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Research Paper

A Scalable Aggregated and Nondestructive Sampling Approach for Food Safety Monitoring of Fresh-Cut Produce During Commercial Processing

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ABSTRACT

Representative sampling is critical for detecting low-prevalence microbial contamination in fresh produce, particularly fresh-cut produce. Conventional grab sampling may miss localized contamination due to heterogeneous microbial distribution. This study evaluated a cloth-based aggregated, nondestructive sampling approach against traditional grab sampling across five fresh-cut produce matrices (onions, lettuce, carrots, tomatoes, and peppers) under commercial processing conditions. Paired samples were collected concurrently, with aggregated cloth and grab sampling conducted side-by-side to reflect current industry practices. Cloth samples were obtained by fitting the cloth onto the distribution cone of a multipocket scale for 2 h, whereas grab samples (150 g) were collected using a gloved hand at approximately 1-min intervals from the product stream during the same 2-h period. Samples were analyzed using a panel of index targets representing indicator microorganisms and microbial groups, and detection frequencies were compared using the exact McNemar test. Cloth sampling yielded significantly higher detection rates for *tet*A (37.5%) and *tet*B (98.4%) in diced carrots ($p < 0.05$), indicating enhanced recovery of low-prevalence targets. For all other evaluated targets and matrices, aggregated cloth sampling demonstrated detection performance equivalent to grab sampling ($p > 0.05$). PCR targeting *Enterobacteriaceae* (EB-set 1) showed near-universal detection across both methods, whereas low-prevalence targets such as *pho*E and *fli*C in certain matrices provided limited discriminatory power, highlighting the importance of target selection. By increasing sampled surface area, aggregated cloth sampling generated representative samples while reducing labor and standardizing sampling procedures. Overall, aggregated cloth sampling was shown to be a scalable alternative to conventional grab sampling under commercial processing conditions.

Fresh produce has been increasingly associated with foodborne illness outbreaks, emphasizing the need for effective monitoring strategies within produce safety programs (Alegbeleye et al., 2018; Guan et al., 2021). Foodborne pathogens such as *Salmonella enterica*, *Escherichia coli* (*E. coli*) O157:H7, and *Listeria monocytogenes* are of particular concern because they may be present at very low prevalence and are often attached to produce surfaces (Guan et al., 2023). As a result, the ability of microbiological testing programs to detect contamination is strongly influenced by the sampling strategy used (den Besten et al., 2025). Representative sampling is therefore essential for maximizing the sensitivity of downstream molecular or culture-based detection methods.

Conventional grab sampling remains the most commonly used approach in produce safety programs. In this method, discrete portions of produce are collected from selected locations and time points within a production lot, with each portion representing the product at the specific place and time of collection. These grab samples may be analyzed individually or combined into small composite samples (e.g. 150 g) to approximate broader representation of the lot (FDA BAM, 2022). However, because each sample represents only a limited frac-

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tion of the production lot, conventional grab sampling may fail to capture contaminated product when pathogens are sporadically distributed, spatially localized, or present at low prevalence (den Besten et al., 2025). Previous studies have shown that increasing the total sampled surface area or aggregating material from multiple product units can improve the likelihood of detecting low-prevalence contamination in food and environmental samples (Harris et al., 2003; Strawn et al., 2013a; Strawn et al., 2013b; Arthur and Wheeler, 2021).

For fresh-cut produce, finished product sampling is typically done by manually compositing picks, which requires a person to maintain sanitary gloves next to weigh scale for extended periods of time in an attempt to collect a representative sample from the thousands of pounds of product that are passing over the distribution cone during a one- or two-hour production interval. The labor-intensive and tedious nature of this process may lead to human error, resulting in cross-contamination or lapses in scheduled sample collection.

Aggregated cloth-based sampling is a composite-sampling approach in which a sterile cloth is brought into contact with a moving stream of finished products for a defined period. As multiple product units contact the cloth, microorganisms and product-associated material are transferred to its surface, thereby integrating microbial material across numerous units and time points and representing a larger portion of the production lot than a discrete grab sample (Portillo et al., 2025). Cloth sampling can efficiently recover microorganisms from surfaces and is widely used in quality control (QC) programs in the beef industry (Arthur et al., 2023) and environmental monitoring programs for food processing facilities (Jones et al., 2020; Keeratipibul et al., 2017). When applied directly to food products, aggregated cloth-based sampling may improve the sample representativeness while simplifying downstream analytical processing (Richards et al., 2026). Because a single cloth can contact a large cumulative product surface area, this approach may provide an equivalent or improved characterization of the microbiological status of a production lot while reducing sampling labor and requiring the same or fewer laboratory analyses.

The effectiveness of aggregated sampling depends on targeting product locations where contamination is most likely to occur and is particularly advantageous when pathogens are primarily surface-associated (Frank, 2001). In many applications, water or buffer solutions are used to facilitate the transfer and recovery of microorganisms from product surfaces (de Oliveira Mota et al., 2021). However, direct validation of cloth-based sampling protocols can be difficult because pathogens in fresh produce typically occur at extremely low concentrations. Prevalence studies using low-frequency genetic markers or surrogate organisms provide a practical alternative for evaluating sampling performance (de Oliveira Mota et al., 2021). Surrogate-based approaches enable characterization of the dynamic equilibrium established during aggregated sampling and can provide insight into the representativeness of composite samples (Wheeler & Arthur, 2018).

In light of these potential advantages, it was hypothesized that aggregated cloth-based sampling would improve food safety systems in the commercial produce industry. This project was designed to determine whether cloth-based composite sampling would provide equivalent or improved detection capability compared with conventional sampling methods. In this study, we evaluated the performance of aggregated cloth-based sampling for fresh-cut produce (diced and slivered) processed on commercial production lines. Paired samples were collected using an aggregated cloth sampler and conventional grab sampling conducted side-by-side, reflecting current industry practice. Six genetic markers were selected as microbial index targets to evaluate the agreement between aggregated cloth sampling and conventional grab sampling across fresh-cut produce matrices. These targets were chosen because they represent naturally occurring microbial populations and food safety-relevant traits, allowing comparison of the two sampling approaches without relying on direct detection of low-prevalence foodborne pathogens. Therefore, the objectives of this study were to (i) assess the performance and representativeness of aggregated cloth-based sampling under commercial processing conditions and (ii) evaluate the equivalence of cloth-based aggregated sampling and conventional grab sampling for detecting low-prevalence genetic markers.

Material and methods

Sample collection by aggregated cloth sampler and tissue grabs. The aggregated cloth sampler (MicroCap™ Sampler, FREMONTA Corporation, San Jose, CA) is a sterile square with a 6- or 8-inch hole in the center designed to fit over the distribution cones of various pocket-scale machines (Fig. 1). The cloth configuration used here was developed in collaboration with produce industry partners to fit the multipocket scale of a commercial fresh-cut processing line and to address practical industry needs for continuous, nondestructive sampling. Because cloth placement, product-flow dynamics, contact time, and naturally occurring low-prevalence contamination are difficult to reproduce accurately at laboratory scale, field evaluation was necessary to assess performance in the intended use setting. The cloth is composed of the same nonwoven food-grade polyolefin fabric as the MicroTally® Swab (USDA FSIS, 2024). The cloth material and processing workflow were informed by previous validation studies of cloth-based sampling in beef-industry applications (Wheeler & Arthur, 2018). Once installed, the cloth sampler collects an aggregated surface sample of the product passing over the scale. Each cloth sampler was retrieved after 2 h for analysis. During the same 2-h time period, a QC staff member stationed at the scale collected onion picks by hand (using gloves) at 1-min intervals to accumulate samples until reaching approximately a 150 g grab sample, which served as a comparison to the aggregated cloth sample. Paired samples were collected concurrently from the same distribution cone and multipocket scale area during the same production period, using both the cloth sampler and grab-sampling method; thus, each pair represented the same underlying sampling unit.

Similarly, paired samples were collected for four additional commodities (cut lettuce, diced carrot, diced tomato, and diced bell pepper) using the same approach. For lettuce, carrot, and bell pepper, the only modification was that the cloth sampler was premoistened with 25 ml of sterile deionized water to facilitate bacterial recovery from the sample surfaces.

Sample processing and DNA extraction. In Figure 2, the onion-sullied cloth sampler was folded and placed into a 32-oz filter bag (MT Filter Bag, FREMONTA Corporation, Fremont, CA), whereas 150 g of cut onion was collected in a separate 32-oz sample bag. For enrichment, 200 ml and 450 ml of ISO-BPW (buffered peptone water) (Criterion, BPW dehydrated, Hardy Diagnostics, Santa Maria, CA) were added to the cloth sampler and grab samples, respectively. The enrichment volumes differed because the two sampling approaches produced different physical sample formats. The 200-ml volume was sufficient to fully wet and process the cloth (Wheeler & Arthur, 2018), whereas 450 ml was required to adequately cover and mix the grab sample per industry practice. Thus, the volumes were selected based on the processing needs of each sample type. Since this study compared the complete sampling-and-processing workflows under commercial conditions, no adjustment was made for enrichment volume. Both samples were homogenized by stomaching at 8.0/S for 60 s (BioBase Bioyu Co., Ltd., China) and then incubated at 35 °C for 20 ± 2 h. Following incubation, 100 ul of the enriched cloth sample or grab sample was transferred to a 1.5-ml microcentrifuge tube. The lysis reagent was maintained in suspension using a magnetic stir bar, and 100 ul of lysis reagent (iQ-check lysis reagent, Bio-Rad Laboratories, Inc., Hercules, CA) was added to each sample while stirring at medium speed. The mixture was homogenized by pipetting up and down. Samples were then incubated in a thermomixer (Hettich AG, Swiss) at 95–100 °C at 1,300 rpm for 15 min. After lysis, the tubes

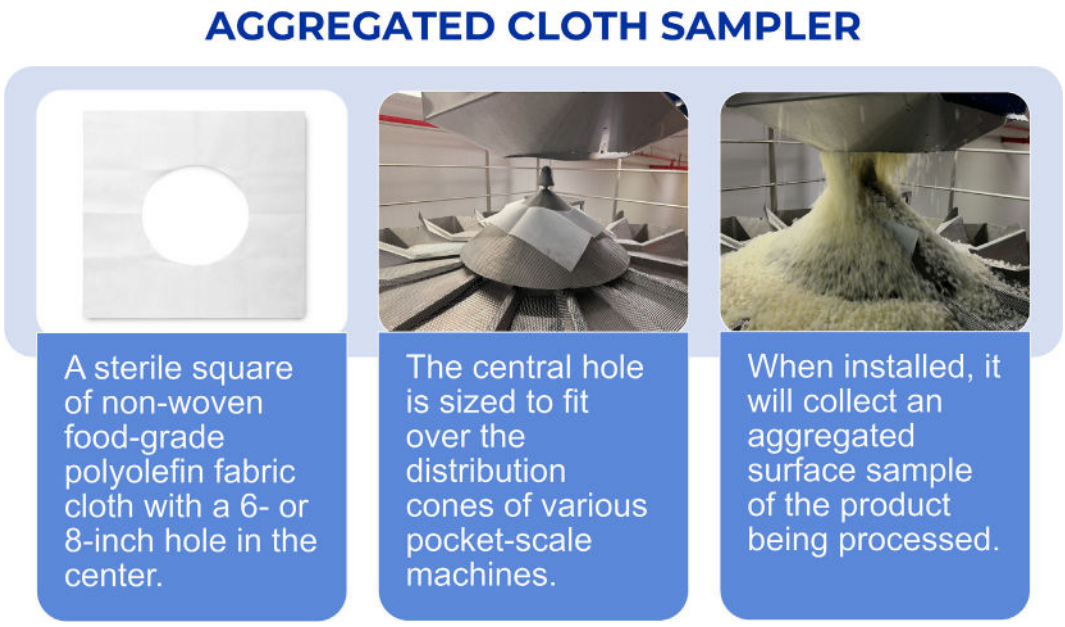

Figure 1. Operational workflow of aggregated cloth sampler for sampling cut onions.

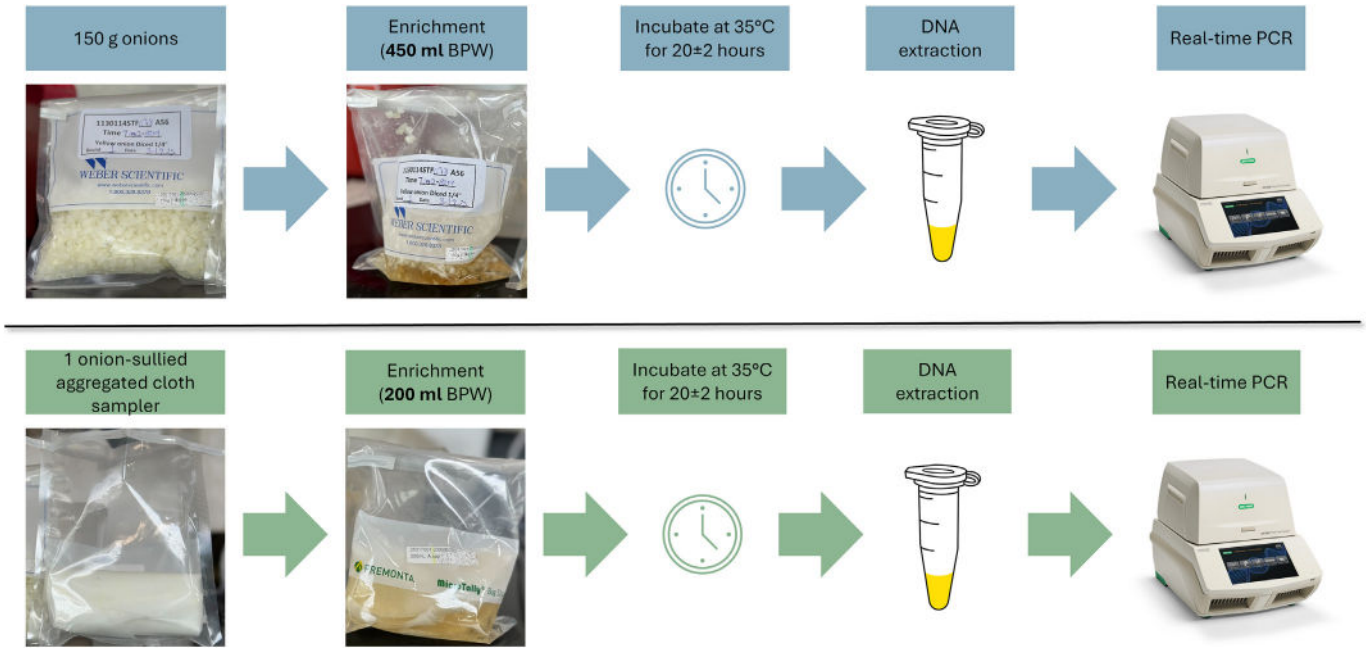

Figure 2. Processing scheme of aggregated cloth samples and tissue grab samples.

were centrifuged at 10,000 × g for 2 min. As a result, the supernatant containing the extracted DNA was used for Real-Time (RT) PCR analysis.

PCR analysis of index targets. The selected index targets were chosen as genetic markers to represent a broad range of microbial groups relevant to fresh produce and to serve as indicators for evaluating the performance of the aggregated cloth sampling relative to conventional grab sampling (Table 1).

The primers and probe of EB-set 1 were designed in this study to target members of the *Enterobacteriaceae* family. The *tet*A and *tet*B genes, which represent tetracycline resistance determinants commonly found in *E. coli* and *Salmonella* (Guarddon et al., 2011), were included as indicators of the enteric Gram-negative bacterial population. The *Lacto* target was selected for the detection of *Lactobacillus* spp. (Delroisse et al., 2008), representing lactic acid bacteria commonly associated with produce environment. In addition, the primers and probe of *ybb*W designed in this study were used to detect generic *E. coli,* which serves as an indicator organism for fecal contamination in irrigation water (Benjamin et al., 2013). The *fli*C gene was reported as the indicator of H7 flagella antigen, which is highly correlated with *E. coli* O157:H7 (Perelle et al., 2004) and found in generic *E. coli* as well. The *pho*E gene was selected as the marker for *Klebsiella pneumoniae* (Shin et al., 2021), a biofilm-forming bacterium associated with poor hygiene practices in food handling (Riwu et al., 2022). The *Leuco-mesen* target was reported to detect *Leuconostoc mesenteroides* (Elizaquível et al., 2008), which is widely distributed in plant-

Table 1
Sequences of the primers and probes were used as index targets in this study

Index targets	Description	Primer/Probe name	Primer/Probe sequence (5′ to 3′)	Reference
EB-set 1	Indicator of *Enterobacteriaceae*	Forward	GGAGACTGCCAGTGATAAAC	This study
		Reverse	GAGGTCGCTTCTCTTTGTATG	
		Probe	ACGTCAAGTCATCATGGCCCTTACG	
*tet*A	Tetracycline resistance gene. Indicator of gram-negative bacteria	Forward	CCGCGCTTTGGGTCATT	Guarddon et al., 2011
		Reverse	TGGTCGCGTCCCAGTGA	
		Probe	TCGGCGAGGATCG	
*tet*B	Tetracycline resistance gene. Indicator of gram-negative bacteria	Forward	AGGCGCATCGCTGGATT	Guarddon et al., 2011
		Reverse	CAGCATCCAAAGCGCACTT	
		Probe	CTTATTGCTGGCTTTTT	
Lacto	Indicator of *Lactobacillus* spp.	Forward	GAGGCAGCAGTAGGGAATCTTC	Delroisse et al., 2008
		Reverse	GGCCAGTTACTACCTCTATCCTTCTTC	
		Probe	ATGGAGCAACGCCGC	
*ybb*W	Indicator of generic *E. coli*	Forward	TCACTGCCATTCTTAACCCG	This study
		Reverse	CCACCGTTTTCTGCTTTCTG	
		Probe	TGGCGATTTGGGCGATTTCACTG	
*fli*C	Indicator of H7 flagella antigen	Forward	CCACGACAGGTCTTTATGATCTGA	Perelle et al., 2004
		Reverse	CAACTGTGACTTTATCGCCATTCC	
		Probe	CCGAAAATACCTTGTTAACTACCGATGCTGC	
*pho*E	Indicator of *Klebsiella pneumoniae*	Forward	TGCAGTACCAGGGTAAAAACGA	Shin et al., 2021
		Reverse	CGCCGTCGCCGTTCT	
		Probe	CCGTGAAGCGAAGAA	
Leuco-mesen	Indicator of *Leuconostoc mesenteroides*	Forward	CCAGTTGTAATGCGTTATTACC	Elizaquível et al., 2008
		Reverse	CACAGCTTGTCCTTATAGAAAA	
		Probe	TTCACTCTTTTCAAGACTTACTG	

associated environments and is commonly found on fruits and vegetables, as well as in fermented plant products (NCBI, 2017; Schifano et al., 2021). Collectively, these index targets encompass both indicator organisms and broader microbial groups relevant to produce microbiota, enabling a comprehensive evaluation of sampling performance across diverse bacterial populations.

For RT-PCR experiments, 5 ul of DNA templates were used in one PCR reaction. A 2X universal probe master mix (Ssoadvanced™ Universal Probes Supermix, Bio-Rad Laboratories, Inc., Hercules, CA) was used for PCR amplification. The primers and probe were synthesized and premixed by Integrated DNA Technologies (IDT) as a 10X stock to be used for each reaction. Additional molecular water was added to make the PCR reaction total volume of 20 ul. The RT-PCR protocol started with 3 min at 95 ˚C and then 40 cycles of 15 s at 95 ˚C and 60 s at 59 ˚C. The extension step was optimized for each target, ranging from 55.7 to 63 ˚C. All PCR reactions were run on a CFX96 Touch Deep Well™ Real-Time PCR Detection System (Bio-Rad Laboratories, Inc., Hercules, CA).

Statistical analysis. Data were recorded as binary outcomes (presence/absence) (Tables S1–5), corresponding to PCR detection (detected vs. nondetected targets). The percentage of positive detections for aggregated cloth samples was reported as %Cloth, representing the proportion of positive samples among all cloth-based samples. Similarly, %Tissue represents the proportion of positive detections obtained from conventional grab samples. Paired samples were collected concurrently from the same location and time period to enable direct comparison between sampling methods. Sample sizes varied across food matrices depending on the manufacturer's processing conditions. Differences between paired proportions were evaluated using the exact McNemar test (binomial test on discordant pairs). All tests were two-sided with a significance level set at $\alpha = 0.05$. All analyses were conducted using standard statistical computing tools (R version 4.5.3).

Results

Cut onions. The first application of the aggregated cloth sampler was in a commercial processing line for cut onions. A total of 233 paired samples were analyzed to compare detection performance between cloth and tissue sampling across six index targets (EB-set 1, *Lacto*, *tet*B, *ybb*W, *Leuco-mesen*, and *pho*E). Detection frequencies (% Cloth and %Tissue) were compared, and statistical significance was evaluated using the McNemar test based on discordant pairs (Table 2).

Across all markers, detection rates were not different ($p > 0.05$) between the two sampling approaches. For *ybb*W, cloth sampling identified more positive samples than grab sampling (81 vs 71), while *tet*B and *pho*E showed similar detection frequencies with minor variation. EB-set 1 exhibited near-universal detection by both methods, with almost all samples testing positive. High detection prevalence was also observed for *Lacto* and *Leuco-mesen*, with only slight differences between methods.

Analysis of discordant pairs (samples positive by one method but negative by the other) showed balanced distributions between sampling methods for all markers. For *ybb*W, 48 samples were uniquely detected by cloth while 38 were uniquely detected by grab sampling ($p = 0.33$). Similar patterns were observed for *pho*E (20 cloth vs. 24 tissue discordant pairs; $p = 0.65$) and *tet*B (10 cloth vs. 14 tissue discordant pairs; $p = 0.54$). For EB-set 1, nearly all samples were positive by both methods (230 concordant positives), with only minimal discordance (2 cloth vs. 1 tissue), reflecting strong agreement between methods ($p = 1.00$). *Lacto* and *Leuco-mesen* also showed comparable distributions of discordant pairs, with no significant differences ($p = 0.28$ and $p = 0.76$, respectively).

Overall, no statistically significant differences were observed between cloth and tissue sampling for any index targets ($p > 0.05$), supporting equivalent detection performance under the conditions tested.

Cut lettuce, diced carrots, diced tomatoes, and diced peppers. To further evaluate the performance of aggregated cloth sampling across additional fresh-cut produce matrices, paired sampling was conducted on cut lettuce ($N = 41$), diced carrots ($N = 64$), diced tomatoes ($N = 72$), and diced peppers ($N = 63$). Detection frequencies (%Cloth and %Tissue) were compared across six index targets (EB-set 1, *tet*A, *tet*B, *ybb*W, *fli*C, and *pho*E), and statistical differences were assessed using the exact McNemar test (Table 3).

Overall, detection rates were comparable between cloth and grab sampling across all matrices and targets, with most paired comparisons showing no statistically significant differences ($p > 0.05$). EB-set 1

Table 2
Detection frequencies (% positive) of selected index targets in cut onions ($N = 233$) using aggregated cloth sampling (%Cloth) and grab sampling (%Tissue), with p values from the exact McNemar test

Food matrix	Presence/N	Index targets					
		EB-set 1	*Lacto*	*tet*B	*ybb*W	*Leuco-mesen*	*pho*E
Cut onions ($N = 233$)	%Cloth	99.6	87.6	10.3	34.8	83.7	25.3
	%Tissue	99.1	84.1	12.0	30.5	82.4	27.0
	p value	1.00	0.28	0.54	0.33	0.76	0.65

Table 3
Detection frequencies (% positive) of selected index targets in different food matrices using aggregated cloth sampling (%Cloth) and grab sampling (%Tissue), with p values from the exact McNemar test

Food matrix	Presence/N	Index targets					
		EB-set 1	*tet*A	*tet*B	*ybb*W	*fli*C	*pho*E
Cut lettuce ($N = 41$)	%Cloth	100.0	17.1	26.8	26.8	9.8	0.0
	%Tissue	100.0	4.9	31.7	29.3	4.9	0.0
	p value	NA	0.18	0.79	1.00	0.69	NA
Diced carrots ($N = 64$)	%Cloth	96.9	37.5*	98.4*	18.8	1.6	0.0
	%Tissue	95.3	20.3	87.5	10.9	0.0	0.0
	p value	1.00	0.01	0.04	0.23	1.00	NA
Diced tomatoes ($N = 72$)	%Cloth	100.0	88.9	90.3	40.3	2.8	6.9
	%Tissue	100.0	80.6	91.7	43.1	11.1	4.2
	p value	NA	0.26	1.00	0.86	0.11	0.63
Diced peppers ($N = 63$)	%Cloth	100.0	31.7	50.8	46.0	11.1	41.3
	%Tissue	100.0	25.4	41.3	44.4	12.7	36.5
	p value	NA	0.22	0.18	1.00	1.00	0.51

NA indicates no discordant pairs for the exact McNemar test (binomial test on discordant pairs).
* indicates statistically significant ($p < 0.05$).

consistently showed near-universal detection (96.9–100%) for both methods across all commodities, indicating broad microbial presence and strong agreement between sampling approaches.

Matrix-specific differences were observed for selected targets. In diced carrots, cloth sampling yielded significantly higher detection rates for *tet*A (37.5% cloth vs. 20.3% tissue; $p = 0.01$) and *tet*B (98.4% cloth vs. 87.5% tissue; $p = 0.04$), suggesting improved sensitivity of cloth sampling for these antimicrobial resistance targets in this matrix. In contrast, no significant differences were observed for *ybb*W, *fli*C, or *pho*E in carrots. For cut lettuce, diced tomatoes, and diced peppers, detection frequencies for all targets were similar between methods, with no statistically significant differences ($p > 0.05$), although minor numerical variations were observed.

The *fli*C marker generally exhibited low detection prevalence across matrices, particularly in lettuce and carrots, while *pho*E was rarely detected except in diced peppers, where moderate detection frequencies were observed for both methods. These trends are consistent with matrix-dependent microbial distributions and target prevalence.

Notably, the index target panel used in this study was partially refined based on initial findings from the cut onion dataset. Specifically, targets demonstrating limited discriminatory power or consistently high detection (e.g., *Lacto* and *Leuco-mesen*) were replaced with *tet*A and *fli*C to improve the relevance and interpretability of the target set across diverse produce matrices. This adjustment enabled better differentiation of sampling performance while maintaining coverage of key indicator groups, including total bacteria (EB-set 1), antimicrobial resistance targets (*tet*A, *tet*B), generic *E. coli* (*ybb*W), and pathogen-associated targets (*pho*E, *fli*C).

Overall, these results further support that aggregated cloth sampling provides detection performance equivalent to conventional grab sampling across multiple fresh-cut produce matrices, with occasional improvements in sensitivity for specific targets and commodities.

Discussion

This study evaluated the performance of cloth-based aggregated sampling relative to conventional grab sampling across multiple fresh-cut produce matrices, including cut onions, lettuce, diced carrots, tomatoes, and peppers, using a panel of index targets representing indicator organisms and microbial groups. Overall, the results consistently demonstrated that cloth sampling provides detection performance equivalent to traditional grab sampling under a commercial processing environment, while providing improved detection for selected low-prevalence targets.

Across commodities, EB-set 1 showed near-universal detection regardless of sampling method, reflecting its role as a broad indicator targeting the *Enterobacteriaceae* family. The high prevalence of this target is expected given the widespread presence of this bacterial group in fresh produce environments and processing systems. Because both methods were applied to the same produce matrices, comparable overall microbial loads were expected. However, the high prevalence of these targets limited their ability to reveal differences attributable specifically to the sampling methods.

In contrast, index targets such as *phoE* and *fliC* represent more specific, pathogen-associated indicators, targeting organisms such as *Klebsiella pneumoniae* and *E. coli* O157:H7, respectively. These targets are not commonly present in fresh produce under typical conditions, resulting in inherently low detection prevalence across matrices. Consequently, although these targets are highly relevant from a food safety perspective and may offer stronger correlation with potential pathogen presence when detected, their low occurrence limits their utility for comparative method evaluation due to insufficient numbers of positive samples.

Together, these findings emphasize that target selection is critical for comparing sampling methods. Targets with excessively high prevalence may provide limited discriminatory power because they are detected consistently by both approaches, whereas targets with very

low prevalence may generate too few positives for meaningful statistical comparison. Therefore, moderate-prevalence index targets are particularly useful because they balance ecological relevance, detection frequency, and sensitivity to potential differences in sampling performance. In this study, these targets generated robust datasets while still allowing comparison of sampling efficiency between methods. Their performance further supports the utility of aggregated cloth sampling for capturing representative microbial populations, particularly when targets are unevenly distributed across produce surfaces.

Moderate-prevalence targets, including *tetA*, *tetB*, and *ybbW*, provided the most informative comparisons across matrices, as they balance detection frequency with ecological relevance. The *tetA* and *tetB* genes encode tetracycline resistance and are commonly associated with antimicrobial-resistant bacteria present in agricultural environments, including those influenced by manure application or environmental reservoirs. Their moderate and variable prevalence makes them useful indicators of microbial populations that are neither ubiquitous nor exceedingly rare, thereby enabling clearer differentiation between sampling approaches. Notably, in diced carrots, cloth sampling achieved significantly higher detection rates for *tetA* and *tetB*, suggesting that increased sampled surface area and continuous aggregation enhance the recovery of heterogeneously distributed or low-abundance targets.

Similarly, *ybbW*, a conserved genetic marker for generic *E. coli*, was included as an index target of fecal contamination and may be relevant to irrigation water quality and produce safety. As a moderately prevalent target, *ybbW* provided useful discriminatory power for comparing the two sampling approaches. Although relatively larger differences in *ybb*W detection were observed between aggregated cloth sampling and grab sampling in some produce matrices, these differences were not statistically significant. The observed variation may reflect the heterogeneous and low-level distribution of generic *E. coli* on fresh-cut produce, combined with differences in the spatial and temporal coverage of the two sampling methods. Aggregated cloth sampling integrates microbial material from product contacting the cloth over time, whereas grab sampling reflects discrete product portions collected at specific time points. Therefore, the matrix-specific differences in *ybb*W detection are likely attributable to sample representation and microbial heterogeneity rather than differences in the produce matrix alone. In addition, because the number of paired samples was limited, increasing the sample size in future studies may reduce the apparent variability between methods and provide greater statistical power to evaluate whether meaningful differences exist.

The refinement of the target panel following the initial onion study further supports the importance of matrix-specific considerations. Targets such as *Lacto* and *Leuco-mesen*, which exhibited limited differentiation in onions, were replaced with targets such as *tet*A and *fli*C to improve sensitivity and applicability across additional produce matrices. This iterative approach highlights that no single target panel is universally optimal; rather, target selection should be guided by expected prevalence, ecological relevance, and the specific objectives of the sampling program.

From an operational perspective, aggregated cloth sampling offers several practical advantages over conventional grab sampling. By enabling continuous, aggregated sample collection over a defined time period, the effective sampling size increases and reduces the likelihood of missing localized contamination events. At the same time, it standardizes sampling procedures, reduces labor requirements associated with repeated manual grabs, and may lower overall testing costs by consolidating multiple sampling events into a single composite sample. The cloth was placed on the central distribution cone from the top without equipment disassembly or modification of the processing line and was removed from the top of the equipment after the 2-h sampling period. This procedure required minimal manual handling and did not interfere with routine processing operations. The 2-h sampling window was selected to align with the facility's standard production schedule and operating procedures. These advantages are particularly relevant for high-throughput processing environments where efficiency and consistency are critical.

This study was designed as a field-based comparison under commercial processing conditions and did not include controlled laboratory validation. The cloth configuration was developed with produce industry partners to fit the multipocket scale of a commercial fresh-cut processing line, where product-contact dynamics, sampling duration, and naturally occurring low-prevalence contamination are difficult to reproduce accurately at laboratory scale. Although the cloth material and processing workflow were informed by previous validation studies in beef-industry applications (Wheeler & Arthur, 2018; Arthur & Wheeler, 2021; Arthur et al., 2023), lab-scale produce-specific inoculation studies are still needed to determine recovery efficiency, analytical sensitivity, and limits of detection. Thus, laboratory validation and commercial field evaluation should be considered complementary in assessing this sampling approach. Another limitation is that this study evaluated naturally occurring microbial index targets rather than direct pathogen detection. Because foodborne pathogens are expected to occur at very low prevalence in commercial produce-processing environments, direct pathogen testing would likely yield few or no positive samples for either sampling method, limiting the ability to meaningfully compare method performance (Zhang et al., 2020; Van Pelt et al., 2018). Therefore, microbial index targets were selected based on the literature to provide measurable, naturally occurring targets for comparing the two sampling approaches. As a result, the findings should be interpreted as a comparison of detection performance for the selected index targets under commercial processing conditions, rather than as a validation of pathogen detection.

Overall, the findings of this study support aggregated cloth sampling as a promising alternative to conventional grab sampling for fresh-cut produce safety monitoring during commercial processing. Future work should expand validation across additional food matrices and identify optimized, produce-relevant index targets with suitable prevalence and discriminatory power. These efforts will further strengthen the utility of aggregated sampling approaches and may support the adoption of aggregated cloth sampling for representative sampling in fresh produce safety systems.

Conclusions

Aggregated cloth sampling performed equivalent to conventional grab sampling across multiple fresh-cut produce matrices and microbial index targets under commercial processing conditions, while providing improved detection for selected low-prevalence targets. By capturing a larger and more representative subset of the microbial population present on the produce overtime, aggregated cloth sampling generated composite samples that better reflect the microbiological status of the production lot, thereby addressing the challenges posed by heterogeneous microbial distribution while maintaining strong agreement with conventional grab sampling. Importantly, this study highlights that appropriate target selection is critical for method evaluation, as targets with excessively high or low prevalence provide limited discriminatory value. In addition to its comparable sampling performance, cloth sampling offers clear operational advantages, including reduced labor, standardized workflows, and potential cost savings. Together, these findings support cloth sampling as a robust, scalable, and practical alternative to grab sampling for representative sampling in fresh produce safety systems under commercial processing conditions.

CRediT authorship contribution statement

Jiewen Guan: Writing – review & editing, Writing – original draft, Formal analysis, Data curation, Conceptualization. **Eric C. Wilhelmsen:** Writing – review & editing, Writing – original draft, Resources,

Conceptualization. **Yongqing Huang:** Writing – review & editing, Methodology, Conceptualization. **Yicun Huang:** Writing – review & editing, Resources, Methodology, Data curation. **Florence Wu:** Writing – review & editing, Resources, Data curation. **Terrance M. Arthur:** Writing – review & editing, Supervision, Project administration, Funding acquisition.

Declaration of competing interest

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Appendix A. Supplementary material

Supplementary material to this article can be found online at https://doi.org/10.1016/j.jfp.2026.100877.

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