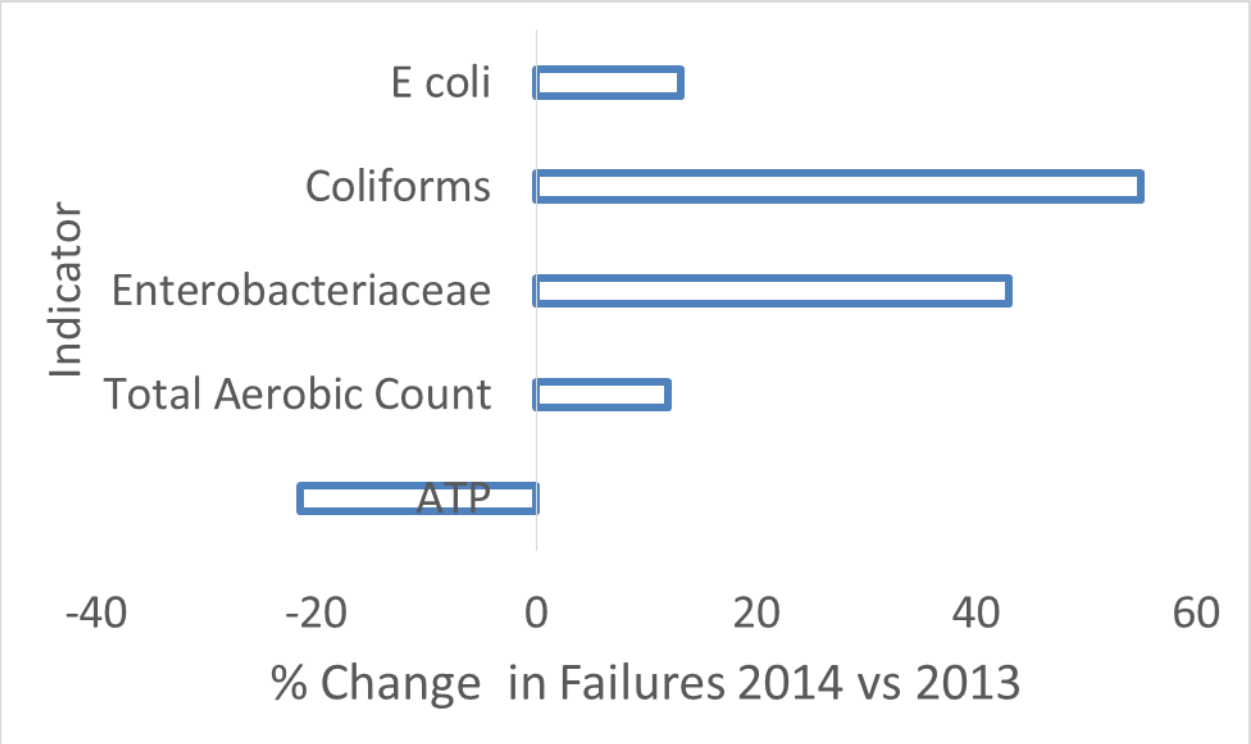

Figure 1: Comparison of % failures of RPC sampled in 2014 vs those screened using the same protocol in 2013.

UNIVERSITY of GUELPH
ONTARIO AGRICULTURAL COLLEGE
Department of Food Science

Discussion

The sampling trails evaluated the visual appearance of RPC then further assessed the sanitary status using ATP swabs in combination with microbial counts. In terms of visual appearance it was evident that approximately 10% of trays had dried on plant material or dust. Labels from previous users were found on an estimated 30% of RPC and were visibly seen on the outside of pallets. On occasion, labels stating "Product of Mexico" were observed underlying the extended distribution lines of RPC.

The microbial counts were selected based on food safety indicators (enterobacteriaceae, coliforms, *E. coli* and *Listeria* spp) and spoilage indicators (Total aerobic count and Yeast & Mould). ATP readings provided a general status of recent contamination derived from microbial and non-microbial sources. From the data it was found that RPC's sampled in Quebec recorded the highest indicator counts along with ATP readings (Table 1). With respect to the latter, over 50% of the RPC's tested had RLU readings over the 3 log limit suggesting inadequate sanitation or post-cleaning contamination (Table 1). Of more concern was the recovery of fecal indicators that included E coli that strongly suggested the trays had been contacted by either contaminated produce and/or water (Table 2). Given the RPC's were freshly delivered it can be hypothesized that contamination was introduced prior to being delivered to the packing house. The TAC and yeast & mould counts were also high again supporting the conclusion that the RPC had either been insufficiently sanitized or subject to post-sanitation contamination (Table 2). No Listeria spp were recovered from any of the tray samples suggesting that *L. monocytogenes* was no present on the RPC's.

The RPC's sampled in Leamington did not harbor *E coli* or other coliforms although high levels of TAC, enterobacteriaceae and ATP counts were recorded (Table 2). The results would suggest that although no indicators were present to suggest potential human pathogens were present, high ATP readings along with TAC and yeast & moulds would suggest inadequate sanitation of the trays or post-cleaning contamination.

UNIVERSITY of GUELPH
ONTARIO AGRICULTURAL COLLEGE
Department of Food Science

The RPC's sampled from Hamilton were found to be the most sanitary with no fecal indicators recovered. Yet, over 20% of the RPC's recorded unacceptable TAC and ATP readings suggesting inadequate sanitation or post-treatment contamination.

From the individual farms it was clear that an unacceptable sanitary standards of RPC's were recorded between different geographical locations over time (i.e. between sampling visits). The results would support the view that the general sanitary quality of RPC's exceeds what would be expected from a sanitary food contact surface. This was confirmed by the pooled data that highlighted a high percentage of fails in terms of ATP and indicator counts (Table 3).

When the indicator counts and ATP readings of RPC were compared between the current and 2013 study it was clear that sanitary standards had not improved (Figure 1). Specifically, the fecal indicator were more prevalent in the current sampling trials compared to the study performed in 2013. Yet, the proportion of RPC's exceeding the 3 log RLU were lower in 2014 vs 2013 although it should be noted that ATP is an indirect approach to assessing the sanitary status of surfaces with more reflective data being obtained using microbial counts.

Conclusions

The results of the study have confirmed that a high proportion of RPC are of poor sanitary status due to inadequate sanitation or post-cleaning contamination. Of concern is the high prevalence of food safety indicators especially E coli which highlights the potential for the presence of enteric pathogens that could encompass viruses, protozoan and bacterial.

Date: October 2014

Analysis performed by: Dr Keith Warriner and Fan Wu

Dr Keith Warriner
Director of the Food Safety and Quality Program
Department of Food Science