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Modeling the formulation pH of elderberry syrup with multiple weak acids

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Abstract: The objective of this work was to develop methods to assess the influence of the ingredients of an acidified elderberry syrup on product pH. A measure of total ingredient buffering (*tBeta*) was defined as the area under the buffer capacity curve of a food mixture or ingredient for pH 2–12. Citric acid (1% w/v), elderberry juice (75% v/v), and malic acid (0.75% w/v) had greater buffering (*tBeta* values of 15.33, 12.00, and 10.95, respectively) than ascorbic acid (0.75%) or lemon juice (3% v/v) (*tBeta* of 5.74 and 3.30, respectively). All other ingredients, including added spices (≤1% each) and honey (25% w/v), had *tBeta* values <2. The observed pH for the syrup mixture (pH 2.67) was within 0.11 pH units of the predicted pH based on combined buffer models of the acid and low acid ingredients (pH 2.78) using Matlab software. A total of 16 model syrup formulations containing elderberry juice with mixed acids (malic, acetic, and ascorbic) and having pH values between 3 and 4 were prepared. The pH values of the formulations were compared to predicted values from combined buffer models of the individual ingredients. Regression analysis indicated an excellent fit of the observed and predicted pH data, with a root mean square error of 0.076 pH units. The results indicated that buffer models may be useful for in silico estimates of how the ingredients in acid and acidified foods may influence pH, thus aiding in product development and safety assessments.

KEYWORDS
acid food, acidified food, buffer modeling, elderberry syrup, pH, *tBeta*

Practical Application: Buffer models using recently developed titration methods for individual acid and low-acid food ingredients can be used to estimate the pH of formulations of these ingredients in silico. The total buffering (*tBeta*) for ingredients or mixtures, along with ingredient concentrations, may be a useful metric for helping to determine which ingredients will have the greatest impact on pH. Such models can aid product development efforts and safety assessments.

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1 | INTRODUCTION

Formulations for acid and acidified foods must maintain a pH at or below 4.6 in order to prevent botulism and to assure the destruction of bacterial pathogens (U.S. Food and Drug Administration, 1979, 2011). To aid in product development and assessing food safety, buffer modeling for food ingredients has been proposed (Price et al., 2020). In this study, buffer models and total buffering (*tBeta*) were used as a means for assessing the buffering and pH impact of individual food ingredients on final product pH of an elderberry syrup. For cold-filled acidified foods that do not receive a heat treatment, the acid ingredients including the primary acidulent(s) and preservative acids must also prevent spoilage organisms from growing in the product. For the development of new acid or acidified products, empirical methods are typically used to determine product pH, with one or more acids being added until the desired pH is reached. During manufacturing, product lots are routinely tested to assure compliance. Due to unknown buffering in many low-acid food ingredients used in acid products, methods for quantitatively assessing pH changes with acid or low-acid food addition to acidic food formulations have not been available.

Recently, buffer models have been used to characterize how acid and low-acid salad dressing ingredients influence product pH (Longtin et al., 2020). Buffer models have also been used to link pH with fermentation acid concentrations during vegetable fermentations, and to help quantify the pH impact of the malolactic reaction of lactic acid bacteria (Breidt & Skinner, 2022). A graphical user interface software program based on ionic equilibria equations (Butler & Cogley, 1998) has been developed to simplify the generation of buffer models and make the technique accessible for a variety of applications (Breidt, 2023).

Food ingredient buffering can be estimated by using a titration method to generate buffer capacity (BC) curves over a pH range of 2–12 (Longtin et al., 2020) where there is little or no buffering from water. Outside of this pH range, buffering is essentially the same for aqueous food products, due to the symmetrical increase in buffering of water at the low and high extremes of the pH scale (Butler & Cogley, 1998). A simplifying assumption for pH modeling of acid or acidified foods was that buffer models can be derived from a series of monoprotic buffers that mimic the buffering of aqueous mixtures having undefined chemical composition (Gordon, 1982; Simms, 1926). For acid or acidified foods, the impact of low-acid ingredients on the finished equilibrium pH can therefore be estimated by the total buffering of the ingredient, which may be approximated by the area under the BC curve. Moreover, the BC curve between pH 2 and 12 essentially defines the pH of the formulation (Price et al., 2020), making pH prediction for ingredient mixtures possible by combining buffer models.

If the composite buffering of low-acid ingredients in a formulated acid or acidified food is taken into account, the pH of the food may be predicted with the addition of mineral acids or weak organic acids. Low-acid food ingredients including amino acids, proteins, nucleic acids, and other compounds may have multiple (undefined) chemical constituents that contribute to buffering below pH 4.6. The concentration of low-acid ingredients in acidified foods affects the pH due to the cumulative effects of buffering of these ingredients. Ingredients with little or no buffering in the pH range of acidified foods such as starches or sugars (Longtin et al., 2020) may thus have little or no impact on the final equilibrium pH other than dilution of the concentration of buffering ingredients even at high concentrations (25% or greater for some added sugars). Salts may also influence pH due to ionic strength effects on the p*K* values of buffers. These effects, however, are usually limited (<0.2 pH units) and are quantifiable (Price et al., 2020).

For the development of an acidified elderberry syrup product, the composite buffering of the acidulent(s) and the buffering of low-acid ingredients must be taken into account in order to achieve a finished equilibrium pH below 4.6. Elderberry products are increasing in popularity and are recognized as having antioxidant activity (Seeram et al., 2001) but may also have some toxicity (Citores et al., 1994). The objective of this study was to determine if buffer models could be used to estimate the influence of pH of both the low-acid (elderberry juice, spices) and acid (honey, lemon juice, malic acid, citric acid, and ascorbic acid) ingredients on product pH. In order to estimate the pH impact of each ingredient (or ingredient mixtures) based on the BC and concentration, a metric for total buffering (*tBeta*) was defined as a function of the area under the BC curve. The *tBeta* value and ingredient buffering were therefore used to estimate the impact of acid or low-acid food ingredients on the final product pH for elderberry syrup formulations.

2 | MATERIALS AND METHODS

2.1 | Ingredients in elderberry syrup

Ingredients for an elderberry syrup formulation typical of commercial products (N. Fragedakis, personal communication) are shown in Table 1. Elderberries were obtained as dried berries (*Sambucus Nigra*) from two commercial sources. Elderberry juice was prepared according to the

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TABLE 1 Elderberry syrup formulation.

Ingredient	Company[a]	Code	Lot or chemical ID
Elderberry juice	Purify Life, Dried	ES1A	N/A
Elderberry juice	Sambucus Berry Co., Dried	ES1B	N/A
Ground ginger	McCormick	ES2	25H 1544
Ground cinnamon	Great Value	ES3	OT 31,062 08,1159
Ground cloves	Great Value	ES4	OT 29,230 171,025
Honey	Great Value	ES5	N/A
Lemon juice	Realemon, Motts	ES6	0309220F2
Citric acid	Sigma–Aldrich	ES7	MKCG2579
L-Ascorbic acid	Sigma–Aldrich	ES8	15313KC
DL-Malic acid	Sigma–Aldrich	ES9	034K0028

[a]Purify Life, Chicago, IL; Sambucus Berry Co., Spokane Valley, WA; McCormick, Hunt Valley, MD; Great Value (Walmart, Inc.), Bentonville, AR; Motts LLP, Plano, TX; Sigma–Aldrich Chemical Co., St. Louis, MO.

TABLE 2 Buffer model data for elderberry syrup ingredients.

Ingredient	Percent[a]	Units/100 mL	Titrant (M)	pH	adj*C* (M)[b]	*tBeta*
Elderberry (ES1A)	75	mL	2	4.41	−0.0230	12.09
Elderberry (ES1B)	75	mL	2	4.54	−0.0273	13.03
Ground ginger	0.5	g	1	4.73	−0.0005	0.50
Ground cinnamon	0.5	g	1	5.40	−0.0001	0.55
Ground cloves	1	g	1	4.47	0.0105	2.58
Honey	25	g	1	4.15	−0.0011	1.49[c]
Lemon juice	3	mL	2	2.82	0.0012	2.11
Citric acid	1 (52.1)	g	2	2.23	−0.0089	15.33
L-Ascorbic acid	0.75 (42.6)	g	2	2.81	−0.0073	5.54
DL-Malic acid	0.75 (55.9)	g	2	2.38	−0.0053	10.95

[a]Units of percent, with concentrations for the acids also in mM units (mM).
[b]Adj*C* in molar units: the negative signs indicate cations; positive values indicate anions.
[c]Excluding the estimated peak for sugar hydroxyls.

supplier's instructions, by adding 100 g of berries to 1 L of water followed by holding at approximately 100°C for 20 min. The solids were removed by straining through a 30-mesh metal screen, and the resulting juice was frozen at −20°C in 50-mL aliquots. The juice was thawed and then combined with syrup ingredients as specified below, with the percentages shown in Table 2. The syrup was titrated immediately or frozen until use.

2.2 | Titration methods

Titrations with sodium hydroxide (NaOH) or hydrochloric acid (HCl) were performed using an automated titrator (Model 902 or 931, Hanna Instruments, Smithfield, RI, USA). Chemicals were obtained from Sigma–Aldrich (St. Louis, MO, USA) unless otherwise stated. In order to generate BC models, titrations of a food ingredient solution or suspension in 50 mL water were done using published titration protocols (Price et al., 2020). Briefly, custom titrator control files were developed in order to generate titration data starting from the initial pH of the solution to pH 1.8 (using 2 M or 1 M HCl) or 12.2 (using 2 M or 1 M NaOH) as specified in Table 2. The titrator was set for dynamic dosing to achieve a continuous set of data points to the specific endpoint, rather than controlled dosing with the supplied neutralization method. Titrant volumes were limited to a range of 0.01–0.1 mL at timed increments of 30 s for pH measurement, using the proprietary dosing algorithm of the manufacturer. Prior to titrations, the titrant acid and base concentrations were standardized to 1 or 2 M, depending on ingredient buffering (see below) to allow the endpoint pH to be reached at either pH 1.8 or 12.2 before a maximum of 50 mL total volume was added. Titrations usually required approximately 30 min. Data files that contained columns with the volume of titrant added

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and the resulting pH were then exported as text files in the manufacturer's format.

2.3 | Modeling BC from titration data

Paired titration data output files for the NaOH and HCl titrations from each sample were imported into Matlab. The titration curves were processed to make BC curves over the pH range of 2–12 as described (Butler & Cogley, 1998; Price et al., 2020) using a stepwise derivative:

$$\beta = \Delta\,(\text{volume of acid or base})\,/\Delta\text{pH}. \quad (1)$$

The BC (β) may be defined as the incremental change in the acid or base concentration divided by the resultant change in pH (Equation 1). A trigonometric least-squares regression method (Price et al., 2020) was then used to generate a continuous model of the BC curve data, using $\alpha = 0.05$, as a multiplier of for the pH range (x) of 2–12:

$$F(x) = B_0 + A_1 \sin(\alpha x) + B_1 \cos(\alpha x) + A_2 \sin(2\alpha x) + B_2 \cos(2\alpha x) + A_3 \sin(3\alpha x) \ldots . \quad (2)$$

Because the regression model was used as a template for the subsequent fitting with an ionic equilibrium model, parsimony was not a concern, and 15 sine and cosine terms (with A and B variables) were used for the regression. A nonlinear optimization algorithm in Matlab (fmincon.m) was then used to fit the regression model (Equation 2) by simultaneously optimizing concentrations C_i in molar units and equilibrium constants K_i for a buffer model derived from ionic equilibrium equations (Butler & Cogley, 1998):

$$\beta = 2.303 \times \left\{ \sum \left(C_i K_i \left[H^+\right] \Big/ \left(\left[H^+\right] + K_i\right)^2 \right) + K_w \Big/ \left[H^+\right] + \left[H^+\right] \right\}. \quad (3)$$

In Equation (3), the equilibrium constant for water was represented by K_w and the hydrogen ion concentration was $[H^+]$. To simplify calculations, concentration and pK values (negative log of the equilibrium constants K_i) for each buffer were independently assigned to monoprotic buffers (Gordon, 1982; Simms, 1926). Initially, seven monoprotic buffers were used for each BC curve that had derived pK values that were evenly distributed across the pH range of 2–12 with concentrations estimated from the corresponding $F(x)$ values (Equation 2). Monoprotic buffers in the optimized model that had similar pK values (within 0.2 pH units) were combined, summing the concentrations in order to generate the final buffer matrix of concentration and pK values.

2.3.1 | Estimating pH from buffer matrices

To estimate the pH from buffer matrices, Equation (4) was solved numerically for $[H^+]$ using Newton's minimization method as suggested by Butler and Cogley (1998) and described by Price et al. (2020):

$$0 = \sum (C_a K_a / (K_a + [H^+])) - \sum (C_b [H^+] / ([H^+] + K_b)) + K_w / [H^+] - [H^+] + \text{adj}C. \quad (4)$$

Acids (C_a and K_a) and bases (C_b and K_b) were modeled differently in Equation (4). With the exception of sugars, which had weakly acidic hydroxyls with pK values above pH 11 (Longtin et al., 2020), buffers with pK values of pH 7 or below were considered acids, and buffers with pK values above pH 7 were modeled as bases. The adj$C$ value estimates for an undefined food ingredient represent both an error in the buffer model and the contributions of salts of acids or bases, and were obtained as described (Price et al., 2020). The negative $\log_{10}$ of $[H^+]$ was then reported as the solution pH.

2.3.2 | Estimating total buffering of food ingredients

The total buffering (*tBeta*) for an aqueous food ingredient was defined as the area under the BC curve (with units of $\beta \times$ pH) for the pH range of 2–12, subtracting the buffering of water:

$$tBeta = 100 \times \left(\int_2^{12} F\left(x_{\text{ingredient}}\right) dx - 0.02 \right). \quad (5)$$

The definite integral of the trigonometric regression model, $F(x_{\text{ingredient}})$, from Equation (2) for a food ingredient was conveniently used in place of Equation (3) for the calculation of *tBeta*. Because the integral of the BC equation for water $\beta = 2.303 \times (K_w/[H^+] + [H^+])$ from pH 2 to 12 was equal to 0.02, the *tBeta* of water based on Equation (5) was zero. The aqueous food ingredients therefore had positive *tBeta* values that were commonly less than 0.25. A multiplier of 100 was arbitrarily included to easily conceptualize and compare *tBeta* values.

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2.4 | Modeling pH of elderberry syrup ingredients

Individual elderberry syrup ingredients were prepared in two identical 50-mL aliquots, one for titration with NaOH and one for titration with HCl, using the formulation concentrations as shown in Table 2. The resulting paired titrator data files were then processed as defined above. To compare *tBeta* measurements and estimate pH values, the buffer models for elderberry syrup ingredient mixtures (excluding honey) were prepared and processed using combined ingredients (elderberry juice, cloves, cinnamon, ginger, and lemon juice) or combined acids (citric acid, malic acid, and ascorbic acid). Because the chemically undefined food ingredients were presented as percentages, the acid concentrations were also presented as percentages. To precisely define the acid concentrations, the percentages were calculated based on molecular weight values of 192.12, 134.09, and 176.12 g/mol for citric acid, malic acid, and ascorbic acid, respectively. BC models for ingredient mixtures were generated using the concentration–p*K* matrices for the two mixed-ingredient buffer models or by combining the matrices from each individual ingredient. Predicted BC curves were then derived from these matrices using Equation (3). Estimates of pH (from Equation 4) and *tBeta* values (Equation 5) were then calculated from the resulting buffer models using Matlab software (Breidt, 2023).

2.5 | Validation of pH estimates from acidified elderberry juice

A dilute elderberry juice (37.5%) was combined with selected citric, malic, and ascorbic acid concentrations up to 0.3% (Table 3) to give pH values between 3 and 4. The observed pH values were recorded using a standardized pH meter (Hanna Instruments). Estimated pH values for the mixtures were precalculated from the combined concentration–p*K* matrices from BC models of each acid with the matrix from the elderberry juice titration. In order to generate concentration–p*K* matrices for different acid concentrations, the concentrations for each buffer in the BC models for 1% citric (52.1 mM), 0.75% malic (55.9 mM), and 0.75% ascorbic (42.6 mM) acids were adjusted. The individual buffer concentrations estimated from the ingredient models (in mM units) were multiplied by the ratio of the diluted acid concentration (from Table 3) to the initial (titrated) acid concentration. The adjusted values were then used to calculate the pH from the combined matrices for the acid–juice mixtures using Equation (4) as described above. Theoretical concentration–p*K* matrices were also generated from published acid p*K* values (Lide, 1995) substituting multiple monoprotic acids at the specified concentration for the polyprotic acids.

2.6 | Software and statistical analysis

A graphical user interface Matlab software program (BufferCapacity3) was used for processing titration data to generate BC curves and estimate pH and *tBeta* values. This software is publicly available (Breidt, 2023). Linear regression for observed and predicted pH values including calculation of the root mean square error (RMSE) for individual measurements of observed and predicted data was done using Excel and JMP software (SAS Institute, Cary, NC, USA).

3 | RESULTS AND DISCUSSION

3.1 | Elderberry juice buffer models

Previous studies that address pH predictions for formulating acid and acidified foods have relied on empirical data using selected acids in studies of specific products (McCarthy et al., 1991; Moreira et al., 1992; Sapers et al., 1984). These studies did not address acidic foods formulated with multiple acids or with varying ingredient concentrations. Recently developed methods using buffer models for food ingredients (Longtin et al., 2020; Price et al., 2020) may be used to estimate novel (untested) formulation pH values. Buffer models of two lots of elderberry juice (ES1A and ES1B; Figure 1) were prepared from titrations using 2 N NaOH or HCl. In general, ES1B had greater buffering capacity than ES1A (*tBeta* 12.09 vs. 13.03) and a higher pH value (4.54 vs. 4.41) as shown in Table 2. The *tBeta* estimate for ES1A is graphically shown (Figure 1) as the dark shaded area under the BC curve. The concentrations of individual buffers from ES1B were greater than those for ES1A, with the exception of buffer 2 (the buffer matrices for the data in Table 2 are shown in Table S1). The largest concentration values for the individual model buffers from ES1A and ES1B were for buffer 7 from each model (33.60 and 42.49 mM, respectively) with a p*K* of 12. Because the p*K* values for the models were limited by pH 12 (the upper limit of the titration data), the buffer peaks were partially masked by the BC of water above pH 12. As a result, these p*K* values were not precisely defined. However, it can be assumed that these buffers represent weakly acidic hydroxyl acids (not strong bases) of sugars in the elderberry juice, as previously observed for weakly acidic hydroxyls from sugars (Longtin et al., 2020). Based on this assumption, the adj*C* values for ES1A and ES1B required to approximate the measured pH were 23.31 and 27.25 mM,

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TABLE 3 The pH of elderberry syrup mixtures with selected acid addition.

Mixture[a]	Citric acid	Malic acid	Ascorbic acid	pH observed[b]	pH predicted[c]	pH theory[d]
1	0.31 (16.1)	0.32 (23.9)	0.29 (16.5)	2.92	2.86	2.93
2	0.34 (17.7)	0	0	3.15	3.11	3.21
3	0.34 (17.7)	0.12 (8.9)	0	3.05	3.00	3.08
4	0.21 (10.9)	0	0	3.40	3.31	3.40
5	0.13 (6.8)	0	0	3.59	3.49	3.58
6	0.15 (7.8)	0	0.31 (17.6)	3.37	3.32	3.37
7	0.13 (6.8)	0	0.13 (7.4)	3.54	3.43	3.40
8	0.10 (5.2)	0.14 (10.4)	0	3.30	3.27	3.34
9	0.13 (6.8)	0.25 (18.6)	0	3.12	3.10	3.16
10	0	0.33 (24.6)	0.33 (18.7)	3.16	3.11	3.15
11	0	0.31 (23.1)	0	3.27	3.19	3.24
12	0	0	0.32 (18.2)	3.72	3.64	3.64
13	0	0.10 (7.5)	0.23 (13.1)	3.51	3.45	3.48
14	0	0	0.12 (6.8)	3.91	3.80	3.83
15	0	0	0.23 (13.1)	3.76	3.71	3.71
16	0	0	0	4.02	3.95	4.03

[a]All concentrations shown as percent and (mM).
[b]The observed pH for 37.5% ES1A and added acids.
[c]The predicted pH using 37.5% ES1A and acid buffer models.
[d]The predicted pH using 37.5% ES1A and published p*K* values.

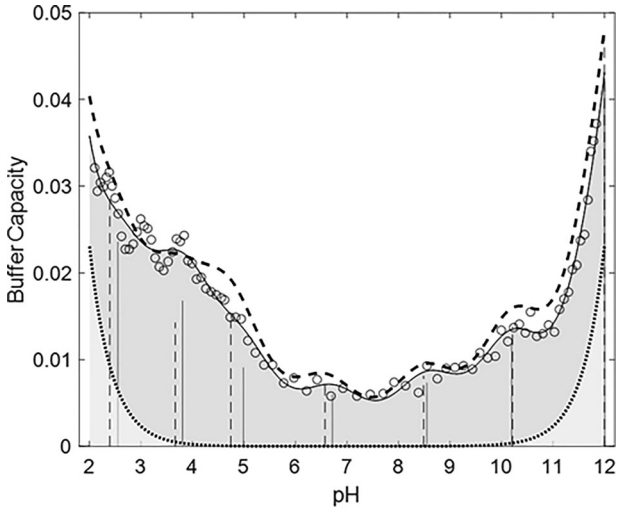

FIGURE 1 Buffer capacity (BC) models of elderberry juice. The buffer models for each elderberry juice sample ES1A (solid lines) and sample ES1B (dashed lines) are shown. The circles represent the titration data for ES1A. For clarity, only the BC data points for ES1A are shown. The dark shaded area represents *tBeta* for ES1A, and the lightly shaded area under the dotted line represents the buffer capacity of water. The vertical lines represent the individual monoprotic buffers for each sample.

respectively (Table 2). The negative sign for the adj*C* values in Table 2 indicates that cation salts may have been present (Price et al., 2020).

The differences in buffering between the two elderberry juice buffer models (Figure 1) were attributed to differences in the concentration of individual buffering components. This result is possibly related to cultivar, growing conditions, or other environmental effects on plant chemistry and physiology. The estimated buffers, however, served to define the BC curves for the two elderberry juices and can thus be used to model the pH of the juice, as well as how pH may change with added ingredients (Longtin et al., 2020; Price et al., 2020).

3.2 | Buffer models of spices and honey

Titrations for the remaining low-acid spices were performed using 1 N NaOH and HCl (Table 2). A comparison of the buffer models for these ingredients with the ES1A buffer model showed that spice ingredients contributed relatively little buffering compared to the other low-acid elderberry syrup ingredients (Table 2; Figure 2). The *tBeta* values for the added ginger and cinnamon (ES2 and ES3) with a concentration of 0.5% were 0.50 and 0.55, respectively. The ground cloves, however, at 1% had a *tBeta* of 2.58, or roughly 20% of the *tBeta* value for the mean buffering of the two elderberry juices.

Honey had a *tBeta* of 1.49 up to pH 10.2 (Figure 2), which was less than that for ground cloves, even though the concentration of honey was 25% (w/v), compared to 1% for

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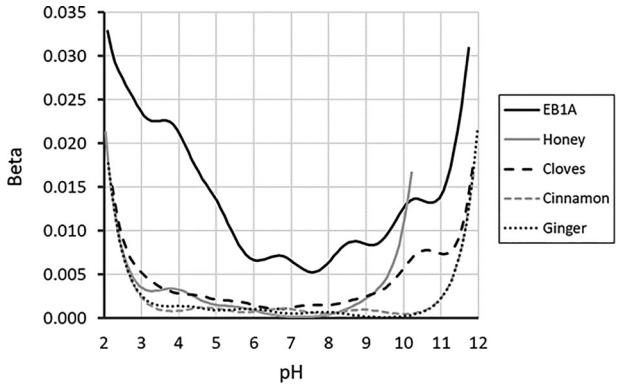

FIGURE 2 Buffer capacity (BC) models of spices and honey. The buffer model for ES1A (black line) was shown for comparison with the syrup ingredients. The buffer models for honey and spices included honey (gray line), cloves (dashed black line), cinnamon (dashed gray line), and ginger (dotted line).

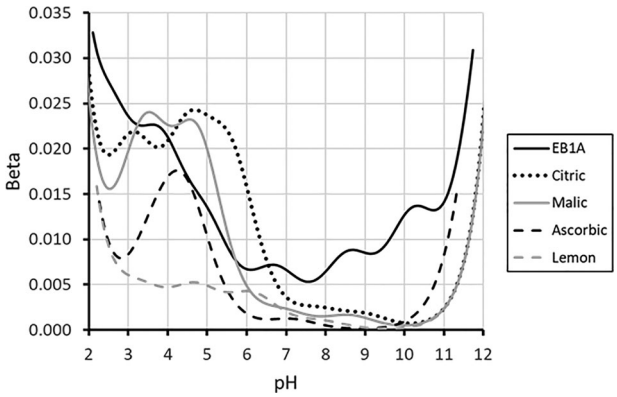

FIGURE 3 Buffer capacity (BC) models of acid ingredients in elderberry syrup. The buffer model EB1A (black line) was included for comparison with the syrup ingredients. Buffer models for the ingredients included citric acid (dotted line), malic acid (gray line), ascorbic acid (dashed black line), and lemon juice (dashed gray line).

ground cloves. A buffer with p*K* 3.89 was also found for honey that influenced the measured pH of 4.13 but was at a concentration of only 5.2 mM. The classification of honey as an acid or low-acid ingredient may vary depending on pH of a particular honey sample. The pH value of honey ranged from 3.42 to 6.10 with a mean of 4.2 for North American samples (White et al., 1962). Regardless of classification, the data indicate that the glucose, fructose, and other sugars in honey do not contribute significantly to buffering or pH changes in the elderberry syrup. For *tBeta* estimates of ingredient mixtures (described below), however, honey was not included due to difficulty of defining an endpoint for the calculated *tBeta* value, based on buffering of the sugar hydroxyls as described (Longtin et al., 2020). Further investigation of *tBeta* calculations for sugars will be the subject of future research.

3.3 | Buffer models of acid ingredients

BC models for each of the acid ingredients are shown in Figure 3. Citric acid at 1% (52.1 mM) had the highest *tBeta* value (15.33) of the acid ingredients, while lemon juice at 3% had the lowest *tBeta* value (2.11) (Table 2). Malic and ascorbic acids both at 0.75% (55.9 and 42.6 mM, respectively) had *tBeta* values of 10.95 and 5.54, respectively. These data indicate that the citric acid had the greatest impact on the pH of the syrup. Examination of the individual BC models for the polyprotic acids, which were titrated in water (with no added salts), revealed that the highest p*K* values of polyprotic malic and citric acids were lower (4.88 and 5.85, respectively) than expected for the corresponding published p*K* values (5.2 and 6.4, respectively) (Table S1). This could be due to the intramolecular interactions between carboxyl groups, ionic strength effects from the anions of each carboxyl group during titration, or possibly differences between the methods reported here and those used for determining the published p*K* values. The acidic p*K* of 4.2 from the BC model for ascorbic acid, however, was similar to the reported p*K* value of 4.1 (Lide, 1995). Previous work has shown that buffer models of monoprotic acids, including acetic acid and lactic acids, had p*K* values that were typically within 0.1 or units of the published p*K* values when adjusted for ionic strength effects (Breidt & Skinner, 2022; Price et al., 2020). Further research will be needed to clarify how titration and buffer modeling methods may influence differences between some (but not all) estimated p*K* values compared to published values.

3.4 | Estimation of pH and buffering from ingredient mixtures

In order to determine if BC models can be used to predict the pH and total buffering derived from ingredient mixture titrations, a combination of selected elderberry syrup ingredients was prepared in water using the concentrations in Table 2, including elderberry juice, ginger, cinnamon, cloves, and lemon juice. The BC curve from formulation one (F1) titration is shown in Figure 4a (solid lines). A model for F1 buffering was then generated using the combined buffer tables from the individual ingredients in F1 (F1 model, Table S2). The predicted BC curve is shown in Figure 4a (dashed lines). The pH from the F1 titration was 3.90, while the F1 model had an estimated pH of 4.04, a difference of 0.14 pH units. The *tBeta* values for the F1 titration data and F1 model were 17.34 and 18.98, respectively (Table S2). The F1 model showed some differences

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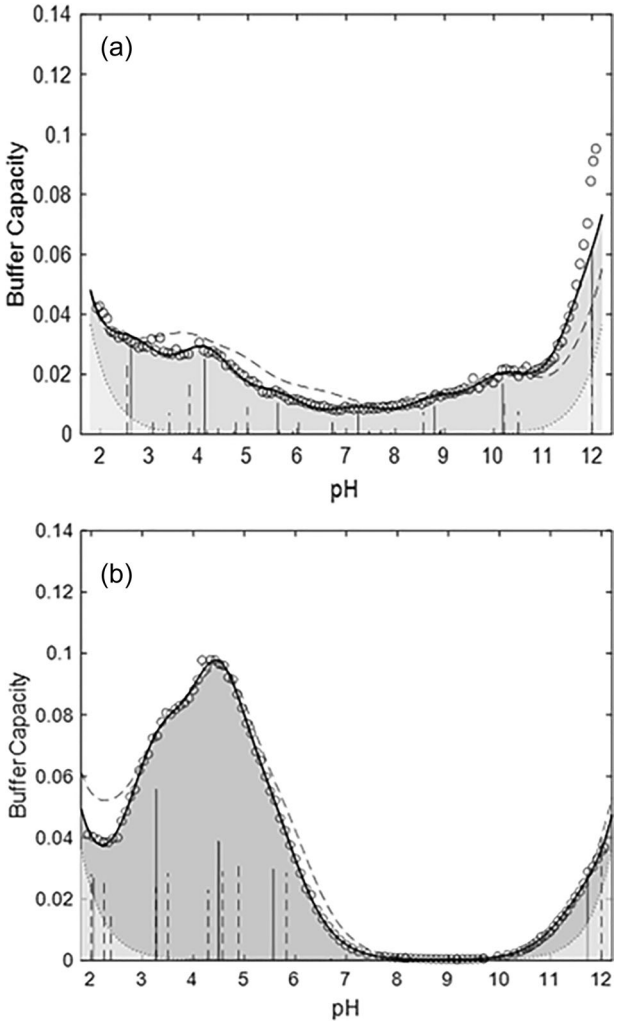

FIGURE 4 Buffer capacity (BC) models of elderberry syrup ingredient mixtures. BC models for the elderberry formulation F1 (a) and the acid mixture F2 (b) are shown. The circles and solid lines represent titration data, and the dashed lines represent predicted BC data from the combined ingredient buffer models. The dark shaded areas represent *tBeta*; the lightly shaded areas and dotted lines represent the buffer capacity of water. The vertical lines represent the individual monoprotic buffers for each mixture.

from the F1 titration data, including slightly greater buffering for the combined ingredient model between pH 3 and 7 (Figure 4a), which likely explained the pH and *tBeta* differences observed.

A titration of a mixture of citric, malic, and ascorbic acids (F2 titration, Table S2) in water using the formulation concentrations (Table 2) had a pH of 2.08 and a *tBeta* of 29.88. A model derived from the combined BC data for the individual acid titrations (F2 model, Table S2) had an estimated pH of 2.22 and a *tBeta* of 32.05. The F2 formulation thus had a 0.14 difference between the observed and predicted pH. The F2 titration and F2 model buffer curves are shown in Figure 4b (solid and dashed lines, respectively), revealing similar BC curves. Subsequently, a mixture of the F1 and F2 ingredients was titrated (F3 titration, Table S2), with a pH of 2.67 and a *tBeta* of 49.64. The F3 titration data were compared to three different models based on combined titration curves: F3 model 1, combining the buffers from the F1 and F2 titrations; F3 model 2, combining the buffers from the F1 titration data and the buffers from each acid titration; and F3 model 3, combining all the buffers from the individual ingredient titrations (Figure 5; Table S2). The F3 models 1–3 had pH values of 2.78, 2.77, and 2.88 and *tBeta* values of 48.18, 50.66, and 51.68, respectively. F3 models 1 and 2 were each within 0.11 pH units of the measured formulation pH of 2.67, while F3 model 3 was 0.215 pH units above the measured value (Table S2). With the exception of F3 model 3, which had 35 individual buffers that were used for calculating the pH, all other models with 24 buffers or less for F1, F2, and F3 had estimated pH values within 0.14 pH units of the measured values. The *tBeta* values for the three F3 titration models were all between 48.18 and 51.67 and approximated the sums of the *tBeta* values from the F1 and F2 models (*tBeta* of 51.03) or the sum of *tBeta* for the F1 and F2 titrations (*tBeta* of 47.22). These data indicate that *tBeta* for mixtures of ingredients was approximately additive.

3.5 | Estimating pH with mixed acid formulations

To determine the accuracy of pH predictions with elderberry juice and different malic, citric, or ascorbic acid combinations, 16 formulations of elderberry juice (37.5%) with varying concentrations of citric, malic, and ascorbic acids (up to 0.3%) were prepared (Table 3). The BC model from a titration of 37.5% elderberry juice was used to calculate estimated pH values using in silico combinations of the buffer tables for the juice and acids. The titrated juice at 37.5% had an estimated pH of 3.95 and a *tBeta* of 6.01 (Table S1). The formulations were chosen to have pH values between 3 and 4. The concentration of individual buffers in BC models from the original titration data for citric, malic, and ascorbic acids at 1% citric acid (52.1 mM), 0.75% malic acid (55.9 mM), and 0.75% ascorbic acid (42.6 mM) was adjusted based on the ratios of the original acid concentrations (from the titration) to the concentrations indicated in Table 3.

A BC curve from a titration of an acid solution of 0.3% of citric, malic, and ascorbic acids (15.6, 22.4, and 17.0 mM, respectively), as used in Mixture 1 from Table 3, was compared to the predicted BC curve derived from the adjusted buffer models for each acid (Table S3; Figure 6). The two models were similar, with observed and predicted pH values of 2.25 and 2.37 and *tBeta* values of 11.12 and 11.66,

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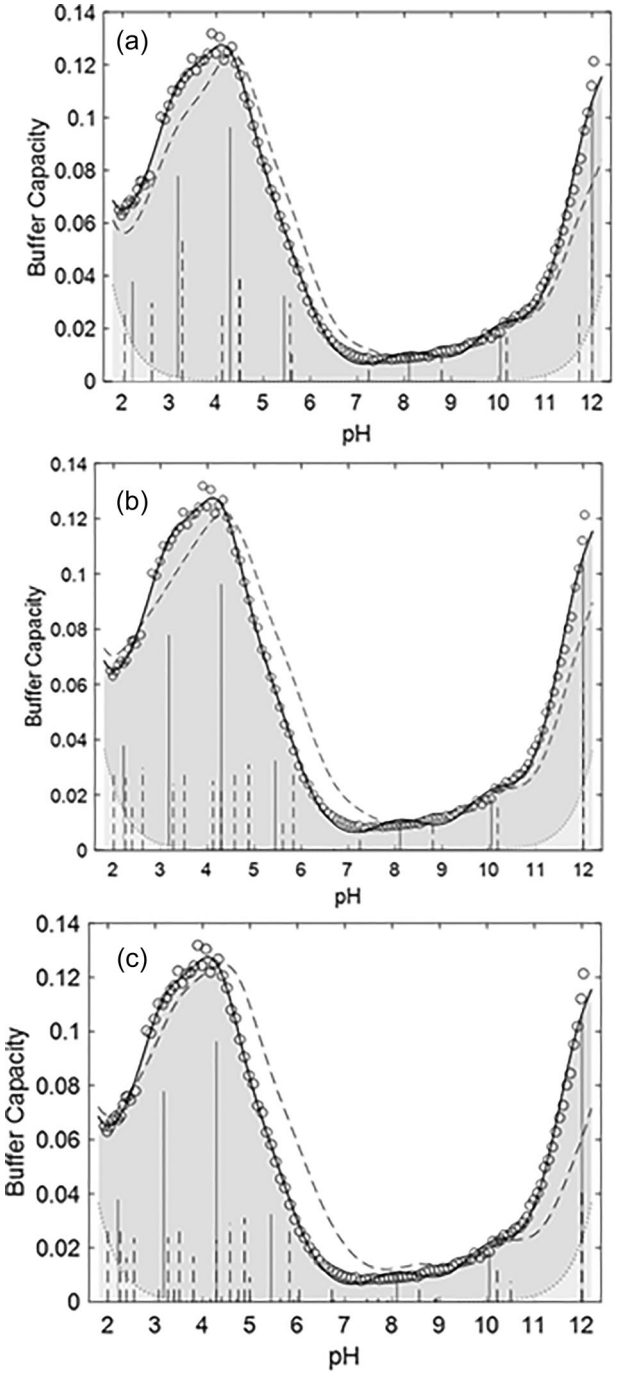

FIGURE 5 Buffer capacity (BC) models of elderberry syrup formulations. The combined BC models for the elderberry syrup formulations are shown. The circles and solid lines represent titration data from the combined syrup ingredients. The dashed lines represent the syrup model combined with the acid model, F3 model 1 (a); the syrup model combined with the individual acid models, F3 model 2 (b); and the combined model with all ingredients, F3 model 3 (c). The dark shaded areas represent *tBeta*; the lightly shaded areas and dotted lines represent the buffer capacity of water. The vertical lines represent the individual monoprotic modeled buffers for each mixture.

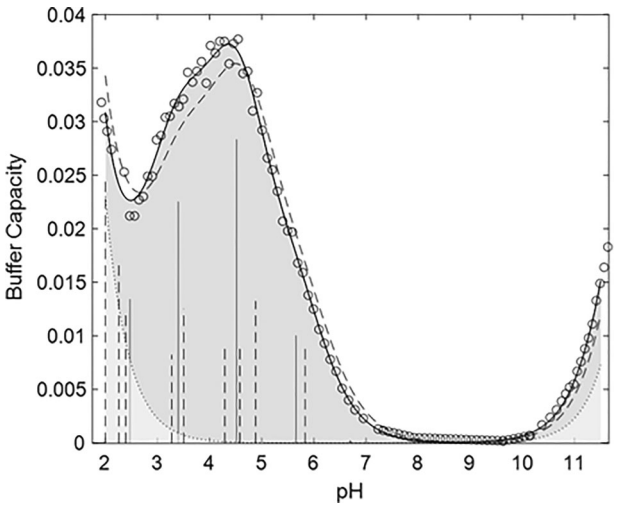

FIGURE 6 Buffer capacity (BC) models of a citric, malic, and ascorbic acid mixture. The BC model from a titration of a mixture of citric acid (0.31%), malic acid (0.32%), and ascorbic acid (0.29%) in water is shown (solid line) with the observed data (circles). The dashed lines represent the model generated by combining buffers for each acid diluted in silico from the original concentrations of citric acid (1%), malic acid (0.75%), and ascorbic acid (0.75%). The dark shaded areas represent *tBeta*; the dotted line and lightly shaded areas represent the buffer capacity of water. The vertical lines represent the individual monoprotic buffers from each model.

respectively (Table S3). The difference in pH (0.12 pH units) may be partially explained by the greater buffering observed for the combined acid model between pH 3 and 4 compared to the titration data. This difference was likely due to accumulated error from the summed buffering from the adjusted BC models of each acid.

The calculated pH values and the measured pH values for each elderberry juice–acid mixture are shown in Table 3. All estimated pH values were within 0.11 pH units of the measured pH values and greater than the observed values. This may be expected because the buffer model of the combined acids in water had a higher pH value than the predicted value (by 0.12 pH units), as described above. Regression analysis of the data (Figure 7) for the observed and predicted values had a slope of 0.91, an intercept of 0.3, and an R^2 value of 0.978. The RMSE was 0.076 pH units. However, if the predicted pH values were generated using the published p*K* values for each acid at the indicated concentrations, the slope, intercept, and R^2 values were 0.95, 0.09, and 0.995, respectively, with an RMSE of 0.045 pH units.

4 | CONCLUSIONS

Buffer models from titration data were found to be useful in estimating the pH of mixtures of acid and low-acid

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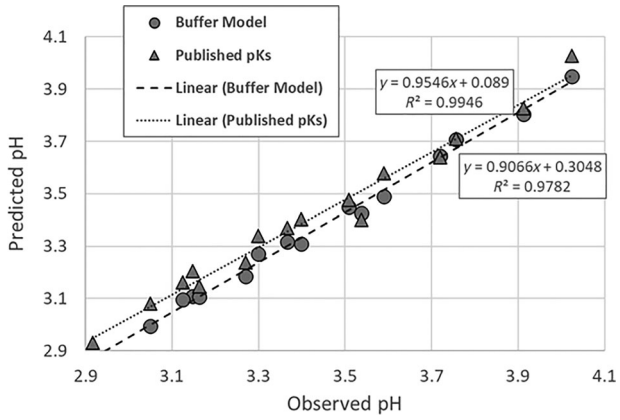

FIGURE 7 Observed and predicted pH for elderberry syrup and added acids. The triangles and dotted linear regression line show the observed and predicted pH for buffer capacity (BC) models using published acid p*K* values. The circles and dashed regression line show the observed and predicted pH for BC models using p*K* values from acid titration data. The inserts show the regression equation and corresponding *R*-squared value.

ingredients. The estimated pH from the F3 (model 3) buffer table was derived from a large number of estimated buffers, including the combined buffering of the individual ingredients ($n = 35$) in the formulation. All other BC models for ingredient mixtures had fewer individual buffers ($n \leq 24$) and had estimated pH values that differed from the measured values by 0.14 pH units or less. These data indicate that BC models may be useful for estimating pH of ingredient mixtures, although it is possible that the accuracy of pH estimations may decrease as the number of ingredients (and total number of ingredient buffers) increases.

The total buffering, expressed as *tBeta* of combined ingredients in elderberry syrup formulations (Figure 5; Table S2), was found to be additive, such as the sum of the *tBeta* from individual ingredients. The magnitude of *tBeta* may therefore be useful for determining the pH impact of ingredients or ingredient mixtures on the formulation pH and may also be useful for estimating pH stability for food products. Manufacturers of acid and acidified foods may use the buffer model data for food ingredients to quantitatively assess how acid or low-acid ingredients will influence pH. Ingredients with greater buffering may be less likely to have pH changes over time, but also may have a greater impact on the finished equilibration pH. Future work may include development of user-friendly software for estimating the pH of acid and acidified foods based on BC models of combined ingredients, to help ensure the safety of these products.

NOMENCLATURE

tBeta total buffering of a food ingredient

AUTHOR CONTRIBUTIONS

Nicholas Fragedakis: Conceptualization; resources; methodology; writing—review and editing. **Caitlin R. Skinner**: Investigation; methodology; writing—review and editing. **Mileah Shriner**: Investigation; writing—review and editing. **Mollie Ruinsky**: Investigation; writing—review and editing. **Seo Young Yang**: Investigation; writing—review and editing. **Robert P. Wine**: Investigation; writing—review and editing. **Lynette Johnston**: Conceptualization; supervision; writing—review and editing; project administration; resources. **Fred Breidt**: Conceptualization; methodology; software; writing—original draft; data curation; formal analysis; project administration; funding acquisition.

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CONFLICT OF INTEREST STATEMENT

The authors declare no conflicts of interest.

DATA AVAILABILITY STATEMENT

Supplementary data are available at USDA Ag Data Commons: (dataset) Breidt, Fred (unpublished). Elderberry syrup buffer modeling data. Ag Data Commons: https://data.nal.usda.gov/dataset/elderberry-syrup-buffer-modeling-data.

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