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Modelling pH Dynamics, SCOBY Biomass Formation, and Acetic Acid Production of Kombucha Fermentation Using Black, Green, and Oolong Teas

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Abstract: Kombucha is a traditional, fermented beverage made with an essential biomaterial known as SCOBY (symbiotic culture of bacteria and yeast). Three different tea types, namely black, green, and oolong, were compared in kombucha fermentation in terms of pH dynamics, the formation of SCOBY biomass, and the production of acetic acid. The rational, exponential, and polynomial models described pH dynamics with good fit, $R^2 > 0.98$. The formation of SCOBY biomass and the production of acetic acid were modelled using sigmoidal functions, with three-parameter logistic and Gompertz models and four-parameter Boltzmann and Richards models. The *F*-test indicated that the three-parameter models were statistically adequate; thus, the Gompertz model was modified to present the biological meaning of the parameters. The SCOBY biomass formation rates ranged from 7.323 to 9.980 g/L-day, and the acetic acid production rates ranged from 0.047 to 0.049% acid (wt/vol)/day, with the highest values from the non-conventional substrate, oolong tea. The correlations between pH and SCOBY biomass or acetic acid using polynomial models enable the prediction of product formation in kombucha processing.

Keywords: kombucha; fermentation; modelling; pH; SCOBY; biomass; acid



Citation: Chong, A.Q.; Chin, N.L.; Talib, R.A.; Basha, R.K. Modelling pH Dynamics, SCOBY Biomass Formation, and Acetic Acid Production of Kombucha Fermentation Using Black, Green, and Oolong Teas. *Processes* **2024**, *12*, 1301. <https://doi.org/10.3390/pr12071301>

Academic Editor: Hah Young Yoo

Received: 22 May 2024

Revised: 14 June 2024

Accepted: 19 June 2024

Published: 22 June 2024



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1. Introduction

Kombucha is a slightly sweet, acidic, and carbonated fermented beverage which has been consumed since 220 B.C. [1]. The global market for kombucha has experienced substantial growth in recent years, with a compound annual growth rate (CAGR) of 15.4% from 2017 to 2023, reaching USD 3.4 billion in 2023, and is anticipated to reach 17.1 billion USD by 2033, with a projected CAGR of 17.4% [2]. The underlying causes that have been spurring the kombucha boom encompass its multitude of potential health benefits, with in vitro and in vivo evidence of its antimicrobial, antioxidant, antiproliferative, anti-inflammatory, and anti-carcinogenic properties, along with its ability to improve intestinal microbiota, regulate nutrient absorption, supply energy, detoxify the body, boost the immune system, and aid in weight loss [1,3–7]. This is associated with the presence of phenolic compounds from tea, organic acids, vitamins, and microbial enzymes produced during fermentation [8,9]. The fermentation process begins with the infusion of sweetened black tea, followed by the incorporation of a biomass cellulose pellicle known as ‘SCOBY’, a symbiotic culture of bacteria and yeast [10]. This is often accompanied by the addition of starter tea, which is previously fermented kombucha, serving to create an acidified environment conducive to SCOBY growth and prevent potential contaminants [11]. SCOBY is a three-dimensional zoogloeal mat with a yellow–brown appearance that consists of acetic acid bacteria (AAB) and osmophilic yeast in a mutually beneficial symbiotic relationship [12,13]. The microbial composition of AAB commonly includes species belonging to *Acetobacter* (*A. aceti*, *A. pasteurianus*, *A. nitrogenifigens*), *Gluconobacter*

(*G. oxydans*), *Gluconacetobacter* (*G. sacchari*), and *Komagataeibacter* (*K. xylinus*, *K. kombuchae*, *K. europaeus*, *K. rhaeticus*, *K. saccharivorans*), while the yeast population typically includes strains of *Schizosaccharomyces* (*S. pombe*), *Zygosaccharomyces* (*Z. bailii*, *Z. lentus*, *Z. bisporus*, *Z. rouxii*, *Z. kombuchaensis*), *Saccharomyces* (*S. cerevisiae*), *Dekkera*, *Brettanomyces* (*B. bruxellensis*, *B. anomalus*), *Candida*, *Kluyveromyces*, and *Pichia* [8,14]. During fermentation, which usually takes place for a duration of 7–10 days at temperatures ranging from 18 to 30 °C [1], the combined action of AAB and yeast leads to both SCOBY biomass formation (Figure 1) and acid production [7]. The resulting acid contributes to the distinct flavour, aroma, and quality of kombucha, while SCOBY formation serves essential roles in propagating and improving the fermentation efficiency. The pH value acts as a key determinant in shaping these processes and the resulting characteristics of the product as it primarily favours the selective growth of specific microorganisms, regulates their activity, and affects their metabolic pathways [6,15]. Hence, it is a standard practice to assess the completion of kombucha fermentation by pH measurement [16] and to ensure it falls within the recommended range of 2.5–4.2, as established by food guidelines [17–19], in accordance with the Food and Drug Administration (FDA) Food Code Model [20], Pennsylvania Department of Agriculture (USA) [17], Centre of Disease Control of British Columbia (BCCDC) plan [21], and Normative Instruction (IN), Brazil [22].



Figure 1. Formation of SCOBY biomass during kombucha fermentation.

The outcome of kombucha fermentation is subject to the influence of numerous factors, which include variables such as the nature and composition of substrates, temperature, fermentation time, and microbial composition [7,14]. The choice of substrates—sugar as the primary source of carbon and tea as the primary source of nitrogen—influences the product yield during kombucha fermentation [12]. While research has examined the impact of various types and concentrations of carbon substrates, such as sucrose [23], molasses [24], glucose [25], brown sugar [26], white refined sugar, coconut palm sugar, and molasses [27], on key properties of kombucha fermentation, those that focus on the influence of nitrogen sources from different tea types are not known. Tea originates from a tea plant or tea shrub, *Camellia sinensis* (L.) Kuntze. The beverage can be produced from its leaves and buds [28,29]. Teas are classified as completely fermented (black tea), partially fermented (oolong tea), and non-fermented (green tea), which results in different compositions [29–31] in terms of polyphenols, amino acids, caffeine, volatile compounds, and minerals [17], which plausibly affect the microbial community and impact the product formation of kombucha fermentation [32]. For instance, certain phenolic compounds such as catechin and gallic acid have been studied for their ability to stimulate bacterial growth, which could eventually affect the fermentation outcomes [33–35]. Among the different tea types, kombucha has been traditionally made using black tea as it is claimed to be a good fermentation medium [36]. As green tea and oolong tea have been demonstrated to possess

higher levels of catechins, total polyphenolic content, and antioxidant activity, as well as greater efficacy in inhibiting pathogenic bacteria compared to black tea [37], these two teas may be a great alternative to black tea in kombucha production.

The modelling of kombucha fermentation enables closer monitoring of the fermentation dynamics during the production process. It also offers opportunities to enhance kombucha fermentation through a better understanding of changes in substrate composition and the quality of the final product. It helps to improve resource utilisation and provide information on microbiota functions and progression under various conditions [38]. The models enable better control of the fermentation factors and assist in process scale-up, process optimisation, and the prediction of fermentation outcomes. For example, modelling techniques have been applied to understand the changes in lactose during milk fermentation by kombucha starter [39], for scale-up of the kombucha fermentation process [40,41], to optimise the liquid fermentation process via cell growth improvement and SCOBY production [42], and to predict the substrate consumption and metabolite production rates in kombucha fermentation using fruit and herbal teas [43]. The widespread utilisation of modelling in numerous research studies shows the growing importance of modelling. Predictive modelling represents a promising area of food fermentation. The sigmoidal models are examples of predictive models that introduce varying degrees of parameter complexity and are commonly used to describe nonlinear relationships within data. For example, the logistic and Gompertz models feature three parameters, while the Boltzmann and Richards models are characterised by four parameters. Models that are capable of interpreting data with biological meaning are exceptionally helpful [44].

With fermented tea and SCOBY biomass emerging to have wider applications beyond food [45–48], such as in the cosmetic and dermatopharmaceutical [49], textile and fashion [50,51], electronic [52], bioelectronics [53], and biomedical industries [54,55], the kombucha fermentation process has received much attention and is in growing demand. This research aimed to model kombucha fermentation using different tea types, namely black, green, and oolong, through measuring pH dynamics, the formation of SCOBY biomass, and the production of acetic acid, and by investigating the relationships between them.

2. Materials and Methods

2.1. Materials

Black tea (Yellow Label Tea, Lipton, Unilever, Bekasi, Indonesia), green tea (Pure Green Tea, Lipton, Unilever, Hefei, China), oolong tea (Legend of Tea, Ipoh, Malaysia), and food-grade fine granulated sugar (Gula Prai, Malayan Sugar Manufacturing Company Berhad, Seberang Perai, Malaysia) were used in kombucha fermentation. SCOBY and starter tea were obtained from a local commercial source (Herbal Remedies, George Town, Malaysia). All chemicals used were of analytical grade.

2.2. Preparation of Kombucha

The kombucha fermentation process was adopted as described in previous studies with slight modifications [23,56], using clean and sanitised utensils to adhere to stringent standards of hygienic conditions. An amount of 1 L of water was heated, and 9% (*w/v*) sugar was added. After the sugar dissolved totally, 0.6% (*w/v*) tea leaves were incorporated and steeped for 10 min. After removing the tea leaves, the solution was cooled to room temperature (29 °C). SCOBY (3% *w/v* on a wet weight basis) and starter tea (10% *v/v*) with pH 2.7 were added to the cooled tea. The beaker was then covered with a clean paper towel secured by a rubber band. The fermentation process was conducted in triplicates over a period of 10 days in a dark environment at a room temperature of 29 °C, where the pH of the fermenting solution was regularly measured using a pH meter (Milwaukee MW-101, Milwaukee Instruments, Rocky Mount, NC, USA) calibrated at pH 4.0 and 7.0.

2.3. Analytical Techniques

SCOBY biomass measurement was performed through regular harvesting. After carefully removing the pellicle of SCOBY formed on the tea's surface, excess moisture was eliminated using paper towels, and the wet weight of SCOBY was recorded, which was expressed in g/L [57,58]. The SCOBY biomass formation was determined by calculating the difference in wet weight before and after the harvesting period.

For acetic acid analysis, 10 mL of sample was extracted daily from the fermentation mixture. The amount of acetic acid was measured by acid-base titration method, with 0.1 M NaOH using phenolphthalein as an indicator [56,57]. The acetic acid concentration was calculated by subtracting the initial acidity from the acidity measurements throughout the fermentation process. The acidity in the fermented kombucha tea was expressed as percent acetic acid using the formula [59]:

$$\% \text{acid (wt/vol)} = \frac{(N)(V_1)(Eq.wt.)}{(V_2)(1000)} \times 100 \quad (1)$$

where N is the normality of titrant, NaOH (mEq/mL), V_1 is the volume of titrant (mL), $Eq. wt.$ is the equivalent weight of predominant acid (60.05 mg/mEq), V_2 is the volume of sample (mL), and 1000 is the factor relating mg to g (mg/g).

2.4. Modelling Study

Table 1 shows all equations used for modelling as a function of time. pH dynamics were modelled using the exponential, polynomial, and rational equations, while SCOBY biomass formation and acetic acid production were modelled using sigmoidal functions, i.e., the logistic, Gompertz, Boltzmann, and Richards equations. All models were fitted by applying the generalised reduced gradient (GRG2) nonlinear optimisation algorithm using solver function in Microsoft Office Excel 365 (Microsoft Corporation, Redmond, WA, USA), with the parameters estimated by minimisation of sum of quadratic differences between observed and model-predicted values to determine the coefficients, including those in the modified Gompertz model [60]. The coefficient of determination (R^2) and residual sum of squares (RSS) between predicted and experimental data were calculated to assess the fitness of the models.

Table 1. Equations used in mathematical modelling against time.

Model	Equation	References
pH dynamics		
Exponential	$y = a \exp(-bt) + c$	[61]
Polynomial	$y = a + bt + ct^2 + dt^3$	[62]
Rational	$y = \frac{a+bt}{1+ct+dt^2}$	[62]
SCOBY biomass formation and acetic acid production		
Logistic	$y = \frac{a}{[1+\exp(b-ct)]}$	[44]
Gompertz	$y = a \exp[-\exp(b-ct)]$	[44]
Boltzmann	$y = \frac{a-b}{1+\exp[(t-c)/d]} + b$	[63]
Richards	$y = \frac{a}{(1+\exp^{b-ct})^{1/d}}$	[62]
Modified Gompertz	$y = A \exp[-\exp(\frac{\mu e}{A}(\lambda - t) + 1)]$	[44]

a , b , c , and d are coefficients; t is the time (days); y is pH, amount of SCOBY biomass (g/L), or acetic acid (% acid (wt/vol)); A is the upper asymptote value; μ is the specific formation rate of SCOBY (g/L-day) or the specific production rate of acetic acid (% acid (wt/vol)/day); λ is the lag phase duration (days); and e is $\exp(1)$.

The model fitting performance was compared and validated for SCOBY biomass formation and acetic acid production using the F -test to test if additional parameter does improve modelling fitting significantly [44,64,65]. The lowest RSS values were from the

Boltzmann model. Hence, it was used as reference RSS_1 , while RSS_2 are those from the three-parameter models to calculate the F -values:

$$f = \frac{(RSS_2 - RSS_1)/(DF_2 - DF_1)}{RSS_1/DF_1} \quad (2)$$

tested against $F_{DF_1}^{DF_2-DF_1}$,

DF_1 represents the number of degrees of freedom, where, in the Boltzmann model, $n - 4$, and DF_2 is from the three-parameter models, $n - 3$.

The Gompertz model (3) was modified to develop an expression for the biological parameters following [44].

$$y = a \exp[-\exp(b - ct)] \quad (3)$$

To obtain the inflection point of the curve, the second derivative is calculated:

$$\frac{dy}{dt} = ac \cdot \exp[-\exp(b - ct)] \cdot \exp(b - ct) \quad (4)$$

$$\frac{d^2y}{dt^2} = ac^2 \cdot \exp[-\exp(b - ct)] \cdot \exp(b - ct) \cdot [\exp(b - ct) - 1] \quad (5)$$

At the inflection point, where $t = t_i$, the second derivative (5) is equal to zero:

$$\frac{d^2y}{dt^2} = 0 \rightarrow t_i = \frac{b}{c} \quad (6)$$

By substituting $t_i = \frac{b}{c}$, the specific formation/production rate, denoted as μ , is derived by calculating the first derivative (4) at the inflection point:

$$\mu = \left(\frac{dy}{dt} \right)_{t_i} = \frac{ac}{e} \quad (7)$$

From Equation (7), the parameter c in the Gompertz equation is written as:

$$c = \frac{\mu e}{a} \quad (8)$$

The description of the tangent line through the inflection point is:

$$y = \mu t + \frac{a}{e} - \mu t_i \quad (9)$$

The lag phase duration, λ , is defined as the t -axis intercept of the tangent through the inflection point:

$$0 = \mu \lambda + \frac{a}{e} - \mu t_i \quad (10)$$

Using Equations (6), (7), and (10) yields:

$$\lambda = \frac{b - 1}{c} \quad (11)$$

From Equations (8) and (11), the parameter b in the Gompertz equation is written as:

$$b = \frac{\mu e}{a} \cdot \lambda + 1 \quad (12)$$

The asymptotic value is reached for t approaching infinity:

$$t \rightarrow \infty : y \rightarrow a \Rightarrow A = a \quad (13)$$

The parameter a in the Gompertz equation is substituted by A , yielding the modified Gompertz equation:

$$y = A \exp \left[- \exp \left(\frac{\mu e}{A} (\lambda - t) + 1 \right) \right] \quad (14)$$

where y is the amount of SCOBY biomass (g/L) or acetic acid (% acid (wt/vol)) at time t , A is the upper asymptote value, μ is the specific formation rate of SCOBY (g/L-day) or specific production rate of acetic acid (% acid (wt/vol)/day), λ is the lag phase duration (days), and t is the time (days).

Finally, data on the tea type for kombucha fermentation in terms of SCOBY biomass formation and acetic acid production and their correlations with pH dynamics were developed for prediction purposes using nonlinear regression approach of third order polynomial equation.

3. Results and Discussion

Figure 2 displays the pH profiles of kombucha fermentation using three different tea types, measured throughout the fermentation period of 10 days. The pH dynamics observed during kombucha fermentation with black, green, and oolong teas were the same, with pH levels dropping from 3.19–3.27 to 2.72–2.79. The pH decreased drastically at the beginning phase of fermentation, which is consistent with the observations of other authors [66,67], showing a pH drop between 0.29 and 0.32 units over the first three days of fermentation. The availability of nutrients stimulates the metabolic activity of microorganisms in SCOBY, resulting in higher acid production as they convert nutrients in the substrates [26]. The fermentable sucrose is rapidly used by yeast, where it is broken down into glucose and fructose. Subsequently, these simple sugars are further metabolised into organic acids by AAB, thereby contributing to the higher acid production and lower pH. However, as fermentation proceeds, the pH change becomes less drastic, with a decrease in pH levels of 0.06–0.08 units observed from day 4 to day 8, showing a decreasing rate of acid production. A slower pH drop may be related to the buffer effect arising from the reactions between synthesised organic acids and minerals from the substrate [24,68]. The decreasing trend of pH approached a straight line, indicating a more stable pH level and suggesting the completion of the fermentation process. Oolong tea kombucha consistently exhibited the lowest pH trend based on Figure 2, reaching a final pH value of 2.72, implying that it undergoes a more pronounced and extensive fermentation process, resulting in a greater amount of generated acid compared to other types of tea. The higher acidity can impart a tangier or sharper taste profile to the final product. The reduction in pH throughout fermentation is beneficial, particularly for polyphenols, which are known to be pH-sensitive, as it helps in preventing their chemical degradation [69,70]. Lower pH levels (pH 3 and below) provide higher stability for catechins [71], which contributes to their preservation and potential health benefits. It was stated that the beneficial properties of kombucha are primarily due to its acidic composition [72]. The acidic environment (pH < 4.5) is also vital for microbiological safety as it stops or severely curtails the growth of pathogenic microorganisms [19]. All kombucha samples maintained pH ranging from 2.5 to 4.2, which was within the acceptable levels, throughout a 10-day fermentation period [17–19], thus supporting the duration of fermentation chosen in terms of suitability and adequacy. Prolonged fermentation could lead to the excessive acidification of the fermented tea due to the continuous metabolism of AAB, which could compromise the health-promoting properties of fermented tea and potentially have detrimental effects on consumers' health [73]. Exponential, polynomial, and rational models were used to describe pH dynamics because these models offer high flexibility and are capable of accommodating a vast variety of patterns [74]. All mathematical models have described pH trends against time effectively with $R^2 > 0.98$, specifically the rational model with the highest R^2 values of 0.99. The R^2 values greater than 0.90 from the fitting of the mathematical models show that the models selected are suitable in explaining high fractions of total variation [65,75],

i.e., whereby the responses are well explained by the factors such that their relationship is strong. The estimated coefficients are listed in Table 2.

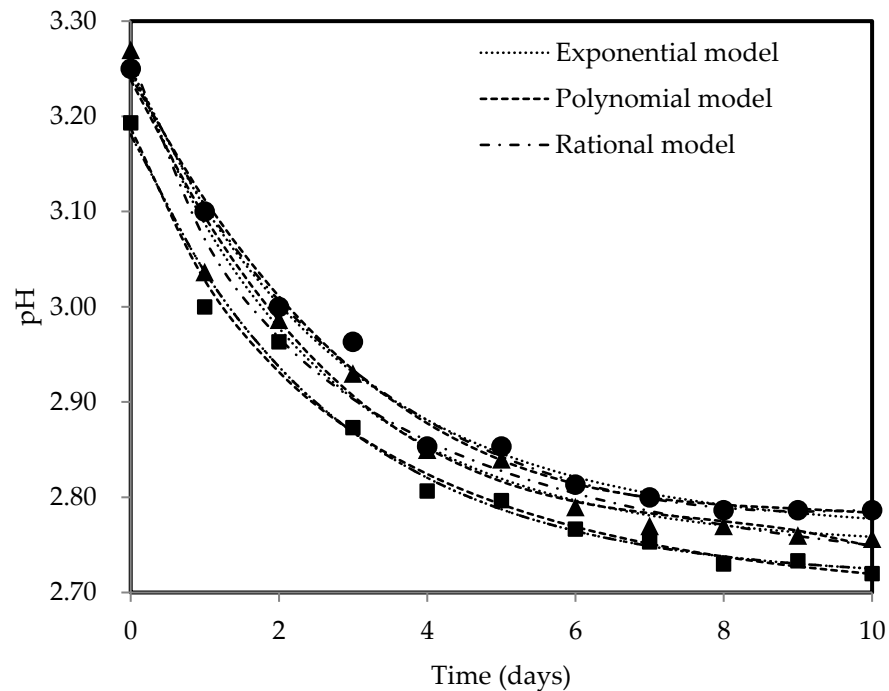


Figure 2. pH profiles for black (●), green (▲), and oolong (■) tea kombucha fermentation with exponential, polynomial, and rational models.

Table 2. Estimated parameters for exponential, polynomial, and rational models of pH dynamics of different kombucha teas.

	<i>a</i>	<i>b</i>	<i>c</i>	<i>d</i>	<i>R</i> ²
Exponential model					
Black tea	0.486	0.354	2.763	-	0.99
Green tea	0.500	0.390	2.748	-	0.98
Oolong tea	0.468	0.368	2.713	-	0.99
Polynomial model					
Black tea	3.242	-0.145	0.016	-0.001	0.99
Green tea	3.238	-0.163	0.020	-0.001	0.98
Oolong tea	3.175	-0.151	0.019	-0.001	0.99
Rational model					
Black tea	3.248	0.298	0.143	-0.002	0.99
Green tea	3.262	1.096	0.419	-0.002	0.99
Oolong tea	3.187	0.778	0.310	-0.001	0.99

The changes in SCOBY biomass and acetic acid show a sigmoidal pattern over time, with both products increasing with fermentation period for all tea types. Figures 3 and 4 present the fitted models of SCOBY biomass formation and acetic acid production, with reasonably good fits of *R*² and RSS values reported in Table 3. The *R*² values higher than 0.90 indicated that the models used are reliable for data prediction purposes when describing process and product changes. The three-parameter models (Gompertz and logistic models) yielded higher RSS values in comparison to the four-parameter models (Boltzmann and Richards models). The measurement error for the computation of *F*-values using the lowest RSS values from the Boltzmann model showed statistical validation of the *F*-ratio test accepting the null hypothesis, *H*₀, where the addition of an extra parameter did not significantly improve model fitting, as presented in Table 4's calculated *F*-values, which were lower than the *F*-table value. As such, the three-parameter models, which are simpler,

were accepted, adhering to the principle of Occam’s razor, favouring a simpler model with acceptable fit [76]. Both Erkmen and Alben [64] and Zwietering et al. [44] recommended a three-parameter model over a four-parameter model due to its simplicity, ease of use, greater stability, and higher degree of freedom in the estimates, which is particularly essential when analysing growth curves with limited measured points. Specifically, the three-parameter model can be interpreted to give a biological meaning to its parameters as compared to the four-parameter model, which presents a challenge in terms of explaining the fourth parameter, a shape parameter, biologically [44,64].

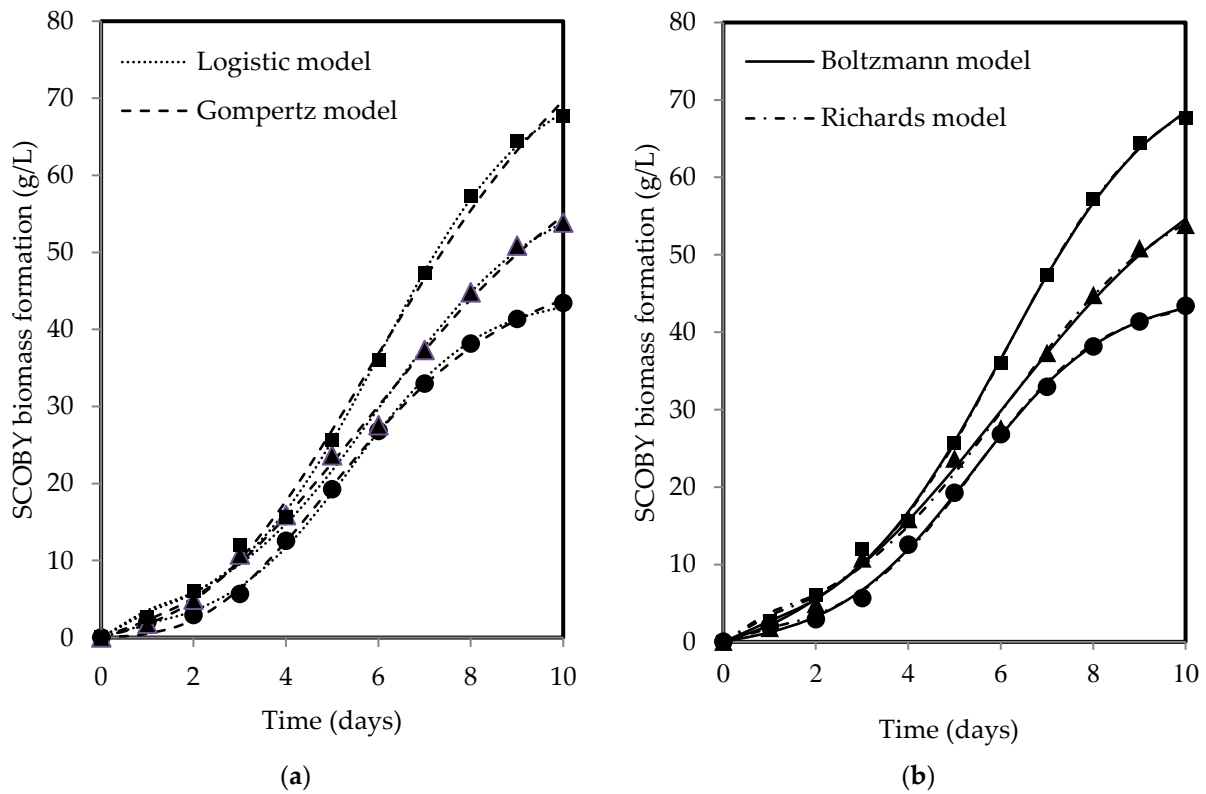


Figure 3. (a) The logistic and Gompertz and (b) Boltzmann and Richards models fitted to describe SCOBY biomass formation during kombucha fermentation using black (●), green (▲), and oolong (■) teas.

Table 3. R^2 and RSS values from models fitting for SCOBY biomass formation and acetic acid production for three tea types of kombucha fermentation.

Models	R^2			RSS		
	Black	Green	Oolong	Black	Green	Oolong
SCOBY biomass formation						
Logistic	0.999	0.995	0.999	3.747	20.305	9.833
Gompertz	0.999	0.997	0.996	3.949	12.024	22.394
Boltzmann	0.999	0.998	0.999	2.526	9.641	8.384
Richards	0.999	0.996	0.999	2.972	10.618	6.438
Acetic acid production						
Logistic	0.996	0.987	0.991	0.0003	0.0009	0.0007
Gompertz	0.997	0.981	0.990	0.0002	0.0014	0.0007
Boltzmann	0.997	0.987	0.991	0.0002	0.0009	0.0006
Richards	0.996	0.987	0.991	0.0002	0.0009	0.0006

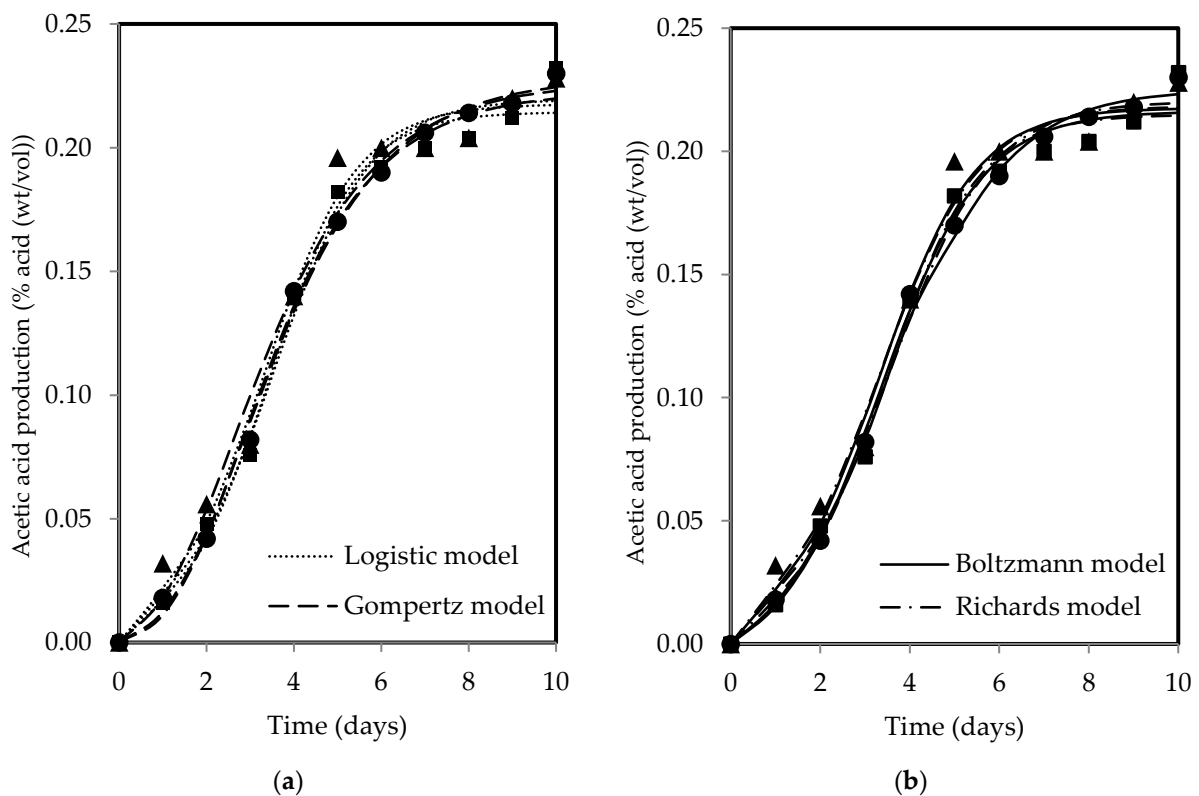


Figure 4. (a) The logistic and Gompertz and (b) Boltzmann and Richards models fitted to describe acetic acid production during kombucha fermentation using black (●), green (▲), and oolong (■) teas.

Table 4. *F*-values of the models.

Models	SCOBY Biomass			Acetic Acid		
	Black	Green	Oolong	Black	Green	Oolong
Logistic	1.933	4.425	0.692	2.638	0.010	0.023
Gompertz	2.255	0.989	6.684	0.239	1.847	0.358

F-table value at $\alpha = 0.05$ is 7.709.

The Gompertz model was modified to ensure its parameters are biologically meaningful, making it more informative. Table 5 shows the values of the biological parameters determined from fitting of the modified Gompertz model. The type of tea exerted an influence on the resultant products, highlighting its role as an essential nutrient supply in kombucha fermentation. This is particularly pivotal as SCOBY cannot produce adequate cellulose independently [12] while also crucially modulating the metabolic activities for acid production. Using black, green, and oolong teas, the formation rates of SCOBY biomass and the production rates of acetic acid in kombucha fermentation were 7.323–9.980 g/L-day and 0.047–0.049% acid (wt/vol)/day, respectively. The oolong tea kombucha exhibited the highest levels of both SCOBY biomass formation and acetic acid production rates when compared to the black and green teas. This suggests that the fermentation process with oolong tea was more effective, as higher product formation rates not only serve as a key indicator of fermentation progress but also reflect a more conducive microbial environment for efficient metabolic processes. This is because microorganisms function most effectively only when they are provided with the right and optimal conditions, such as available nutrient content, appropriate pH, and favourable temperatures for metabolising their substrates [77]. However, the exact reasons for deviations in fermentation dynamics among different tea types remain unclear because of the complexity of compounds such as polyphenols and their biotransformation, which resemble a metabolic labyrinth driven by

microorganisms [78,79]. The microbes responsible for SCOBY biomass formation include *Komagataeibacter xylinus*, *Komagataeibacter rhaeticus*, and *Komagataeibacter hansenii* [47,80], while acid production is attributed to AAB and yeasts such as *Brettanomyces bruxellensis* and *Zygosaccharomyces bailii* [81].

Table 5. Parameters of modified Gompertz model: SCOBY biomass formation and acetic acid production rate (μ), lag phase duration (λ), and upper asymptote value (A).

Parameters	Black	Green	Oolong
SCOBY biomass formation			
μ (g/L-day)	7.323	7.393	9.980
λ (day)	2.313	1.935	2.308
A (g/L)	49.759	74.807	92.870
Acetic acid production			
μ (% acid (wt/vol)/day)	0.048	0.047	0.049
λ (day)	1.126	0.871	1.164
A (% acid (wt/vol))	0.229	0.227	0.223

Unlike the Gompertz model, the modified Gompertz model presented parameters which can be linked to the physical process, such as μ representing the formation and production rates of products when describing the growth dynamics of the kombucha fermentation process. This is especially valuable in light of the increasing demand for SCOBY biomass in various industries and the critical role of acid production in flavour development, safety, and quality control. SCOBY biomass has received growing interest beyond kombucha fermentation because of its unique qualities of microfibrillar nanostructure, high mechanical strength, high elasticity, thermal stability, excellent biological affinity, high liquid absorption capacity, biodegradability, high crystallinity, and high degree of polymerisation [82–85], all of which are highly valuable for industrial applications. Unlike plant cellulose, SCOBY biomass does not require extensive pre-treatment as it contains cellulose, which does not have lignin, hemicellulose, pectin, arabinose, and other plant-derived components [82,85,86]. The purification of plant cellulose requires the use of chemicals such as sodium hydroxide and sulfuric acid which increase biological oxygen demand (BOD) and chemical oxygen demand (COD) in aquatic ecosystems, contributing to environmental pollution [82,86]. Hence, promoting the productivity of SCOBY biomass is helpful in offering an environmentally friendly, sustainable, and renewable alternative while also tapping into the potential for additional profits.

Figure 5 presents a correlation that allows for the prediction of SCOBY biomass formation and acetic acid production from pH with best-fit polynomial models, Equations (14) and (15), respectively. SCOBY biomass formation and acetic acid production decreased with pH increase, showing negative correlations and suggesting that microorganisms responsible for the fermentation process are less active at higher pH levels. This is consistent with earlier research, which indicated that elevating pH results in the diminution of SCOBY biomass formation [25]. Along with the decline in production, there was an emergence of mouldy growth and other contaminants when the pH was increased to 5 [25]. At pH 6, the only observation was the presence of mould growth embedded within the nanofibrils, with no discernible formation of SCOBY [25]. Typically, AAB is known to grow well within a pH range of 5.0–6.5 [87]. However, owing to the symbiotic relationship with other microorganisms present, it adapts more effectively to lower pH levels in kombucha fermentation [38]. Hence, AAB in kombucha showed the capability to thrive and generate SCOBY biomass at pH 3.0 or lower as fermentation progressed [23,25,67]. The count of AAB also increased consistently under acidic conditions, which increased the production efficiency of SCOBY biomass [15,38]. These bacteria use ethanol generated by yeast cells as a substrate for acetic acid production. The acetic acid produced by AAB, in turn, has been shown to stimulate yeast activity in ethanol production, demonstrating their synergistic

growth and thereby facilitating SCOBY biomass formation [25,88,89]. The changes in hydrogen ion concentration from the acids not only inhibit the undesirable growth of microbial contaminants but also influence the growth of fermenting microorganisms and the formation of products. Therefore, monitoring pH levels is significant for quality assurance, as deviations from the optimal range may lead to the reduced efficiency of fermentation. The primary corrective action if the pH exceeds the limit is to continue the fermentation process and remeasure the pH [18]. If the pH does not drop to 4.2 or below within seven days, the fermentation temperature is likely too low or the culture is contaminated, and it should be discarded [18]. Alternatively, if the pH drops below the specified limit, the high acidity should be diluted by adding freshly brewed tea.

$$\text{SCOBY biomass} = -7.48(\text{pH})^3 + 65.47(\text{pH})^2 - 191.01(\text{pH}) + 185.79, R^2 = 0.98 \quad (15)$$

$$\text{Acetic acid} = 8.53(\text{pH})^3 - 72.063(\text{pH})^2 + 202.08(\text{pH}) - 187.87, R^2 = 0.97 \quad (16)$$

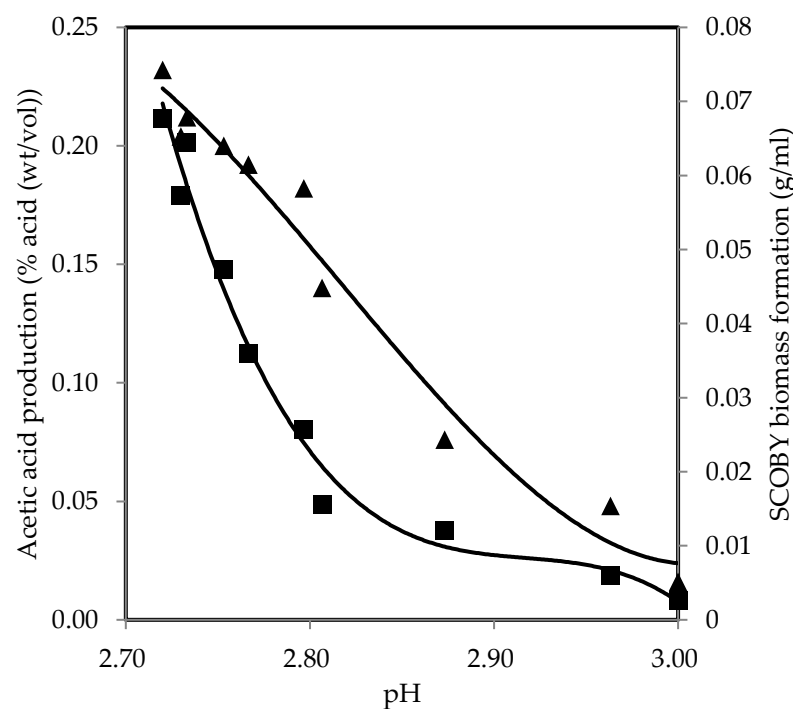


Figure 5. SCOBY biomass formation (■) and acid production (▲) as a function of pH values during oolong tea kombucha fermentation.

4. Conclusions

Modelling was used to evaluate the impact of different tea types—black, green, and oolong—on pH, SCOBY biomass formation, and acid production in kombucha fermentation. With pH decreasing significantly and being well described using rational, exponential, and polynomial models, the products of SCOBY biomass and acetic acid increased throughout the course of fermentation. The sigmoid curve models—logistic, Gompertz, Boltzmann, and Richards—were all well fitted to describe the products adequately. From the modified Gompertz model, the SCOBY biomass formation and acetic acid production rates were the highest in non-conventional kombucha using oolong tea at 9.980 g/L-day and 0.049% acid (wt/vol)/day, respectively, compared to green tea at 7.393 g/L-day and 0.047% acid (wt/vol)/day, respectively, and black tea at 7.323 g/L-day and 0.048% acid (wt/vol)/day, respectively. With pH used as the main monitoring parameter in kombucha processing, the developed polynomial models correlating to product formation provide avenues for estimating product formation for production needs. The present study enabled the prediction of pH, SCOBY biomass formation, and acid production with different models, which

is useful for improving the quality of kombucha fermentation and providing information on how the process can be made efficient. Future research should take into account the effects of other factors such as temperature, substrate concentration, the surface area of fermentation media, and microbial composition.

Author Contributions: Investigation, methodology, formal analysis, writing—original draft preparation, A.Q.C.; writing—review and editing, N.L.C.; supervision, N.L.C., R.A.T., and R.K.B. All authors have read and agreed to the published version of the manuscript.

Funding: This research received no external funding.

Data Availability Statement: Data are contained within the article.

Acknowledgments: This research did not receive any specific grant from funding agencies in the public, commercial, or not-for-profit sectors. A.Q.C acknowledges financial support from Universiti Putra Malaysia through Graduate Research Fellowship for her course in UPM.

Conflicts of Interest: The authors declare no conflicts of interest.

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