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Effect of fermentation time on physicochemical and sensory quality of kombucha tea

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Abstract
While industrial soft-drink consumption continues to rise, a contrasting trend has emerged: fermented beverages like kombucha are gaining popularity among health-conscious consumers who value probiotic content and low sugar. This research investigated how fermentation duration (0, 5, 10, 15, and 20 days) influenced the physicochemical properties, organic acid profile, microbial dynamics, and sensory acceptability of kombucha prepared from black tea using a Symbiotic Culture of Bacteria and Yeast (SCOBY) at the Bogor Institute of Agricultural Technology, Bogor, West Java during February-March 2023. A single-factor completely randomised design with four replications was adopted. As fermentation progressed, pH declined from 6.8 to 2.7, total acidity rose from 0.08 to 1.28%, total sugars dropped from 10.1 to 3.2%, and total phenolic content increased from 84 to 213 mg GAE L^{-1}. Acetic acid was the dominant organic acid (42.1% of total), followed by glucuronic acid (28.3%). Sensory evaluation showed that 10-day kombucha scored highest for overall acceptability (8.1 on a 9-point scale), offering a balanced sweet-sour profile. Fermentation beyond 15 days produced an excessively vinegary taste that panellists rejected. Ten days appears to be the optimum fermentation window for a palatable, antioxidant-rich kombucha from black tea under Bogor's ambient conditions.

Keywords: Fermentation time, physicochemical properties, kombucha, SCOBY, organic acids, sensory quality, black tea, antioxidant, total phenolics, probiotic beverage

Introduction
Kombucha is a mildly acidic, effervescent drink produced by fermenting sweetened tea with a symbiotic culture of acetic acid bacteria (mainly *Komagataeibacter xylinus*) and osmophilic yeasts (*Saccharomyces* spp., *Zygosaccharomyces* spp.) [2]. In contrast to most commercial soft drinks, which rely on added carbonation and artificial flavours, kombucha derives its fizz and flavour from microbial metabolism a distinction that appeals to consumers seeking 'living' beverages [3].

The fermentation time is the single most controllable variable governing the chemical and sensory character of the final product. Short fermentation yields a sweet, tea-like drink low in organic acids, while prolonged fermentation produces a sour, vinegar-like liquid unsuitable for direct consumption [4]. Published recommendations for fermentation duration vary widely from 7 to 21 days depending on temperature, tea type, sugar concentration, and SCOBY activity [5, 6]. Little work has been done to systematically map these changes for black tea kombucha prepared under the warm, humid conditions of West Java, Indonesia.

This research tracked physicochemical and sensory parameters at five-day intervals over a 20-day fermentation to identify the duration that maximises both nutritional value and consumer acceptability.

Preparation and fermentation protocol
Commercial Java CTC black tea (Tong Tji) was brewed at 1% (w/v) in boiling filtered water for 10 minutes and strained. Sucrose (food grade) was dissolved at 10% (w/v) while the tea was still warm. After cooling to 30 °C, the sweetened tea was transferred to sterilised wide-mouth glass jars (2 L capacity). A mature SCOBY pellicle (approximately 150 g) along with 200 mL of previously fermented kombucha (starter liquid) was added to each jar [7]. Jars were covered with muslin cloth secured by rubber bands to allow air exchange while

excluding insects. Fermentation proceeded at 28 ± 2 °C (ambient temperature in the laboratory during February-March 2023). Samples were drawn aseptically at 0, 5, 10, 15, and 20 days for analysis [8].

Material and Methods

Material

The research was conducted at the Food Microbiology and Hygiene Laboratory, Bogor Institute of Agricultural Technology, Bogor (6.59°S, 106.80°E). The SCOBY culture was obtained from the Department of Microbiology, Bogor Agricultural University, and maintained through weekly sub-culturing in sweetened black tea. Folin-Ciocalteu reagent, gallic acid standard, and DPPH (2,2-diphenyl-1-picrylhydrazyl) were procured from Sigma-Aldrich, Singapore. HPLC-grade standards for acetic, glucuronic, lactic, and citric acids were from Merck Indonesia [9, 10].

Methods

pH was measured with a calibrated digital meter (Eutech pH 700). Total titratable acidity was determined by titration against 0.1 N NaOH using phenolphthalein indicator and expressed as % acetic acid [11]. Total sugars were estimated by the phenol-sulphuric acid method. Total phenolic content was measured by the Folin-Ciocalteu assay and expressed as mg gallic acid equivalents (GAE) L^{-1} [12]. Antioxidant activity was assessed by the DPPH radical scavenging assay. Organic acid profiling was done by HPLC (Shimadzu LC-20A, C18 column, 0.01 N H_2SO_4 mobile phase, UV detection at 210 nm) [13]. Viable counts of acetic acid bacteria and yeasts were determined on glucose-yeast extract-$CaCO_3$ agar and YM agar, respectively. Sensory evaluation used a 15-member trained panel scoring colour, aroma, taste, effervescence, and overall acceptability on a 9-point hedonic scale [14]. Data were analysed by one-way ANOVA; means compared by Duncan's test at $p \leq 0.05$.

Quality control

The pH meter was calibrated daily with buffers at pH 4.0 and 7.0. HPLC columns were flushed with mobile phase for 30 minutes before each run, and a mixed organic acid standard was injected at the start and end of each batch. All microbiological media were sterility-checked. Sensory sessions were held between 10:00 and 11:30 AM in a well-lit, odour-free room [15].

Results

Table 1: Ranking of fermentation durations by overall sensory acceptability score

Rank	Ferm. day	Overall score	Colour	Taste	Effervescence
1	10	8.1	7.8	8.2	7.6
2	15	6.4	7.1	6.0	7.2
3	5	6.2	7.4	5.8	5.1
4	0	5.3	7.6	4.2	2.8
5	20	4.1	5.8	3.6	7.8

Table 2: Physicochemical changes in kombucha at different fermentation periods

Day	pH	Acidity (%)	Total Sugar %	Phenolics (mg GAE/L)	DPPH (% inhib.)	Cellulose (g/L)
0	6.80	0.08	10.1	84	21.3	0
5	4.62	0.34	7.4	128	38.7	1.8
10	3.41	0.71	5.1	183	54.2	4.3
15	3.02	1.02	3.8	207	61.8	6.1
20	2.73	1.28	3.2	213	63.4	7.8
CD ($p \leq 0.05$)	0.18	0.06	0.43	11.2	3.8	0.47

pH fell sharply during the first 10 days (from 6.8 to 3.4) and then declined more slowly. Total sugars were consumed at an average rate of 0.5% per day in the first 10 days as yeasts converted sucrose to glucose and fructose, which acetic acid bacteria further oxidised to organic acids (Table 2). Phenolic content nearly doubled by day 10, likely through microbial biotransformation of tea catechins, and plateaued thereafter. The 10-day sample struck the best balance: sufficient acidity for tartness, enough residual sugar for body, and high phenolic content without the harsh vinegar note of later samples.

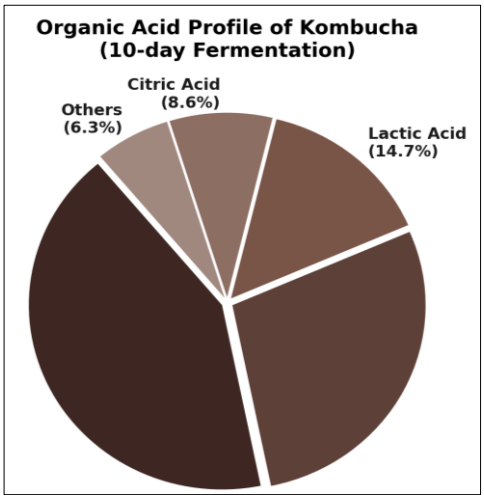

Fig 1: Organic acid composition of kombucha at 10 days of fermentation

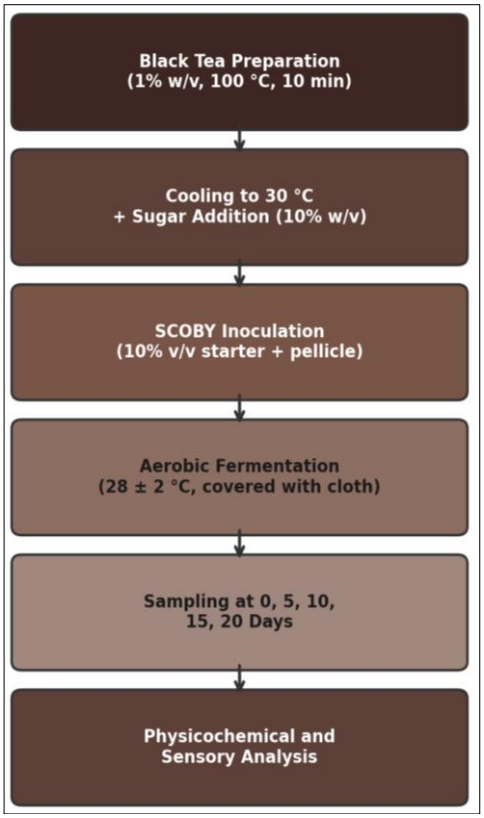

Fig 2: Flowchart of the kombucha preparation and sampling protocol

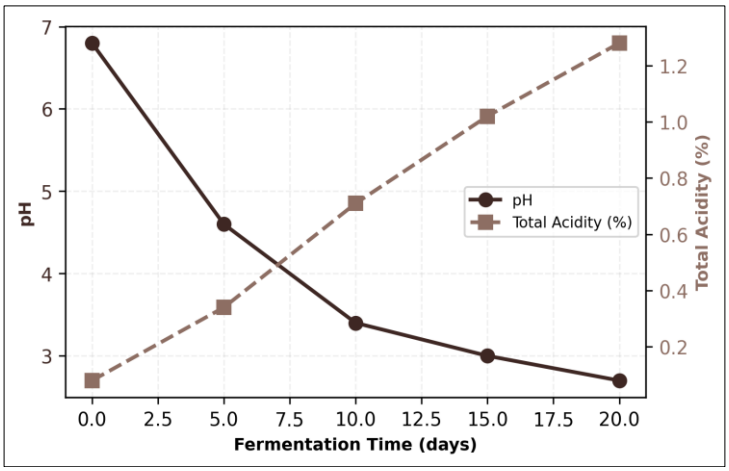

Fig 3: Changes in pH and total acidity during 20-day kombucha fermentation

Interpretation

Day 10 emerges as the sweet spot across all measured dimensions: antioxidant activity reached 54.2% DPPH inhibition (vs. 21.3% at day 0), sensory overall acceptability peaked at 8.1, and microbial counts of both acetic acid bacteria (4.7×10^6 CFU mL^{-1}) and yeasts (3.2×10^6 CFU mL^{-1}) were at their highest, suggesting an active probiotic load for the consumer.

Discussion

The rapid pH decline between days 0 and 10 reflects vigorous acetic acid fermentation, which is consistent with the observations of Jayabalan *et al.* [4] who reported a pH drop to 3.5 by day 9 in green tea kombucha at 30 °C. The mechanism involves sequential metabolism: yeasts first hydrolyse sucrose and produce ethanol, which acetic acid bacteria then oxidise to acetic acid using oxygen diffusing through the muslin cover [16]. This explains why cellulose pellicle thickness and acidity rose in parallel the pellicle is produced by the same *Komagataeibacter* species responsible for acetic acid synthesis.

The doubling of phenolic content by day 10 likely involves microbial esterases cleaving ester bonds in tea polyphenols, liberating free catechins and gallic acid that register more strongly in the Folin-Ciocalteu assay [17]. Chakravorty *et al.* [18] reported similar increases and attributed the antioxidant gain to both liberated phenolics and microbial metabolites such as D-saccharic acid 1,4-lactone. For small-scale producers in Java, these data mean that a 10-day ambient fermentation is enough to achieve functional-food levels of antioxidants without specialised temperature control [19].

Conclusion

This research aimed to determine the fermentation duration that maximises both nutritional quality and sensory acceptability of black-tea kombucha under Bogor's ambient

conditions. A 10-day fermentation produced the highest overall acceptability score (8.1), substantial antioxidant activity (54.2% DPPH inhibition), and a balanced organic acid profile dominated by acetic and glucuronic acids. Extending fermentation beyond 15 days rendered the beverage unpalatably sour. Producers should therefore target a 10-day window, with daily pH monitoring as a practical endpoint indicator (target pH 3.3-3.5). Future work could explore flavouring additions (ginger, lemon, fruit juices) to broaden consumer appeal and test shelf stability of bottled kombucha under refrigerated and ambient storage [20].

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