

Chemical Profile, Antioxidants, and Enzymatic Activity of the Kombucha Tea Beverages as a Potential Anti-obesity Beverage

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ABSTRACT

This study aimed to investigate the chemical profile, antioxidant activity, and enzyme inhibition of black tea kombucha and kombucha without tea, as well as the relationship between these three parameters. Both kombuchas of different fermentation time (1, 7, 14, 21 days) had been analysed. The pH and total soluble solids (TSS) of both samples decreased gradually with fermentation time. The scavenging activity (DPPH), total phenolic content (TPC), and total flavonoid content (TFC) of black tea kombucha were significantly higher than kombucha without tea. The porcine pancreatic lipase (PPL) inhibition of both samples in the study increased gradually with fermentation time. Significant differences ($p < 0.05$) were observed in pH, TSS, and PPL inhibition from the beginning to the end of fermentation in black tea kombucha, while for kombucha without tea, significant differences ($p < 0.05$) were observed in pH, TFC and PPL inhibition. Moreover, significant differences ($p < 0.05$) were observed in all parameters except pH between the two samples at the end of fermentation. The Pearson correlation revealed kombucha's lipase inhibition was correlated with its chemical profile, not with antioxidant activity. These findings indicated the anti-obesity potential of kombucha, and it was highly associated with kombucha's chemical profile.

INTRODUCTION

Kombucha, a fermented tea beverage, has been consumed for centuries. Its preparation involves adding a symbiotic culture of bacteria and yeast (SCOBY) to sweetened tea, undergoing fermentation for seven to fourteen days [1]. The fermentation process produces organic acids, enzymes, bioactive compounds, and vitamins, which are believed to have beneficial health effects. Antimicrobial, antioxidant, hepatoprotective, anti-hypercholesterolemic, anticancer, anti-inflammatory, and other health promoting effects were found in kombucha in preclinical research [2]. Chemical composition and bioactive compound content vary based on factors such as tea type, fermentation conditions, and SCOBY strain. This research focuses on kombucha brewed from *Camellia sinensis* black tea leaves, due

to its richness in bioactive components, which associated with potential anti-obesity effects [3]. Understanding kombucha's chemical profile, antioxidant, and enzymatic activity may unlock its potential as a functional food or nutraceutical for managing obesity. This research aims to assess kombucha's inhibitory effects on pancreatic lipase, emphasizing its anti-obesity potential.

MATERIALS AND METHODS

The kombucha were produced according to the instructions provided by Releaf Kombucha, Penang, Malaysia and research publications [1,4]. Black tea kombucha were made with sweetened tea, SCOBY, and starter tea in a 90:3:7 ratio, with initial pH 4.5, and left for fermentation at 25 °C. The kombucha

were collected 200mL on days 1, 7, 14, and 21 of the fermentation process and stored at 4 °C. Kombucha without tea, serving as controls, were prepared by substituting sweetened tea with sweetened water and undergoing the same fermentation period. All experiments were in triplicate.

pH was determined using a pH meter. Total Soluble Solids (TSS) were measured using a hand refractometer. Organic acids were analysed using high-performance liquid chromatography (HPLC) with a UV detector. Antioxidant properties were evaluated using DPPH method. Total phenolic content was determined using Folin-Ciocalteu assay. Total flavonoid content was determined using aluminium chloride colorimetric method. Enzymatic activity was determined using porcine pancreatic lipase (PPL) inhibition assay. One-Way ANOVA was used to analyse data. Mean differences were tested to compare significant differences between black tea kombucha and control

(kombucha without tea) under different fermentation times (day 1, 7, 14, 21).

RESULTS AND DISCUSSION

From **Table 1**, significant changes ($p < 0.05$) were observed for all parameters, except for DPPH, TPC, and TFC for black tea kombucha and control, and TSS for control. Black tea kombucha outperformed the control in all parameters except PPL inhibition, attributable to the nutrient-rich black tea aiding fermentation and its high antioxidant content.

Figs. 1, 2, and 3 show chromatograms with comparable peaks, indicating ineffective separation, possibly due to system or column clogging, causing poor peak shape or split peaks. The mobile phase, KH_2PO_4 buffer, is crucial for separation but can precipitate if not handled properly. Bacterial growth or contaminants in the mobile phases can also lead to clogging.

Table 1. Alteration of the chemical, antioxidant, and enzymatic activity parameters during fermentation of black tea kombucha and control. Values are expressed as mean \pm SD of triplicates measurement. Superscripts with different letters are significantly different at $p < 0.05$ in the same row.

Parameters	Fermentation time (day)							
	Black tea kombucha				Control			
	1	7	14	21	1	7	14	21
pH	3.56 \pm 0.01 ^a	3.34 \pm 0.08 ^a	3.22 \pm 0.10 ^b	3.00 \pm 0.14 ^c	3.57 \pm 0.02 ^a	3.39 \pm 0.07 ^a	3.25 \pm 0.07 ^b	3.17 \pm 0.13 ^b
TSS (° Brix)	9.1 \pm 0.1 ^a	8.8 \pm 0.2 ^a	8.5 \pm 0.2 ^b	8.2 \pm 0.2 ^c	9.1 \pm 0.1 ^a	9.0 \pm 0.0 ^a	8.9 \pm 0.1 ^a	8.8 \pm 0.2 ^a
DPPH	91.35 \pm 6.64 ^a	81.66 \pm 2.71 ^a	78.77 \pm 3.10 ^a	80.48 \pm 8.91 ^a	12.44 \pm 2.61 ^b	15.69 \pm 11.03 ^b	16.64 \pm 5.93 ^b	18.95 \pm 11.21 ^b
TPC	0.7858 \pm 0.0389 ^a	0.7207 \pm 0.1583 ^a	0.8308 \pm 0.0844 ^a	0.7950 \pm 0.0238 ^a	0.0831 \pm 0.0135 ^b	0.1260 \pm 0.0582 ^b	0.1282 \pm 0.0589 ^b	0.1247 \pm 0.0511 ^b
TFC	0.4646 \pm 0.0757 ^a	0.3987 \pm 0.0658 ^a	0.4104 \pm 0.0386 ^a	0.4560 \pm 0.0191 ^a	0.0078 \pm 0.0012 ^b	0.0383 \pm 0.0051 ^b	0.0416 \pm 0.0068 ^b	0.0621 \pm 0.0067 ^b
PPL inhibition	31.31 \pm 7.01 ^a	64.41 \pm 7.99 ^b	75.17 \pm 2.86 ^b	78.08 \pm 2.74 ^b	34.95 \pm 0.66 ^a	68.52 \pm 3.89 ^b	76.13 \pm 6.13 ^b	89.82 \pm 4.38 ^c

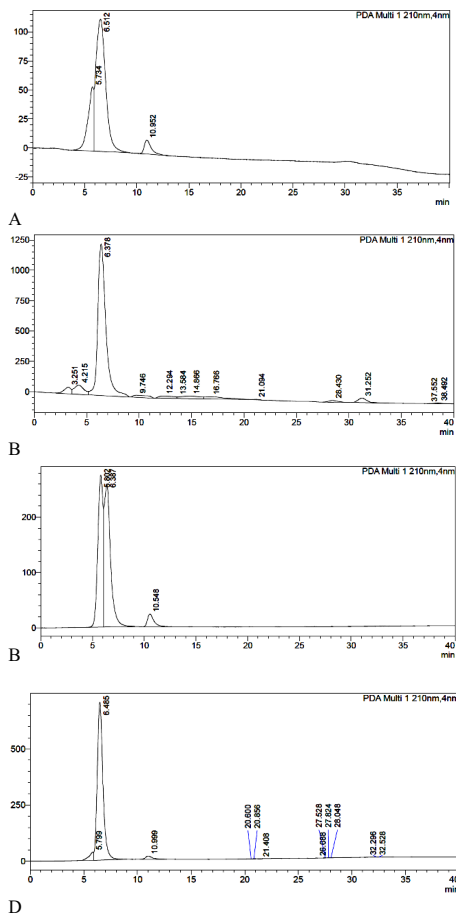


Fig. 1. HPLC chromatograms of four standard organic acids: acetic acid (A), lactic acid (B), gluconic acid (C), glucuronic acid (D).

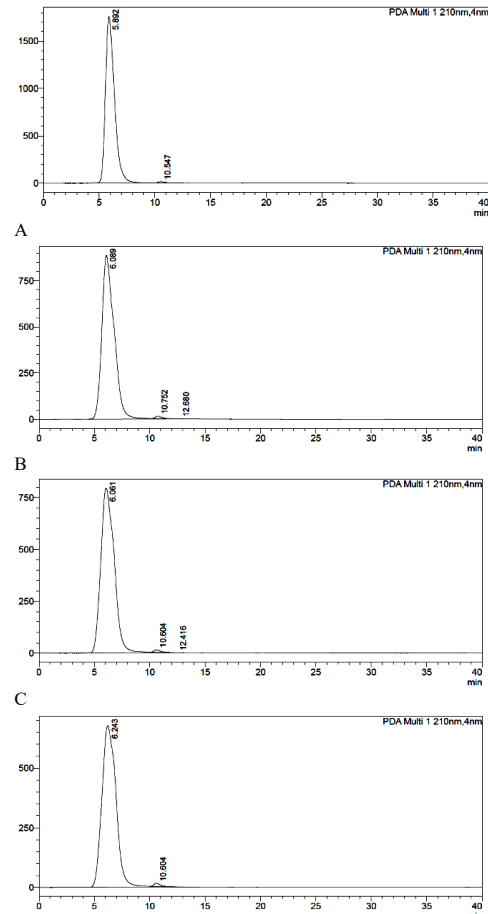


Fig. 2. HPLC chromatograms of organic acids in black tea kombucha for each fermentation period: day 1 (A), 7 (B), 14 (C), 21 (D).

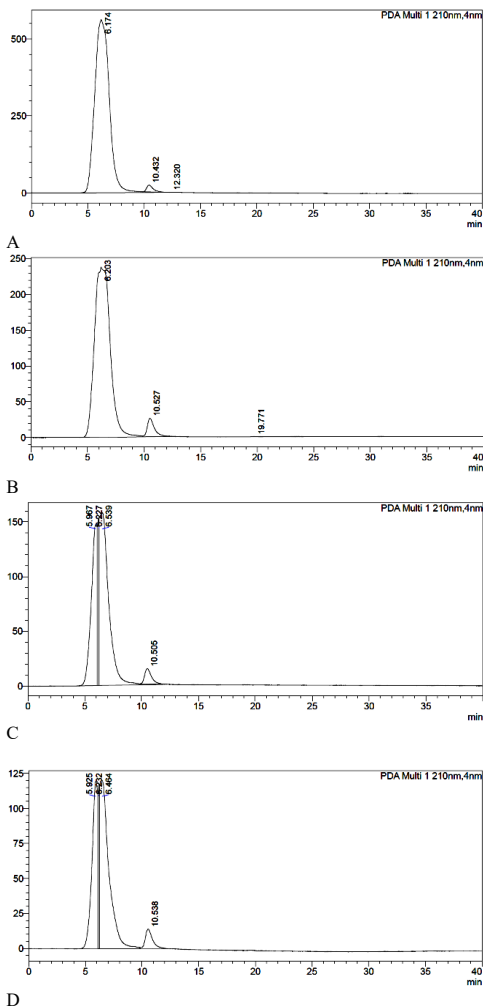


Fig. 3. HPLC chromatograms of organic acids in control for each fermentation period: day 1 (A), 7 (B), 14 (C), 21 (D).

Table 2 reveals strong negative linear relationship between lipase inhibition and both pH and TSS in kombucha, suggesting that higher acidity and lower sugar content during fermentation may contribute to increased bioavailability and activity of lipase inhibitors. In addition, the weak negative correlation between PPL inhibition and antioxidant parameters (TPC, TFC, DPPH), as shown in Table 2 are not statistically significant, suggesting that the relationship may be substrate-dependent and not directly linked in the fermentation process.

Table 2. Correlations between chemical profile, antioxidant, and enzymatic activity in black tea kombucha and control.

Factors	pH	TSS	DPPH	TPC	TFC
PPL inhibition	-0.814**	-0.588**	-0.165	-0.110	-0.098

** . Correlation is significant at the 0.01 level (2-tailed).

CONCLUSION

Throughout a 21-day fermentation process, both black tea kombucha and kombucha without tea experienced a continuous decrease in pH and TSS, suggesting ongoing fermentation. Black tea kombucha possessed higher antioxidant activity, TPC, and TFC compared to kombucha without tea, with fermentation time showing no significant impact on antioxidants. Despite this, both types of kombucha displayed a high lipase inhibition capability, which increased over time. The correlation analysis indicated that kombucha's PPL inhibition was associated with its chemical

profile rather than its antioxidant activity. This highlights kombucha's potential as an anti-obesity agent, emphasizing the need for a deeper study of its chemical profile to identify the potential elements.

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