### **Systematic Review**

# A Review of Coffee Kombucha Brewing and Health Effects as a **Fermented Beverage**

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#### ABSTRACT

**Background:** Coffee kombucha, or what many kombucha brewers call koffucha, is one of the new varieties of kombucha, by replacing the tea infusion with coffee extract. However, the method of making koffucha still varies, like the coffee varieties, coffee extraction method, SCOBY and starter ratio, sugar ratio, fermentation time, and second fermentation. This review aims to examine koffucha brewing methods and its potential health benefits.

Methods: This study reviewed the study about koffucha from previous literature using PRISMA method. The literature was gathered through a systematic search of PubMed, Scopus, and Google Scholar databases. Search terms included "coffee" and "kombucha," combined using Boolean operators.

**Results:** The optimum method for koffucha brewing is 12 days of fermentation at room temperature 25°C with a 10% sugar ratio. Robusta is considered better at total phenolic content, antioxidant level, and low sugar koffucha. Those who prefer low caffeine could opt for arabica with a lighter roasting level instead. The starter ratio and coffee ratio still vary and could not be concluded.

Conclusion: The fermentation of coffee might increase its health properties, including antimicrobial, antifungal, anti-aging, and anti-neurodegenerative. In vivo study showed no sign of toxicity of koffucha. Koffucha shows potential as a functional beverage, but standardized methods and further validation are needed.

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### INTRODUCTION

Fermented foods and beverages have become trending for their healthy properties, especially kombucha as fermented tea beverage. Kombucha was first discovered in East Asia around 220 BC during Tsin Dynasty and famous for its detoxifying and energizing benefits, but later being named kombucha from "Kombu", the Japanese physician's name who treated the digestive problems of Emperor Inkyo around 414 AD, and "cha", the Japanese word for tea (Jayabalan, Malbaša, & Sathishkumar, 2016). Original kombucha was made using sweet tea leaves infusion, added with a symbiotic culture of bacteria and yeast (SCOBY) and starter tea from previous brewing (Jayabalan et al., 2016; Karyantina, Surulloh, & Suhartatik, 2024).

SCOBY of mainly consists Acetobacteraceae, Gluconobacter. (Komagataeibacter Komagataeibacter xvlinus. Komagataeibacter Komagataeibacter rhaeticus, Komagataeibacter saccharivorans, and Komagataeibacter kombucha) bacteria; and Zygosaccharomyces, Candida, Torulaspora, Pichia, Brettanomyces, Schizosaccharomyces and Saccharomyces yeast (Zofia et al., 2020). Many health benefits are claimed by consuming kombucha regularly, including antimicrobial, antioxidant, hepatoprotective, anticancer, anti-inflammatory, antidiabetic, lowering cholesterol, enhancing liver detoxification, and immunomodulatory effects (Jayabalan et al., 2016; Zofia et al., 2020).

Nowadays, people can make their own kombucha at home. Many varieties have been made from kombucha, including another leaf infusion, flower tea, fruits, and coffee. Some also add second fermentation by adding more sugar or fruits, then undergo anaerobic fermentation to give a refreshing effervescent effect and taste (Emiljanowicz & Malinowska-Pańczyk, 2020; Le, 2017). Coffee kombucha, or what many kombucha brewers call koffucha, is one of the new varieties of kombucha, by replacing the tea infusion with coffee extract (Kusdiana, 2020; Le, 2017).

However, the method of making koffucha still varies, like the coffee varieties, coffee extraction method, SCOBY and starter ratio, sugar ratio, fermentation time, and second fermentation. Koffucha studies were still limited, hence the researchers still study the proper method and recipe for making koffucha and its benefits. This study collects data from previous researchs and compares the benefit results to determine the better brewing method. This study aims to assess the optimum koffucha-brewing method reviewed from its active compounds and health benefits, by reviewing the study about koffucha from previous literature. The researcher hoped it would give more insight into making koffucha better, especially for home brewers and markets.

#### MATERIALS AND METHOD

Articles were screened based on Preffered Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) method. The literature was gathered through a systematic search of PubMed, Scopus, and Google Scholar databases. Search terms included "coffee" and "kombucha," combined using Boolean operators. Studies published between 2015 and 2025 in English were considered. Coffee beans extract kombucha was the main topic for this study, so cascara coffee and coffee leaves extract kombucha study was excluded. Another addition to the koffucha besides its main ingredients and articles unrelated to the health properties were also excluded. Key findings were extracted and synthesized into thematic categories, including active compounds, health benefits, and gaps in current research. Articles were collected and managed with Microsoft Excel and Mendeley Reference Manager tools.

PRISMA flowchart showed in Figure 1. Descriptively, 21 articles from Pubmed, 60 from Scopus, and 831 from Google Scholar were collected. 68 duplicates were removed. 394 articles older than 2015 were removed. A total of 447 articles were screened, and 433 were excluded due to irrelevance to coffee kombucha and its health-related properties. From 14 articles, one reported a backslopping method that may disrupt the original kombucha brewing method, two articles were not relevant, and three articles only reported hedonic tests. The final report used was eight articles.

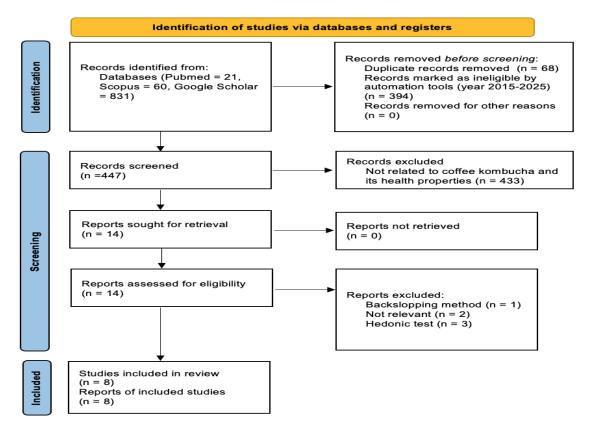


Figure 1. Identification of studies using PRISMA method

### RESULTS

#### **Koffucha Brewing Method Profiles**

Eight studies about koffucha were found with many varieties of its recipe. The recipe and key findings were compared in table view (Table 1). Most studies used arabica and robusta coffee beans, while only one study used liberica. All studies used the immersion coffee extraction method. Three studies used green (unroasted) coffee, two used roasted, while the rest were unknown. The coffee ratio ranged from 0.125% to 7.5%. The sugar ratio was between 5% and 10.5%. The starter ratio varies from 3% to 10%, but one remained unknown. Fermentation time varies from 1 to 28 days. All studies did not use second fermentation. All studies used room temperature of about 25°C, and one study observed different temperatures.

#### pH Level

A significant decrease in pH level was observed in fine-ground koffucha from 5.0 to 4.1 on day 7 of fermentation, compared to coarse-ground and instant coffee (Watawana, Jayawardena, & Waisundara, 2015). Meanwhile, Karyantina et al. (2024) found the highest pH of koffucha was 3.42 in the Arabica koffucha 1%, while the lowest pH was 2.74 in Liberica koffucha 3%. All types of coffee and coffee concentrations fit into the pH level food safety issues. The higher the coffee concentration, the lower the pH (Karyantina et al., 2024). Miranda et al. (2023) showed the pH ranged from 4.52 (day 0 of fermentation) to 3.25 (day 21 of fermentation), reducing rapidly in the first 6 days followed by a slight decrease until the end of fermentation. The graphic from the study seemed to touch pH 3.5 by day 12 of fermentation (Miranda et al., 2023).

 Table 1. Koffucha Brewing Method Profiles

No	Author	Coffee Type	Roasting Level	Grind Size	Coffee Extraction Method	Coffee Ratio	Sugar Ratio	Starter Ratio	Fermen- tation Time	Fermen- tation Tempera- ture	Key Findings
1.	Zofia et al. (2020)	Arabica	Green (not roasted)	-	Immersion with 95°C water, stirred until cooled down (25°C), filtered	3%	10%	6%	7; 14; 28 days	Room temperature (± 25°C)	Total phenolic content, total flavonoid content, ROS level, SOD activity, cytotoxicity, anticollagenase activity, antielastase activity, TEWL and skin hydration activity, SPF
2.	Pavlović et al. (2023)	Arabica	-	-	Immersion with boiling water, stirred at room temperature for 30 minutes, filtered	2%	5%	-	21 days	Room temperature	Chemical composition, antioxidant activity, anti-diabetic activity, anti-neurodegenerative activity
3.	Watawana et al. (2015)	-	Roasted	Coarse; fine; instant	Immersion with boiling water for 5 minutes, filtered,	1%	10%	3%	1–7 days	Room temperature	pH level, total phenolic content, antioxidant activity, anti-diabetic activity

No	Author	Coffee Type	Roasting Level	Grind Size	Coffee Extraction Method	Coffee Ratio	Sugar Ratio	Starter Ratio	Fermen- tation Time	Fermen- tation Tempera- ture	Key Findings
4.	Savitri et al. (2024)	Arabica; Robusta	Light; medium; dark roasted	Fine	cooled down (24 ± 3°C) Immersion with boiling water until dissolved, cooled down	7.5%	10.5%	9.1%	7; 14 days	Room temperature	pH level, total vitamin C, caffeine content, lactic acid bacteria content
5.	Karyantina et al. (2024)	Arabica; Robusta; Liberica	-	-	Immersion with boiling water for 5 minutes, filtered, cooled down (25°C)	1%; 2%; 3%	10%	10%	12 days	Room temperature	Antioxidant activity, total phenolic content, total tannin, total sugar, pH level
6.	Narko et al. (2020)	Robusta	Green (not roasted)	-	Immersion with boiling water until dissolved, cooled down, strained	0.125%	10%	9.1%	18 days	Room temperature (25°C)	Toxicity
7.	Narko et al. (2020)	Robusta	Green (not roasted)	-	Immersion with boiling water until dissolved, cooled down, strained	0.125%	5%; 7.5%; 10%	9.1%	6; 12; 18 days	25°C; 31°C; 37°C	Caffeine content, chlorogenic acid content

No	Author	Coffee Type	Roasting Level	Grind Size	Coffee Extraction Method	Coffee Ratio	Sugar Ratio	Starter Ratio	Fermen- tation Time	Fermen- tation Tempera- ture	Key Findings
8.	Miranda et al. (2023)	Arabica	Medium	Fne	Immersion for 5 minutes, filtered with Whatman 1 filter paper, cooled down (25°C)	2%	5%	10%	0; 6; 12; 15; 21 days	25 ± 3°C	pH level, total phenolic content, 5-CQA content, antimicrobial and antifungal activity, in vivo toxicity

#### **Total Phenolic Content**

Fermentation reduced the total phenolic content of green arabica koffucha from  $630.05 \pm 5.20$  mg GAE/g DW unfermented coffee to  $106.76 \pm 3.72$  mg GAE/g DW on day 7 of fermentation but then began to multiply at day 14 and day 28 of fermentation. From Zofia et al. (2020) research, 28 days of fermentation were the highest total phenolic content (Zofia et al., 2020). In contrast, Watawana et al. (2015) research showed increasing total phenolic content at day 7 fermentation, with the highest total phenolic content from fine ground koffucha (26.4 mg GAE/g). Specifically, the CGA was significantly increased after day 4 of fermentation at fine ground koffucha. On day 7 of fermentation, both fine ground and coarse ground koffucha reached the same level, 12.9 and 13.2 mg/L, respectively (Watawana et al., 2015).

Meanwhile, Pavlović et al. (2023) research did not find a significant difference in CGA content from arabica coffee and koffucha of 20 days of fermentation, 41.33 and 43.52 mg/L, respectively (Pavlović, Stajić, Gašić, Duletić-Laušević, & Ćilerdžić, 2023). Koffucha studied by Karyantina et al. (2024) showed the highest total phenol yield was 9.10 mg GAE/ml in the Liberica koffucha 1%, while the lowest result was 6.66 mg GAE/ml in the Arabica koffucha 1% (Karyantina et al., 2024).

Green robusta koffucha studied by Narko et al. (2020) showed the lowest CGA level was 105.15 mg/L obtained at a condition of 10% sugar concentration, an incubation temperature of 25 °C, and an incubation time of 6 days, and the highest CGA level was 144.37 mg/L which was obtained at a condition of sugar concentration of 5%, an incubation temperature of 25 °C, and an incubation time of 18 days (Narko et al., 2020). Miranda et al. (2023) did not show a significant difference in the total phenolic content and 5-CQA, the main class of CGA, from day 0 to day 21 of fermentation (Miranda et al., 2023). Another phenolic content, the caffeic acid, significantly increased only on fine ground koffucha from day 6 of fermentation onward, with the final day 7 concentration was 28.4 mg/L (Watawana et al., 2015).

## **Total Sugar Content**

Karyantina et al. (2024) stated that the highest total sugar content of koffucha was 15.32% in the Arabica koffucha 2%, while the lowest total sugar content was 10.09% in the Robusta koffucha 3% and Liberica koffucha 2%. This might be caused by the sugar content from each type of coffee being different from the beginning. The sugar content in Arabica coffee beans is 6.0-9.0 g/100 g, Robusta coffee beans is 0.9-4.0 g/100 g, and Liberica coffee beans is 8 g/100 g (Karyantina et al., 2024).

#### Lactic Acid Bacteria Content

A study by Savitri et al. (2024) showed an increasing level of lactic acid bacteria on day 7 of fermentation and decreasing at day 14 of fermentation. Robusta koffucha had more lactic acid bacteria content and remained stable at a medium roast. Arabica koffucha had lower lactic acid bacteria but was more stable at any roasting level (Savitri et al., 2024).

### **Antioxidant Activity**

Green arabica koffucha antioxidant activity from Zofia et al. (2020) reduced at day 7 of fermentation but increased to almost the same with unfermented coffee at day 28 of indicating strong antioxidant properties using 1,1-diphenyl-2picrylhydrazyl (DPPH) inhibition method (Zofia et al., 2020). Arabica koffucha from

Pavlović et al. (2023) showed 60% DPPH inhibition activity, lower than unfermented one (80.57%) at 10mg/mL concentration. Beta-carotene bleaching inhibition also showed the same effect, arabica koffucha could inhibit 91.41%, 15% more than unfermented one (Pavlović et al., 2023). Meanwhile, in Watawana et al. (2015) research, antioxidant activity started to significantly increase from day 6 of fermentation onward on fine ground koffucha and day 7 of fermentation on coarse ground koffucha (Watawana et al., 2015).

Karyantina et al. (2024) found the highest antioxidant activity was Liberica koffucha at 1% (74.86%) while the lowest was Robusta koffucha at 3% (47.43%). The researchers also stated that the decrement in antioxidant activity might be caused by a decrease in nutrients needed for microbial growth. The treatment of coffee and coffee concentration also had a significant effect on the antioxidant activity of koffucha (Karyantina et al., 2024). Savitri et al. (2024) study showed total vitamin C was increased on day 7 of fermentation but decreased on day 14 of fermentation (Savitri et al., 2024).

## Reactive Oxygen Species (ROS) Activity

The research conducted on fibroblasts and keratinocytes cell lines showed that the concentration of 1000 µg/mL increased the production of ROS, significantly after day 14 of fermentation of green arabica koffucha (Zofia et al., 2020).

## Superoxide Dismutase (SOD) Activity

Green arabica koffucha on day 14 of fermentation at the concentration of 500 and 1000 μg/mL shows a higher activity of the SOD enzyme compared to the green coffee extract (Zofia et al., 2020).

## **Toxicity**

The highest increase in keratinocytes and fibroblasts cell line proliferation was observed on day 14 of fermentation at the concentration of 250 and 500 µg/mL. However, after day 28 of fermentation, it slightly reduced the proliferation and viability of skin cells, up to 25% for keratinocytes in the Neutral Red test, which indicates a cytotoxic effect. Lactate dehydrogenase (LDH) cytotoxicity assay also gave similar results (Zofia et al., 2020).

In vivo toxicity was also studied by Narko et al. (2020) against zebrafish embryos. It showed that green robusta koffucha considered safe with an LC50 value was 1294.29 ppm, far above the safe category threshold (Narko, Wibowo, Damayanti, & Wibowo, 2020). Toxicity tested on Galleria mellonella larvae treated with all samples survived and did not show melanization, therefore, the study from Miranda et al. (2023) does not show toxicity (Miranda et al., 2023).

## **Antimicrobial and Antifungal Activity**

Koffucha showed bactericidal activity against Staphylococcus aureus from day 6 of fermentation and against the other strains on day 12 of fermentation. Koffucha also showed fungicidal activity against Candida albicans, Cryptococcus gattii, and Candida neoformans on day 12 of fermentation, and inhibitory activity against C. neoformans on day 12 and 15 (Miranda et al., 2023).

### **Anti-Aging Effect**

One of the causes of aging on the skin is the degradation of collagen and elastin by collagenase and elastinase enzymes, respectively. Green arabica koffucha at day 14 of

fermentation and 1000 μg/mL concentration was able to inhibit collagenase activity by about 30% and elastinase activity about 25% (Zofia et al., 2020).

## **Anti-Diabetic Activity**

Arabica koffucha from Pavlović et al. (2023) study did not inhibit the activity of alpha-amylase, or their activities were negligibly low. Alpha-glucosidase inhibition was also poor, but it should be emphasized that the fermented coffee extract was two-fold more active than its unfermented extract (Pavlović et al., 2023). This result was contrary to the result from the Watawana et al. (2015) study which found that there was a significant increase in antidiabetic activity after day 5 of fermentation onward, with the highest value being on fine ground koffucha. Alpha-amylase was inhibited better than alpha-glucosidase (Watawana et al., 2015).

### **Anti-Neurodegenerative Activity**

Arabica koffucha from Pavlović et al. (2023) showed 45.05 ± 3.46% acetylcholinesterase (AchE) inhibition at 10 mg/mL concentration, which was higher than the unfermented one (26.31  $\pm$  1.19%). Meanwhile, tyrosinase (TYR) inhibition at 10 mg/mL concentration showed moderate inhibition at  $34.67 \pm 1.41\%$ , which was also higher than the unfermented one (28.80  $\pm$  0.46%) (Pavlović et al., 2023). This suggests that koffucha fermentation could increase anti-neurodegenerative activity.

#### DISCUSSION

The recommended kombucha's pH level for food safety issues was between 2.5 and 3.5, while preventing the growth of pathogenic bacteria should be maintained between 2.5 to 3.3. It reached that level of pH on day 14 of fermentation to all types of koffucha (arabica and robusta, all types of roasting) (Savitri et al., 2024). While fermentation from Watawana et al. did not meet the criteria of pH level of kombucha on day 7 of fermentation, 3% starter, and low amount of coffee and starter, day 12 and day 14 of fermentation showed proper pH from Savitri et al. (2024), Karyantina et al. (2024), and Miranda et al. (2023) studies, though the coffee ratio was different, ranging from 1% until 7.5%.

It indicates that the minimum period for brewing koffucha to proper pH was 12 days of fermentation, and a 5–10% ratio of sugar and starter, regardless of the amount of coffee extracted and coffee types. Total phenolic content was compared due to chlorogenic acids (CGA) as the most abundant phenolic compounds in coffee bean extracts (Jayabalan et al., 2016; Tajik et al., 2017). CGA has an antioxidant, anti-diabetic, anti-carcinogenic, anti-inflammatory, and anti-obesity effects on the body (Miranda et al., 2023; Tajik et al., 2017).

In addition, caffeine content should be considered. The maximum daily caffeine that humans consumed according to the Indonesian National Standard was 0.9-2% w/w (Savitri et al., 2024). Watawana et al. (2015) study showed increasing in caffeine content from fine ground koffucha to 15.1 mg/L on day 7 of fermentation. The coarse ground koffucha's caffeine content did not significantly increase (Watawana et al., 2015). Savitri et al. (2024) study showed that arabica has lower caffeine content than robusta, and the darker the roasting, the more caffeine contained. If fermentation went, there was a slight increment in caffeine content.

This study showed that the caffeine content was still fulfilling the Indonesian National Standard (Savitri et al., 2024). The study by Narko et al. (2020) showed from green robusta koffucha, the lowest caffeine content was 21.98 mg/L obtained at a condition of 7.5% sugar concentration, an incubation temperature of 31°C, and day 12 of fermentation, while the highest caffeine concentration was 29.34 mg/L obtained at a condition of 10% sugar concentration, an incubation temperature of 25°C, and a fermentation time of 6 days (Narko et al., 2020).

Another addition was tanning content. Karyantina et al. (2024) observed the highest tannin content was 0.16% in the Liberica koffucha 3%, while the lowest tannin content was 0.02% in the Arabica koffucha 1%. The higher the coffee concentration, the higher the tannin content tends to be. Koffucha with 3% coffee from all types of coffee beans reached the level of 0.14-0.16% tannin content (Karyantina et al., 2024). Flavonoids are also one of the phenols that possess several medicinal benefits, including anti-cancer, antioxidant, anti-inflammatory, and antiviral properties.

They also have neuroprotective and cardioprotective effects (Narko et al., 2020). Fermentation reduced the total flavonoid content of green arabica koffucha from 156.84  $\pm$  4.11 mg QE/g DW unfermented coffee to 17.02  $\pm$  2.33 mg QE/g DW at day 7 of fermentation but then multiplied greatly at day 14. From Zovia et al. research, 28 days of fermentation was the highest total flavonoid content (Zofia et al., 2020).

The data of total phenolic content gives various results, from increasing, and decreasing, to no difference at all. Many of the studies suggest brewing above 7 days of fermentation to give an increment of total phenolic content. Liberica coffee was the best in total phenolic content, followed by robusta and arabica. Increasing the coffee ratio gives a higher tannin composition (Karyantina et al., 2024). Those who want low caffeine intake should take Arabica coffee with a lighter roasting process, coarser ground coffee, and a minimum recommended day of fermentation (Savitri et al., 2024).

Although there is only one study about total sugar content, it suggested that those who want low sugar koffucha could take robusta coffee with a minimum sugar ratio recommended. Be aware that liberica coffee also contains higher sugar than robusta. Lactic acid bacteria and their fermentation help to prevent toxin formation and microbial spoilage, extend shelf life, change the taste of the original ingredients, improve the digestibility of foods and nutritional value, and offer health benefits (Savitri et al., 2024). It suggested that better lactic acid bacteria content was below 14 days of fermentation due to decrement of lactic acid bacteria after 14 days of fermentation, with robusta coffee at medium roast.

DPPH inhibition of around 70% was considered an antioxidant (Miranda et al., 2023). This study's results vary from each other, with green arabica koffucha being considered low antioxidant (only about 40%). Arabica and liberica coffee are considered high antioxidants at 1–3% coffee ratio and 12 days of fermentation. Robusta also gives high antioxidant buat at a coffee ratio of 1-2% only. Nutrients for microbial growth should be considered, with a recommendation of 10% sugar from this study. The fermentation time recommended was around 7–14 days, but the data was still limited.

Study from Zofia et al. (2020) was limited to fibroblasts and keratinocytes cell lines only, suggesting that the production of ROS applied to the topical use of koffucha. This data also suggested koffucha fermentation process might increase the SOD activity. Green arabica koffucha cytotoxicity was strictly dependent on the fermentation time. All toxicity from vivo research was considered safe until 21 days of fermentation, while longer fermentation could reduce the proliferation and viability of skin cells. All of the toxic research still has not conducted on humans. In Vivo studies are still limited to toxicity in zebrafish embryos and Galleria mellonella larvae.

Miranda et al. (2022) suggested optimal minimum fermentation time of koffucha for antimicrobial and antifungal activity was 12 days. Anti-diabetic activity from Pavlović et al. (2023) study did not inhibit the activity of alpha-amylase, or their activities were negligibly low, contrast with the study from Watawana et al. (2015). This study should be researched more since there are only two data with opposite results. The optimum incubation temperature was 25°C, while the incubation period was 18 days, based on CGA and caffeine content only (Miranda et al., 2022; Narko et al., 2020). Almost all research used the immersion method of coffee. Some studies reported that espresso and cold brew method coffee contains more antioxidant properties than the immersion method (Le, 2017). This method might increase the antioxidant properties after koffucha fermentation.

This review is limited by the limited availability of peer-reviewed studies specifically addressing coffee kombucha (koffucha). Most of the existing literature is based on in vitro or animal studies, with a lack of clinical trials in humans. Additionally, the variation in brewing methods, ingredients, and fermentation parameters across studies hinders the ability to establish standardized conclusions.

### CONCLUSION

From this literature study, it can be concluded that fermentation of coffee might increase its health properties, including antimicrobial, antifungal, anti-aging, and antineurodegenerative. In vivo study showed no sign of toxicity of koffucha. The optimum method for koffucha brewing is 12 days of fermentation at room temperature 25°C with a 10% sugar ratio. Robusta is considered better at total phenolic content, antioxidant level, and low sugar koffucha, but those who prefer low caffeine could opt for arabica with a lighter roasting level instead. The starter ratio (3–10%) and coffee (0.125–10.5%) ratio still varies and could not be concluded.

The researcher suggested studying more about koffucha in the future, including other coffee brewing methods, the variance of coffee beans, roasting level, grind size, ratios, and fermentation time, to give more actual data to be compared. Koffucha's benefit to the body, including antidiabetic and other health properties could be studied to give more valuable data. In vitro and in vivo studies could be conducted to gain more data about the impact of koffucha on health.

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