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Vegetable fermentations brined with low salt for reclaiming food waste

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Abstract: Fermentation of eight vegetables was studied as an alternative for reclamation of surplus volumes. Fermentation performance was predicted by comparing the amounts of acid that could be produced from the intrinsic sugar content with that buffered by the fresh vegetable matrices prior to reaching an inhibitory pH for fermentative microbes (3.30). Native fermentations were brined with 345.0 mM sodium chloride, 40.0 mM calcium chloride, 6.0 mM potassium sorbate, and vinegar to adjust the initial pH to 4.70. High-performance liquid chromatography analysis, pH, and carbon dioxide measurements and spiral plating on selective media were employed to monitor the progress of fermentations. The average colony counts for yeast and/or molds and *Enterobacteriaceae* declined to undetectable levels from 3.6 ± 1.5 log CFU/ml within 7 days of fermentation. The fermentation of sugars produced lactic, acetic, succinic, and/or malic acids, and ethanol. As predicted, the fermentation of vegetables with low sugar content, such as broccoli, green leaf lettuce, and green pea proceeded to completion. The fermentation of vegetables with a moderate sugar content, such as green bell pepper, red ripened tomato, and green bean were incomplete at pH 3.1 ± 0.2. The fermentation of high sugar vegetables including sweet potato and corn were expected and observed to be incomplete. It is concluded that the intrinsic sugar content and buffer capacity of surplus vegetables are relevant parameters in obtaining complete fermentations.

KEYWORDS
fermentation, food waste, reclaim, vegetable

Practical Application: Vegetables are the second most wasted commodity in the United States and a substantial constituent of the global food waste. Development of fermentation to reclaim surplus vegetables from farms, grocery stores, and farmer's markets offers opportunities to ameliorate economic losses and environmental impact and add value to waste. The research described here suggests that a fraction of vegetables could be fermented in cover brines while others, with high sugar content, need specialized handling. Evidently, optimization of vegetable fermentation with starter cultures and added buffers represent an opportunity to stimulate complete bioconversions useful for reclaiming surplus volumes.

1 | INTRODUCTION

Vegetable production occupied 4.4 million acres in the United States in 2017, with an associated sales value of 19.6 billion US dollars (USDA-National Agriculture Statistical Service, 2019). It is estimated that 44.4% of the US annual vegetable production is lost or wasted, only marginally surpassed by the fresh fruit category (USDA-Economic Research Service, 2020). This represents a serious compromise in the efficient use of energy, land, and freshwater (Buzby et al., 2014). Vegetable waste is not only a concern in the United States but globally given their short shelf-life (Gustavsson et al., 2011). In 2018, 50% of the US vegetable production consisted of tomato, onion, and sweet corn. Sweet corn and tomato, along with snap bean and cucumber, also occupy the largest portion of harvested land (2.38 million acres; USDA-National Agriculture Statistical Service, 2020). Head lettuce, tomato, and onion had the highest market value, adding to 32% of the $19.6 billion sales value.

The estimates of loss from farm to retail for sweet yellow corn, ripened tomato, cucumber, onion, and head lettuce were 78, 38, 60, 55, and 46%, respectively, in 2017 (USDA-Economic Research Service, 2020). These estimates are inclusive of losses from farm to retail, consumer level (at retail and restaurants), and non-edible portions. Such losses are associated with rejection of vegetables for food safety regulations (industry or government) and quality standards, decomposition, damage due to the malfunction of processing equipment, unpurchased holiday foods, and consumers dislikes (Muth et al., 2019). More specifically, vegetable losses at the farm level occur due to pests, climatic changes, or lack of labor. At the retail level, losses are associated with overstocked product display to encourage purchases, consumer expectations of cosmetic perfection, wholesale pack sizes that are too large as compared to store needs, expired sell by dates, damaged products, unpopularity, and low staffing (Gunders, 2012).

The reclamation of surplus vegetables at the farm and retail levels using fermentation could add value to waste. Preservation by fermentation can increase value of finished products, prolong shelf-life, and improve nutrient content (Chang & Chang, 2010; Di Cagno et al., 2008; Gunders, 2012). The fermentation of vegetables can impact the reduction of edible vegetable waste while creating profitable secondary uses. Vegetables that have been inadequately stored, failed to meet quality standards, or surpassed harvest time due to labor shortages can be reclaimed through the fermentation process to generate diverse value-added products. The conversion of sugars naturally present in vegetables to organic acids by lactic acid bacteria denies an energy source to spoilage microbes and lowers the pH of the vegetable tissue. It is this rapid reduction in pH that inhibits organisms of public health significance from compromised fresh vegetables, extending their shelf-life, and reinstituting the lost margin of safety (Breidt & Caldwell, 2011). Vegetable pieces, trims, and nubs, as well as rejects at processing facilities, can be redirected to a preservation process that enables their use in soups, sauces, and smoothies and/or as natural sources of colors, exotic flavors, and texture enhancers. Additionally, the changes of the fermented vegetable sensorial characteristics via fermentation can increase the economic value of end products. For example, one pound of fresh green cabbage was sold in the United States at an average of $0.62 in 2016, while its derivative sauerkraut had a value of $1.15/lb. (USDA-Economic Research Service, 2016). Conversion of vegetables to value-added food products represents a profitable alternative that reduces the impact of food waste on the utilization of energy and natural resources.

This study evaluated the preservation of eight of the most produced and wasted vegetables in the United States, including sweet yellow corn, green leaf lettuce, green bean, broccoli, red ripened tomato, green bell pepper, green pea, and orange sweet potato. Such groups of vegetables

TABLE 1 Content of glucose, fructose, sucrose, and malic acid in the fresh vegetables and amounts utilized in fermentations

(A)	Glucose			Fructose		
Vegetable	Reference mean (mM)	Measured from the fresh vegetable (mM)	Percent utilized	Reference mean (mM)	Measured from the fresh vegetable (mM)	Percent utilized
Green leaf lettuce	20.82	11.61 ± 3.62	97.40	22.20	23.29 ± 3.48	97.67
Broccoli	31.36	2.12 ± 1.06	80.3	46.35	0.93 ± 0.25	79.31
Green bell pepper	61.34	44.50 ± 1.13	81.59	64.94	50.06 ± 3.15	70.98
Red ripened tomato	87.70	75.20 ± 2.75	65.06	94.92	88.10 ± 5.10	63.06
Green bean	84.65	49.28 ± 1.13	93.77	69.66	46.88 ± 3.44	68.22
Orange sweet potato	60.50	101.52 ± 8.59	49.12	38.02	72.29 ± 6.15	55.17
Green pea	3.33	11.20 ± 2.15	95.94	10.82	13.91 ± 1.09	98.42
Sweet yellow corn	190.39	38.16 ± 2.98	5.81	107.68	32.50 ± 0.96	None

(B)	Sucrose			Malic acid	
Vegetable	Reference mean (mM)	Measured from the fresh vegetable (mM)	Percent utilized	Measured from the fresh vegetable (mM)	Percent utilized
Green leaf lettuce	None	17.75 ± 2.27	97.69	13.74 ± 6.77	95.23
Broccoli	0.29	1.24 ± 0.70	70.10	23.23 ± 5.59	91.52
Green bell pepper	5.84	29.27 ± 1.09	33.30	None	None
Red ripened tomato	0.29	19.97 ± 3.37	24.92	6.76 ± 2.27	27.22
Green bean	10.66	2.14 ± 1.25	ND[a]	14.12 ± 4.96	87.00
Orange sweet potato	76.25	19.03 ± 1.76	ND	43.53 ± 5.40	77.05
Green Pea	72.89	14.53 ± 2.26	98.41	7.23 ± 4.48	100
Sweet yellow corn	25.85	40.58 ± 3.85	None	13.80 ± 3.40	None

Note: The fermentations of sweet yellow corn were unique in producing propionic acid to 25.60 ± 11.92 mM.
[a]ND, not determined.

represent a range of natural sugar content (Table 1). It was hypothesized that the performance of fermentation could be predicted based on the intrinsic glucose, fructose, and sucrose content in the vegetables. The prediction was based on an estimation of the concentration of organic acids that could be produced from the documented sugar content and the volume of acid needed to reduce the pH of a particular vegetable slurry to 3.3, as determined by titration. These predictions were compared to the profiles of native fermentations. The long-term goal of this research is to identify fermentation parameters requiring optimization for the complete conversion of sugars to acids or ethanol, which is critical in the stability of products during elongated-bulk storage.

2 | MATERIALS AND METHODS

2.1 | Predicting the performance of vegetable fermentations

Data of the intrinsic amounts of glucose, fructose, and sucrose on each of the eight vegetables included in the study were obtained from the USDA National Nutrient Database for Standard Reference Release 28. The reported amounts were converted to millimolar concentrations assuming a density of 1 mg/cm^3. The calculated molar concentrations were utilized to estimate the maximum lactic acid production from fermentation or lactic acid equivalents. Such calculated yield assumed a complete homofermentation in the absence of alcoholic fermentation. The estimated yield for a heterofermentation could be estimated by dividing the lactic acid equivalents by two.

2.2 | Titration of vegetable slurries to determine amounts of acids needed for dropping the pH to 3.0

It was of interest to understand how much acid could be tolerated by the vegetable matrices prior to a reduction in pH to 3.3. A pH of 3.3 in vegetable fermentations is inhibitory for the prevailing lactobacilli such as *Lactiplantibacillus plantarum* (Di Cagno et al., 2008; Kotzekidou & Roukas, 1986; Oguntoyinbo et al., 2016; Wouters et al., 2013). Growth of *L. plantarum* is inhibited

at pH 3.3 and continued acid production can result in an eventual decrease in pH to 3.0 (McDonald, Hassan, et al., 1991). Spoilage of fermented vegetables typically develops in batches that reach a pH of 3.3, prior to the complete disappearance of simple sugars (Franco et al., 2012; Johanningsmeier & McFeeters, 2013).

Three lots of each vegetable were secured from the local retail market (Raleigh, NC) and used to determine the amounts of acids needed to drop the pH to 3.0 by titration. Triplicate samples of 100.0 ± 1.0 g of vegetable slurries were titrated with 7 M lactic acid (Sigma Aldrich, St. Louis, MO, USA), 7 M acetic acid (Sigma-Aldrich), or a mixture of both acids at equal concentrations of 7.01 M. Slurries were prepared in triplicate for each vegetable. Each vegetable sample (~200 g) was blended in a NutriBullet (Homeland Houseware, Pacoima, CA, USA) for 30 s to prepare slurries. The vegetable slurries used for titration consisted of 50.0 ± 1.0 g of a blended vegetable and 50.0 ± 0.2 g of distilled water. A total volume of 100.0 ± 1.2 g of each vegetable slurry was used for titration with acids. Aliquots of 100.0 ± 0.1 μl of an acidic solution were added at 20-min intervals to each of the 24 samples of vegetable slurries until a final pH of 3.0 was reached. The 20-min intervals were needed to achieve equilibration in between acid additions. The vegetable slurries were constantly stirred at medium speed to accelerate and ensure equilibration of the homogenate after each acid addition. For thick slurries, such as those produced from sweet potato and green beans, the mixing was done manually with a spatula for 30 s. The pH of the slurries was measured with an Accumet Research 25 pH meter (Fisher Scientific, Carlsbad, CA, USA) equipped with a Gel-Filled Pencil-Thin pH Combination Electrode (Accumet, Fisher Scientific). A total of 72 samples were titrated. Titration data were used to calculate the molar concentration of each acid needed to achieve a pH of 3.3 in a vegetable matrix. A logarithmic mathematical model was used to get a trendline that fitted a scatter plot of pH versus acid concentration. The trendline was used to define the amounts of acid reduced in each vegetable matrix to decrease the pH to 3.3 and 3.0. Such concentrations were used to estimate the amounts of acids that could be produced in fermentations prior to bacterial and metabolic inhibition.

2.3 | Vegetable fermentations

Triplicate lots of each vegetable were secured locally and rinsed with distilled water upon arrival in the laboratory. The vegetables were visually inspected to remove spoiling pieces. The size of fermentation vessels was adjusted to accommodate for the natural shape and volume of each vegetable. Vegetables were diced, cut or blended, as needed, to enable equilibration with the cover brine. The seeds, placenta, calyx, pedicel, and septa were removed from the green bell peppers by cutting around the stem. The coreless green bell pepper pericarps were sliced in quarters, and each piece was cut in half to generate eight pieces. Green bell peppers were packed in jars of 924 ml in a 50:50 ratio. Broccoli florets with about 1 cm stem were brined in jars of 1310 ml. Broccoli florets were added to 20% of the jar volume and top off with 80% cover brine. Sweet potato slices and tomato cubes, both of 1.27 cm, were manually cut and individually packed in jars of 504 ml at a 50:50 ratio. Green beans, green leaf lettuce, and green peas were brined whole. Green leaf lettuce was packed in jars of 3885 ml to 10% of the total volume and top off with 90% cover brine. Green beans and green peas were packed in jars of 1310 and 504 ml, respectively. Such vegetables were packed to 40 and 50% of the volume of the jars and top off with 60 and 50% cover brine, respectively. Corn kernels were blended prior to fermentation as described above for titrations and packed in jars of 504 ml using a 50:50 ratio.

Fermentation cover brines were formulated with NaCl (Morton Canning and Pickling Salt, Chicago, IL, USA), anhydrous calcium chloride ($CaCl_2$; Brenntag, Durham, NC, USA), and potassium sorbate (Mitsubishi International Ingredients, Atlanta, GA, USA) equilibrating to 345.0, 40.0, and 6.0 mM, respectively. The total volume of the vegetables and cover brine was used to calculate the amounts of ingredients needed per jar to appropriately manage the effect of equilibration. Potassium sorbate was incorporated into the cover brine formulation to prevent growth of yeast and molds known to induce instability in reduced NaCl vegetable fermentations (Guillou et al., 1992; Pérez-Díaz et al., 2015). The initial pH prior to fermentation was adjusted to 4.7 with acetic acid in the form of 20% vinegar (200 grains). The volumes of acetic acid needed to adjust the initial pH to 4.7 were determined by the titrations described above. Green bell pepper, broccoli, orange sweet potato, green leaf lettuce, green beans, green peas, and sweet yellow corn were acidified with 5.71, 9.43, 36.96, 7.76, 11.82, 45.0, and 9.41 mM acetic acid, respectively. Tomatoes were not acidified as such vegetable is naturally acidic with a pH of 4.58 ± 0.05. The pH of fresh cover brines prepared to pack green leave lettuce, broccoli, green bell pepper, red ripened tomato, green bean, orange sweet potato, green pea, and sweet yellow corn were 4.70, 4.90, 5.34, 6.75, 4.70, 4.40, 3.69, and 5.00, respectively. All brines were equilibrated with the vegetables.

Jars were closed with commercial metal lug caps. Each lid was equipped with a rubber septum in its center to allow for sampling of cover brine using a 10 ml syringe attached to a 18G × 1 1/2″ needle (Becton Dickinson). The jars were incubated at 30°C for 21 days. Cover brine samples were aseptically collected from jars on days 2, 7 or 10, 14 or 16,

and 21 for biochemical and microbiological analyses performed as described below.

2.4 | Microbiological analysis of vegetable fermentations

Aseptically collected cover brine samples and fresh vegetable homogenates were serially diluted in 0.85% NaCl solution. Fresh vegetable samples were sliced with a sterile knife, when needed, using aseptic techniques and blended using a Waring Commercial Blender 700S (Torrington, CT, USA), equipped with a sterilized glass vessel, for 90 s at medium speed. Vegetable slurries were homogenized for 1 min at maximum speed using a Seward Stomacher 400 (Bohemia, NY, USA) and 6″ × 4.5″ filter stomacher bags. Samples plated on MRS agar (Difco Laboratories) supplemented with 1% of a 0.1% cycloheximide solution (Oxoid, Basingstoke, England) were used for the enumeration of presumptive lactobacilli. Yeast and mold agar (YMA) supplemented with 0.01% chloramphenicol (Sigma-Aldrich, St. Louis, MO, USA) and 0.01% chlortetracycline (Sigma-Aldrich) was used for the enumeration of presumptive yeasts. Violet, red bile agar (Becton Dickinson and Co., Franklin Lakes, NJ, USA) supplemented with 1% glucose (VRBG) was used for the enumeration of presumptive *Enterobacteriaceae*. An automated spiral plater (Autoplate 4000; Spiral Biotech, Norwood, MA, USA) was used for the inoculation of all culture media. MRS plates were incubated at 30°C anaerobically for 48 h in a Coy Anaerobic Chamber (Grass Lakes, MI, USA). YMA plates were incubated aerobically at 30°C for 48 h. VRBG plates were incubated at 37°C aerobically for 24 h prior to the enumeration of colonies. Purple and pink colonies on VRBG were counted and recorded as presumptive *Enterobacteriaceae*. Colonies from agar plates were enumerated using a Flash & Go Automated Colony counter (cat. 90006010, IUL Instruments, Barcelona, Spain).

Isolated colonies (25 ± 7) on MRS plates inoculated with cover brines from each vegetable fermentation were streaked for purity on the same selective medium. The colonies were randomly selected from MRS agar plates that presented the highest lactobacilli colony counts from each vegetable fermentation. Frozen stocks were prepared for each purified colony in MRS broth containing 1.63 M glycerol (Sigma-Aldrich) and stored at −80°C. A total of 174 pure cultures were identified using the partial sequence of the 16S rRNA gene. Pure cultures grown in MRS broth were used to extract total genomic DNA using an InstaGene Matrix DNA extraction kit (Bio-Rad) following the manufacturer's instructions. Extracted DNA was used for the partial amplification of the 16S rRNA gene for identification of the isolates. The PCR mixture contained 2× master mix (Bio-Rad), 1 µl of the extracted genomic DNA, and 0.6 µM of primers 8f (5′-AGAGTTTGATCCTGGCTCAG-3′) and 1492r (5′-GGTTACCTTGTTACGACTT-3′) (Wilson et al., 1990). The PCR steps consisted of 4 min at 94°C followed by 30 cycles of 1 min at 94°C, 2 min at 57°C, and 2 min at 72°C, with a final extension step of 7 min at 72°C. Amplicons were sequenced by Eton Bioscience Inc. (Durham, NC). Sequence data were formatted and analyzed using BioEdit software (www.mbio.ncsu.edu/bioedit). Only bases that had quality scores greater than or equal to 20 were used for the alignment. Alignment of 16S rRNA gene sequences was performed with the basic local alignment search tool (BLAST) (Altschul et al., 1990; Sayers et al., 2020) using the 16S ribosomal RNA sequence database to determine the identity of the isolates. The partial 16S rRNA gene sequences for the lactic acid bacteria isolated from green bean, green leaf lettuce, green bell pepper, sweet yellow corn, green pea, broccoli, orange sweet potato, and red ripened tomato can be located using accession numbers MW234137 to MW234152, MW234153 to MW234170, MW234171 to MW234194, MW234195 to MW234218, MW234219 to MW234248, MW234249 to MW234274, MW234275 to MW234291, and MW234292 to MW234303, respectively.

2.5 | Monitoring of fermentation biochemistry and pH measurements

The fresh vegetable homogenates prepared for the microbiological analysis were also used for pH measurements. The vegetable homogenates and fermentation cover brine samples were spun for 10 min at 15,294 *g* at room temperature (Eppendorf Centrifuge 5810R, Fisher Scientific, CA, USA) to remove cells and particulate, so that clarified supernatants could be used for pH measurement. The pH of supernatants was measured with an Accumet Research 25 pH meter (Fisher Scientific) equipped with a Gel-Filled Pencil-Thin pH Combination Electrode (Accumet Fisher Scientific).

The vegetable homogenates were subjected to two freeze (−20°C) and thaw cycles prior to chemical analysis and centrifugation to enable the release of sugars into the liquid phase. The frozen and thawed homogenates and fermentation cover brine samples were clarified as described above for pH measurements. The concentrations of organic acids and ethanol were measured by high performance liquid chromatography (HPLC) analysis using a 30-cm HPX-87H column (Bio-Rad Laboratories, Hercules, CA, USA) for the separation of components and a Shimadzu HPLC system (UFLC, Shimadzu Scientific Instruments, Durham, NC, USA) with accompanying software. Separation was

TABLE 2 Comparison of the amounts of lactic and acetic acids that can be added to the vegetables before a pH of 3.0 is reached; theoretical production based on sugar content and actual amounts produced in native vegetable fermentations

Vegetable	Amounts of acids needed for acidification to pH 3.0 (mM)*			Calculated lactic acid equivalents (mM)	Acid produced (mM)		Ethanol produced (mM)
	Lactic acid	Lactic and acetic acids	Acetic acid		Lactic acid	Acetic acid	
Green leaf lettuce	135 ± 15	98 ± 7	255 ± 15	86.04	31.6 ± 10.6	33.0 ± 12.2	None
Broccoli	173 ± 13	189 ± 7	225 ± 20	156.59	11.4 ± 1.4	5.2 ± 0.6	18.0 ± 0.1
Green bell pepper	64.8 ± 3.0	91 ± 7	189 ± 20	275.93	102.7 ± 14.4	18.0 ± 7.2	None
Red ripened tomato	65.0 ± 3.0	77 ± 7	>130	366.41	110.0 ± 43.6	17.0 ± 10.7	None
Green bean	57.6 ± 1.5	154 ± 14	463 ± 14	351.27	155.7 ± 15.8	43.5 ± 10.9	28.5 ± 8.6
Orange sweet potato	72.3 ± 2.0	350 ± 10	699 ± 28	502.05	122.2 ± 37.3	19.5 ± 8.0	None
Green pea	NA	326 ± 7	1251 ± 100	319.87	148.8 ± 6.4	44.0 ± 4.7	21.4 ± 10.8
Sweet yellow corn	210 ± 14	133 ± 7	90 ± 5	699.57	108.0 ± 61.6	45.6 ± 9.1	None

performed at 65°C using 0.015 N H_2SO_4 for the mobile phase with a flow rate of 0.9 ml/min. An ultraviolet light detector (210 nm, RID-10A, Shimadzu) was used for quantification of malic, succinic, lactic, propionic, butyric, and benzoic acids. In addition, acetic acid, ethanol, and glycerol were quantified using a refractive index detector (SPD-20A, Shimadzu) that was connected in series.

The concentrations of glucose, fructose, and sucrose were determined from fermentation supernatants or fresh vegetable homogenates using an enzymatic kit (K-SUFRG 04/18; Megazyme, Bray, Ireland) and following the manufacturer's instructions.

2.5.1 | Statistical analysis

Significant differences among colony counts and pH measurements were determined by a two factor ANOVA test without replication and a post-hoc Tukey test using Microsoft Excel where lot and time were considered as factors. Significant differences as a function of time are presented in the graphs and were determined at a p-value $\leq$ 0.05.

3 | RESULTS AND DISCUSSION

We predicted the feasibility of achieving complete fermentations by comparing the amounts of acids produced from the intrinsic sugar content of each vegetable with that needed to reduce the pH of the matrices to pH 3.3 (Figure 1). Therefore, it was determined that vegetables containing relatively low sugar amounts, such as green leaf lettuce and broccoli (Table 1), could proceed to completion, given that more lactic acid was needed to drop the pH of such matrices to 3.3 than the amounts that could be produced from the intrinsic sugar content (Table 2). Vegetables containing moderate amounts of sugars, such as green bell peppers, green peas and green beans (Table 1), were expected to sustain incomplete fermentations as the theoretical production of lactic acid amounted to 315 ± 42 mM (Table 2), which is at least twice the concentration of acid needed to reduce the pH to 3.3 (Table 2). Similarly, it was speculated that vegetables containing a relative high content of sugars, such as sweet potato and sweet yellow corn would not be completely fermented. While most of the predictions were confirmed by the observations made from native vegetable fermentations, it was observed that sugar content varies within vegetable type. This is particularly exemplified by green peas. Such vegetable was expected to contain 3.33, 10.82, and 72.89 mM of glucose, fructose, and sucrose, respectively, based on the published data (Table 1). However, the concentrations measured in the sample studied were at 11.20 ± 2.15, 13.91 ± 1.09, and 14.53 ± 2.26 mM (Table 1). The documented sugar content of broccoli also substantially differed from that measured from the samples included in this study (Table 1). Such variations may be related to variety, physiological stage, and sources (Kurina et al., 2021; Lu et al., 2002).

This research systematically evaluated native vegetable fermentations brined with low salt concentrations (2% NaCl) to extend shelf-life of surplus volumes. The cover brine formulation used is safe for consumption and allows fermented vegetables to be stored in their own fermentation liquor in finished products without desalting. About 1% acetic acid prevents the proliferation of *Clostridium botulinum* and the consequent production of toxin in cucumber fermentations brined with 6% NaCl (Ito et al., 1976; Koukou et al., 2021). Potassium sorbate was incorporated into the fermentation cover brines anticipating that the activity of the indigenous yeasts and molds could induce undesirable chemical and physical changes in the vegetables (Etchells et al., 1961; Garcia et al., 2021; Guillou et al., 1992; Mehyar et al., 2011; Pérez-Díaz et al.,

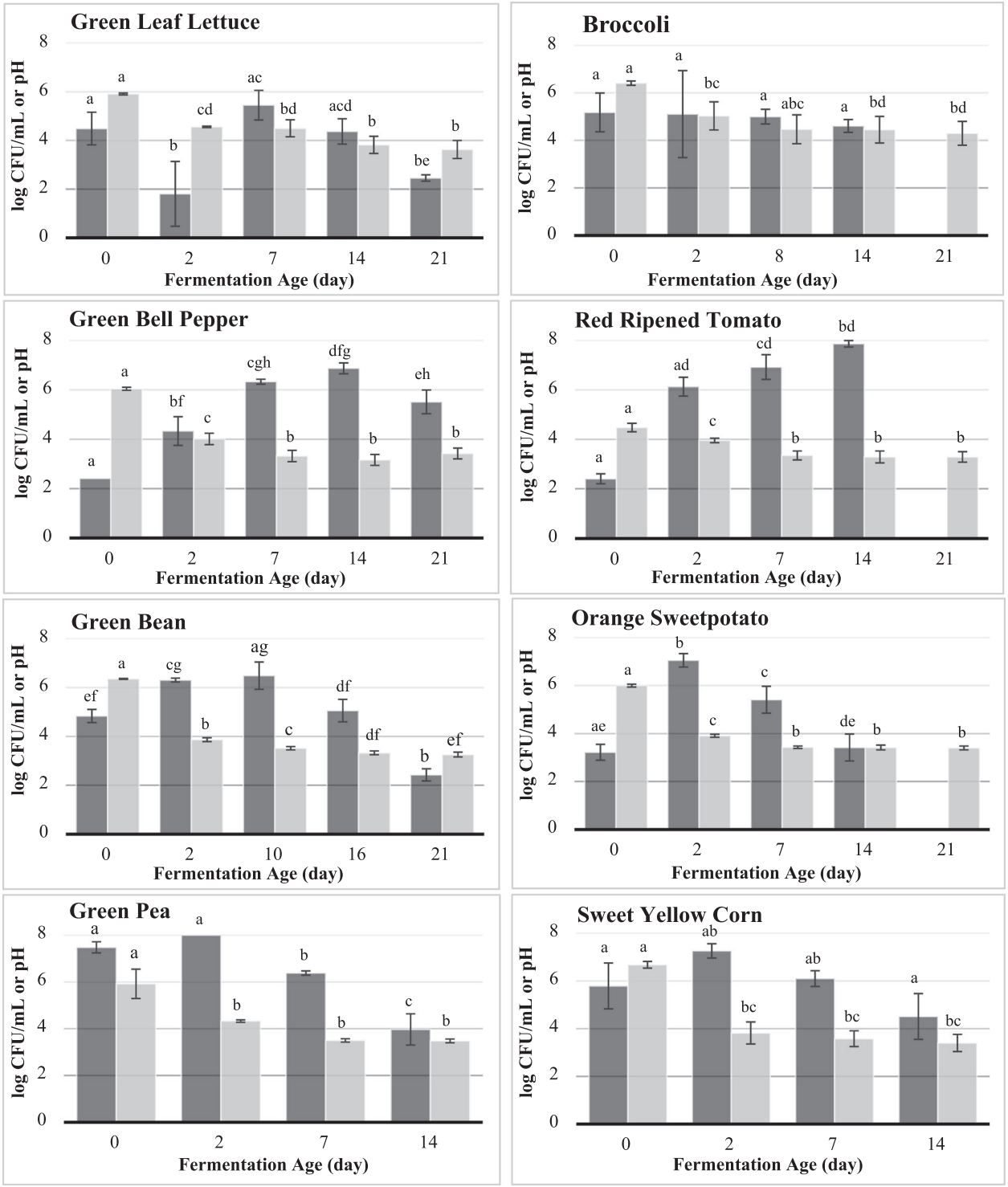

FIGURE 1 Changes in colony counts for lactobacilli (■) and pH (□) during fermentation of the eight selected vegetables. Data for 0 day were obtained from fresh vegetables. Statistical difference among time within a variable (pH or colony counts) is denoted by different letters

2015; Stratford et al., 2020). Yeast growth is sporadically observed in vegetable fermentations particularly under aerobic conditions and with shredded substrates (Etchells et al., 1947). Potassium sorbate is effective against most respiring fungi in fermented products (Etchells et al., 1961; Stratford et al., 2020). Table 3 presents the decline in the number of viable yeasts and molds in the eight vegetable fermentations tested to undetectable levels after 7 days of incubation, except in one lot of diced, red ripened tomato that remained at 4.3 log CFU/ml after 14 days. Such observation confirms the effectiveness of incorporating the preservative in the fermentation cover brine against

TABLE 3 Colony counts (log CFU/ml) for yeast and molds from YMA plates and presumptive *Enterobacteriaceae* from VRBG plates for fresh vegetable homogenates and cover brine samples collected on days 2, 7 or 10, and 14 or 16 of fermentations

	YMA				VRBG		
Vegetable	**Fresh vegetable**	**Day 2**	**Days 7 to 10**	**Days 14 to 16**	**Fresh vegetable**	**Day 2**	**D 7 or 10**
Green leaf lettuce	3.8 ± 0.2	TFTC[a]	TFTC (10)	TFTC (16)	4.8 ± 0.2	1.9 ± 0.7	TFTC (10)
Broccoli	1.9 ± 0.1	TFTC	TFTC (8)	NA	TFTC	TFTC	TFTC (7)
Green bell pepper	TFTC	3.3 ± 1.2	TFTC (7)	NA[b]	2.1 ± 0.7	3.3 ± 1.2	TFTC (7)
Red Tomato	1.3	0.9 ± 0.8	2.5 (7)	4.3 (14)	>4	>4	TFTC (7)
Green bean	3.8 ± 0.6	0.9	0.7 (10)	TFTC (16)	5.5 ± 0.8	2.1 ± 1.1	TFTC (10)
Orange sweet potato	1.8	1.2 ± 1.1	TFTC (7)	NA	2.4 ± 0.9	TFTC	TFTC (7)
Green pea	3.7 ± 0.9	1.7	TFTC (7)	NA	2.5 ± 2.3	1.5 ± 0.1	TFTC (7)
Sweet yellow corn	5.9 ± 1.3	5.2	TFTC (7)	NA	4.1 ± 0.1	3.0 ± 3.0	TFTC (7)

Note: The number in parentheses in the last column corresponds to the sampling day. Presentation of a number without a standard deviation corresponds to the colony counts observed in one sample out of the three included in the experiment given that the remaining two samples did not generate colonies on plates.
Abbreviations: VRBG, violet, red bile agar supplemented with 1% glucose; YMA, yeast and mold agar.
[a]TFTC means too few colonies to count, often representing no colonies on plates.
[b]NA means the counts are not available.

yeast and molds. The vegetable fermentations conducted in closed jars were microbiologically stable for up to 4 months. This result agrees with those reported by McFeeters and Pérez-Díaz (2010) for the preservation of cucumbers with potassium sorbate in closed jars. Furthermore, Table 3 shows that the low salt vegetable fermentations sustained a rapid reduction in the concentration of *Enterobacteriaceae*. This is partially attributed to the initial addition of acetic acid as vinegar in cover brines to adjust the pH to 4.7 (Etchells et al., 1947; Jiang et al., 2020; McDonald, Fleming, et al., 1991). Acetic acid (67 mM) inhibits the growth of *Enterobacteriaceae* in cucumber fermentations brined with 1.94 M NaCl (McDonald, Fleming, et al., 1991).

Our results demonstrate that fermentation of vegetables containing low amounts of sugars proceeded as predicted. Green leaf lettuce fermentations resulted in a reduction in pH from 5.9 ± 0.04 to 3.63 ± 0.37 (Figure 1), a pH slightly higher than that which is inhibitory for the fermentative lactic acid bacteria. Sugars were below the limit of detection (Table 1), but an incomplete acid production was observed, suggesting that microbial metabolic activities stalled during incubation (Table 2). The concentration of presumptive lactobacilli reached a maximum level of 5.4 ± 0.6 log CFU/ml by day 7 of the fermentation and declined after this point (Figure 1). *Leuconostoc* and *Pediococcus* were isolated from these fermentations but not *Lactiplantibacillus* (Figure 2), suggesting that the native lactic acid bacteria were inefficient in completing the fermentations. *Leuconostoc* and *Pediococcus* are sensitive to a pH of 4.5 and below (Feiner, 2006; Fitzsimons et al., 1992). Yang et al. (2010) observed an incomplete lettuce fermentation when the vegetable was fermented with bacteria from residual fermented cabbage. The mixed vegetable fermentation had a minimum pH of 3.6 resulting from the production of 25 mM lactic acid by a population of lactic acid bacteria of less than 4.0 log CFU/ml at the highest point during a 60-day incubation period. It remains necessary to determine if the chemical constituents of green leaf lettuce are inhibitory to microbial growth and/or metabolic activity and if an acid-resistant starter culture can assist in driving the complete conversion of sugars.

Contrary to green leaf lettuce, the lactobacilli native to broccoli drove the fermentations to completion, with final pH values reaching 4.3 ± 0.5 (Figure 1). Colony counts for presumptive lactobacilli remained at 4.5 ± 0.5 log CFU/ml (Figure 1) and included *Lactiplantibacillus*, *Leuconostoc*, and *Pediococcus* (Figure 2). The low densities of lactic acid bacteria in broccoli fermentations may have been related to the intrinsically low sugar concentrations in the tissue (Table 1). Sources of energy to support bacterial growth were reduced in the broccoli fermentations after 4 days of incubation at 30°C, with residual sugar amounts being within the standard deviation of the measurements and converted to lactic acid, acetic acid, and ethanol (Table 2).

Different from green leaf lettuce and broccoli, green pea was expected to be rich in sucrose, a disaccharide. The USDA Standard Reference reports that green pea naturally contains 3.33 mM glucose, 10.82 mM fructose, and 72.89 mM sucrose (Table 1). However, the samples used in this study had an intrinsic sucrose content of 14.53 ± 2.26 mM (Table 1). Therefore, sufficient buffer capacity existed in the vegetable matrix to sustain a complete bioconversion of the sugars before the pH became inhibitory for lactobacilli, consequently enabling a complete fermentation. The nearly neutral intrinsic pH of green pea (6.0 ± 0.6) declined to 3.7 ± 0.1 after 14 days of fermentation at 30°C (Figure 1). Presumptive homofermenting

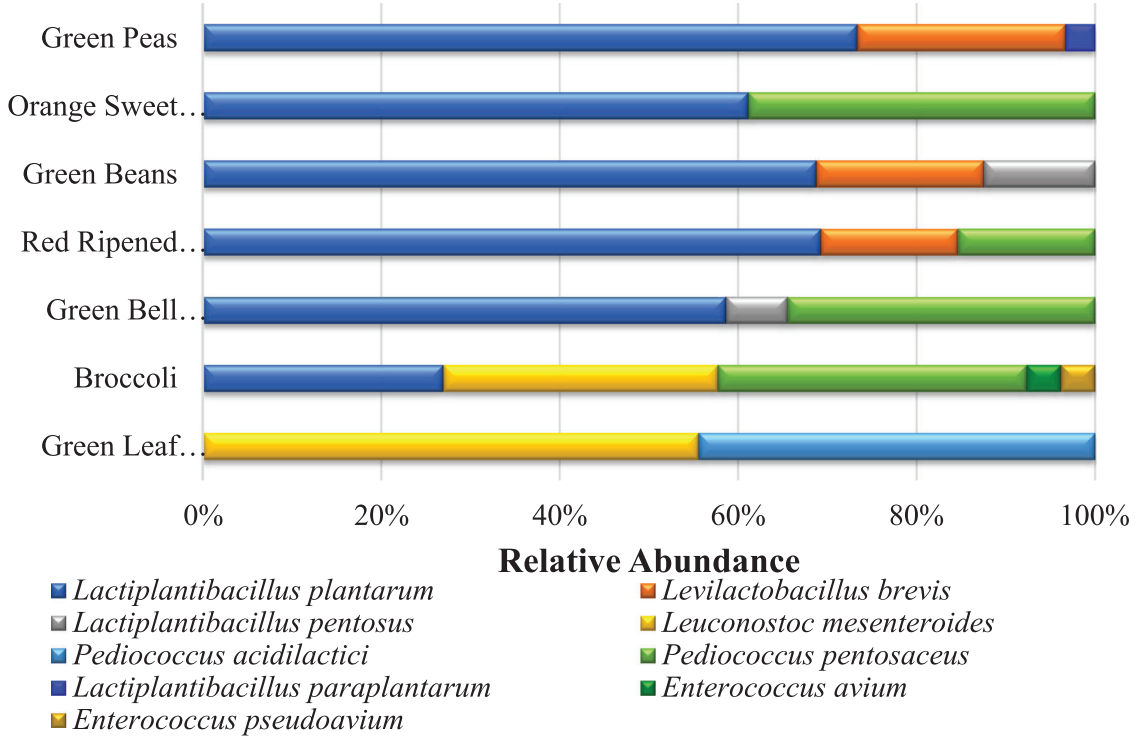

FIGURE 2 Abundance of the bacterial species isolated from MRS plates inoculated with fermented vegetable homogenates and identified based on the partial sequencing of the 16S rDNA

lactobacilli reached colony counts of 8.0 ± 0.1 log CFU/ml after 2 days, declining to 4.0 ± 0.7 log CFU/ml after 14 days (Figure 1). The robust fermentations of green pea were dominated by lactobacilli (Figure 2).

Green bell pepper and green bean have intermediate levels of glucose (61.3 and 84.6 mM, respectively), fructose (65.0 and 70.0 mM, respectively), and some sucrose (6.0 and 11.0 mM, respectively), which is evidently higher than those found in green leaf lettuce, broccoli, and green pea (Table 1). It is estimated that the intrinsic sugar content in green bell pepper and green bean is sufficient to sustain the production of about 276 and 351 mM lactic acid, respectively, in a homofermentation or half of such amount from heterofermentation (Table 2). The titration data (Table 2) suggest that there is insufficient buffer capacity in the fresh vegetables to support a complete fermentation. This prediction is supported by the observations made from the laboratory scale fermentations. An end point pH of 3.1 ± 0.2 was measured from fermented green bell peppers and green beans (Figure 1), while residual sugars were still available in the vegetable matrices (Table 1). The sugars were mainly converted to lactic and acetic acids, but malic acid (11.01 ± 3.89 mM) and succinic acid (6.20 ± 0.41) were also produced in green bell pepper fermentations, and succinic acid (14.03 ± 1.54 mM) and ethanol (28.48 ± 8.58 mM) were produced in green bean fermentations (Table 2). The population of presumptive lactobacilli increased to a maximum of 6.5 ± 0.5 log CFU/ml after 14 days of the fermentation in both systems (Figure 1). *Lactobacillus* prevailed in these fermentations and was followed by *Pediococcus* in green bell pepper fermentations (Figure 2).

Tomato is rich in citric acid and naturally acidic as evidenced by an intrinsic pH of 4.6 ± 0.5 (Figure 1). While the glucose and fructose content of tomato varies widely, they were not expected to contain sucrose or starch (Table 1). The experimental lots, however, contained 20.0 ± 3.4 mM sucrose (Table 1). Tomato could sustain the production of 366 mM lactic acid from natural sugars (Table 2), which significantly overpowers the intrinsic buffer capacity of the acidic vegetable (Table 2). Tomato fermentations resulted in the incomplete utilization of the sugars (Table 1), production of acetic and lactic acids, and end point pH of 3.3 ± 0.2 (Table 2 and Figure 1). The fermentation of red ripened tomato was unique in producing enough carbon dioxide to cause effervescence (data not shown). Others have observed that the fermentation of green tomato is prevailed by heterofermentors of the *Leuconostoc* genus, which could explain carbon dioxide production (Paramithiotis et al., 2014). In the fermentations studied here, yeasts and molds remained viable (4.3 log CFU/ml) after 14 days of incubation, despite the incorporation of potassium sorbate in the cover brine (Table 3). A maximum concentration of 7.9 ± 0.1 log CFU/ml was determined for presumptive lactobacilli after 14 days of

fermentation (Figure 1). The population of fermenting microbes was dominated by the facultative heterofermentor *L. plantarum*, the heterofermentor *Levilactobacillus brevis*, and the homofermentor *Pediococcus pentosaceous* (Figure 2). The metabolic activity of yeasts and heterofermentative lactic acid bacteria can contribute to the production of carbon dioxide in red ripened tomato fermentation. It is apparent that there is insufficient buffer capacity in tomato to accommodate for the acid that could be produced from the natural sugars prior to the inhibition of lactic acid bacteria at a pH of 3.3.

Among the eight vegetables tested, sweet yellow corn and sweet potato contain substantial amounts of glucose, fructose, and sucrose (Table 1). In addition to 12.7 g of starch, sweet potato contains an average of 60.5 mM glucose, 38.0 mM fructose, and 76.3 mM sucrose (Table 1), which could yield 502.1 mM lactic acid (Table 2). Sweet yellow corn contains 190.4 mM glucose, 107.7 mM fructose, and 25.9 mM sucrose (Table 1), potentially producing 699.6 mM lactic acid (Table 2). Such amounts of lactic acid are likely to overwhelm the buffer capacity of any vegetable matrix and clearly surpass those of sweet yellow corn and sweet potato (Table 2). We observed incomplete fermentations for blended sweet yellow corn kernels and sweet potato slices (Tables 1 and 2). Substantial amounts of sugar remained in the sweet potato and sweet yellow corn fermentations after 21 days at an ending pH of 3.4 ± 0.4 (Figure 1). The density of presumptive lactobacilli increased to nearly 7.2 ± 0.3 log CFU/ml (Figure 1) and included *Lactiplantibacillus* and *Pediococcus* in sweet potato (Figure 2). Isolated colonies from sweet yellow corn fermentation brines were not identified.

4 | CONCLUSION

It is concluded that the completion of a vegetable fermentation can be predicted from lot specific sugar content and buffer capacity. However, the preservation of vegetables by fermentation is also dependent on the intrinsic chemical composition of the matrices and the indigenous microbiota. Production of organic acids other than lactic and acetic acid and/or ethanol occurred in the fermentation of vegetables containing moderate and high amounts of sugars. This observation suggests that there is a tendency in fermentation systems with moderate or high sugar levels to diversify acid production, perhaps to gain microbial viability for an extended time. The complete fermentation of vegetables containing moderate sugar levels, such as green bell peppers and green beans, seems to depend on added buffer capacity, which could be achieved by supplementing fermentation cover brines with calcium chloride and vinegar or calcium acetate as proposed by Fleming et al. (1978). In the case of vegetables containing relatively high sugar, such as sweet potato or sweet yellow corn, fermentation could be achieved by adding a mixed starter culture of lactic acid bacteria and yeast in addition to a buffer. Specific to red ripened tomato is the need to increase the initial fermentation pH and/or add a buffer to enable the complete fermentation of the vegetable. The optimization of vegetable fermentation has potential as an alternative to reclaim surplus vegetables for reducing food waste.

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AUTHOR CONTRIBUTIONS

Connor Little: data curation; investigation; visualization; writing – review & editing. **Viviana Cruz-Martinez**: data curation; investigation; visualization. **Datricia Pearl St. Fort**: investigation; visualization. **Christian Pagan-Medina**: investigation; supervision. **Clinton A. Page**: data curation; investigation; writing – review & editing. **Yobet Perez-Perez**: data curation; visualization. **Michael E. Taveirne**: resources; supervision; writing – review & editing. **Alice M. Lee**: resources; supervision; writing – review & editing. **Nancy Arroyo-Gonzalez**: resources; supervision; writing – review & editing. **Cariluz Santiago-Ortiz**: resources; writing – review & editing. **Ilenys M. Perez-Diaz**: conceptualization; data curation; formal analysis; funding acquisition; investigation; methodology; project administration; resources; supervision; visualization; writing – original draft; writing – review & editing

CONFLICTS OF INTEREST

The authors declare no conflict of interest.

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