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Functional Food Potential of Green Tea Waste (Used Tea Leaves) Through Lactic acid Fermentation

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Authors' contributions

This work was carried out in collaboration between both authors. Author KS designed the study, performed the statistical analysis, wrote the protocol and wrote the first draft of the manuscript. Author YN managed the analyses of the study. Both authors read and approved the final manuscript.

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ABSTRACT

Aims: Fermented functional foods were developed to utilize green tea waste (used tea leaves) and their antioxidative activity was examined.
Methodology: The initial tea waste was prepared by steeping regular tea leaves in deionized water at 90°C for 60 s. This procedure was repeated to obtain the fifth and tenth sequential extracts. Subsequently, lyophilized tea leaves were rehydrated with potable water and inoculated with *Lactococcus lactis* subsp. *cremoris*. Following thorough mixing, the inoculated sample was fermented under anaerobic conditions at 25°C in the absence of light. To monitor the progress of fermentation, the pH levels of the infusion were measured on days 1, 3, 7, and 14. Additionally, the antioxidative activity of the fermented tea leaves and their infusions was assessed using the 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging assay.

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Results: After one week of lactic acid fermentation, the pH of the infusions from the fifth and tenth tea leaf extracts decreased significantly compared to other samples. However, within two weeks, all sample infusions reached a pH below 4, suggesting that catechins in the used tea leaves may inhibit lactic acid production during the early stages of fermentation. Despite the multiple extractions, the tea leaves from the fifth and tenth extracts retained elements that supported fermentation. Furthermore, the infusion prepared from the tea leaves after the tenth extract continued to exhibit antioxidative activity, indicating that beneficial properties persist even after repeated extractions**.**

Conclusion: Tea waste can be effectively utilized for lactic acid fermentation. Moreover, the resulting infusion may provide health benefits, making it a potential functional food.

Keywords: Tea waste; used tea leaves; Camellia sinensis; fermentation; lactic acid bacteria; antioxidant activity; functional food.

1. INTRODUCTION

Green tea (*Camellia sinensis*), a staple agricultural product of Japan, has been used since ancient times. The tea offers numerous health benefits due to its rich composition of bioactive compounds. The primary components—catechins, theanine, and caffeine—have been extensively studied [1–5]. Recent scientific research has elucidated the functions of various components of green tea, leading to its widespread consumption worldwide. With increased tea consumption, the large quantity of tea waste (used tea leaves) produced suggests that effective utilization would be beneficial. Traditionally, green tea waste has been used as fertilizer or feed, and major tea beverage companies have developed various industrial tea waste products [6]. Tea waste is recycled into items such as wallpaper, pens, paper, cardboard, artificial turf, and cushioning materials. These products exhibit the antioxidant properties of the catechins that remain in the waste, which probably contributes to their effectiveness. Additionally, tea waste is used in cooking because it retains some valuable nutrients, which may include insoluble ingredients that are not extracted when the tea is brewed [1,2].

Green tea waste may thus have beneficial functions, one of which is fermentation—a method that is traditionally used to make Japanese foods such as soy sauce, miso, and sake. While various fermented teas are made using microorganisms such as yeast, fungi, or lactic acid bacteria (LAB), each of which has its own characteristics [7–10], they are typically made from unused green tea leaves, not from tea waste. We have previously developed a fermented tea treated with LAB; however, the fermenting ability of repeatedly brewed tea leaves remains unknown. LAB have been shown to increase intestinal acidity, suppress putrefaction, and improve constipation by supporting peristaltic movement in the intestines [11–13]. Further, studies have revealed that LAB can improve immune function, lower triglyceride and blood cholesterol levels [14–17], and affect brain function [18]. Thus, LAB are drawing increasing attention due to their numerous health benefits.

In this study, we aimed to explore the further utilization of tea waste by developing a functional food through lactic acid fermentation. We measured the antioxidant activity of the fermented tea waste to assess its effectiveness as a functional food.

2. MATERIALS AND METHODS

2.1 Preparation of Green Tea Waste

Five grams of regular green tea were subjected to a series of extractions. The initial extraction was performed by steeping the leaves in deionized water at 90 °C for 60 s. This procedure was repeated to obtain the fifth and tenth sequential extracts. The samples were lyophilized using a freeze dryer for subsequent use in fermentation.

2.2 Lactic Acid Fermentation and Measurement of Fermentation Progress

Five grams of dried tea leaves were rehydrated with 100 mL of potable water and inoculated with approximately 1×10^6 colony-forming units (CFU) of *Lactococcus lactis* subsp*. cremoris* (*L. cremoris*) purchased from (Konno Co., Ltd., Akita, Japan). After thorough mixing, the inoculated sample was fermented under anaerobic conditions at 25 °C in the absence of light. Oxygen-absorbing and carbon dioxide-generating agents were used to create anaerobic conditions (AnaeroPack, Mitsubishi Gas Chemical, Tokyo, Japan). Regular green tea leaves (unused tea leaves) were fermented under the same conditions as a control, for comparison with the fermented tea waste. The fermentation progress of the infusion was monitored on days 3, 7, and 14 via pH measurement. It has been established that the pH of the infusion decreases with the progression of lactic acid fermentation [19].

2.3 Antioxidative Activity

After fermentation, the leaves were separated from their infusions and the antioxidative activity of each was measured. The leaves were dried, and 1 g of powdered leaves was steeped in 100 mL of 90 °C water for 1 min to prepare a liquid sample.

The liquid sample was added to a stable radical solution of 2,2-diphenyl-1-picrylhydrazyl (DPPH) dissolved in 50% ethanol. The amount of DPPH remaining after the reaction was determined using spectrophotometry (wavelength 520 nm). The DPPH radical scavenging rate, which indicates the strength of antioxidative activity, was calculated using the following formula:

$$\text{DPPH radical scavenging rate (\%)} = 100 \times [(A\ \text{blank} - A\ \text{sample}) / A\ \text{blank}]$$

2.4 Statistical Analysis

Data were expressed as the mean ± standard error of the mean (SEM). Statistical analysis was performed using Student's *t*-tests and one-way analysis of variance (ANOVA).

3. RESULTS AND DISCUSSION

LAB have gained attention for their beneficial effects, especially concerning the brain–gut axis [18,19]. Although several teas fermented with LAB exist, there has been no research on fermented teas made from tea waste using LAB.

In this study, we developed a novel fermented functional food using green tea waste with *L. cremoris* added for lactic acid fermentation. In most cases, plant-derived LAB such as *Lactobacillus plantarum* are used for lactic acid fermentation. Instead, we used *L. cremoris*, a unique bacterium that produces exopolysaccharides, which protect bacterial cells in harsh environments, promoting their proliferation [14–17,20]. In a previous study, we observed a decrease in pH as fermentation with *L. cremoris* progressed, indicating successful fermentation [20].

Fig. 1 shows changes in the pH of tea waste infusions during fermentation. The infusions made from the fifth and tenth brews promoted lactic acid fermentation, despite having lost many of the beneficial components that were initially present in the tea leaves. The differences in fermentation progress observed may be attributable to the catechins in the tea leaves, since they are known to inhibit LAB activity [20]. The control (unused tea leaves) and the first-brew tea waste likely contained large quantities of catechins [21], while the fifth and tenth brew tea waste probably contained fewer catechins, allowing for more rapid fermentation. The repeated brewing process may also have created a more conducive environment for LAB activity, possibly due to damaged leaf tissue and the elution of insoluble components. The progress of fermentation may be influenced by the remaining components in the tea waste, since 80% of the water-soluble components are extracted in the third brew [22]. Finally, the pH of the infusions decreased to around 4 within two weeks, indicating that all samples had undergone successful fermentation, despite variations in the fermentation rate.

To assess the potential of fermented tea waste as a functional food, we measured its antioxidative activity (Fig. 2). In Fig. 2A, the control (unused tea with fermentation) exhibited the highest antioxidative activity because of the presence of catechins. Catechins are well-known antioxidants with numerous health benefits and are used in a variety of products [1–5]. The catechins were not degraded by the LAB during fermentation (20) but were instead extracted into the infusion due to their water solubility, maintaining strong antioxidative activity. As shown in Fig. 2, increased brewing reduced the catechin content, leading to a decrease in antioxidant activity. However, the tenth brew infusion still exhibited slight antioxidant activity despite its low catechin content. In contrast, the antioxidant activity of the leaves was much lower than that of the infusions (Fig. 2B), suggesting that the antioxidative components, including catechins, were primarily extracted into the infusion during fermentation.

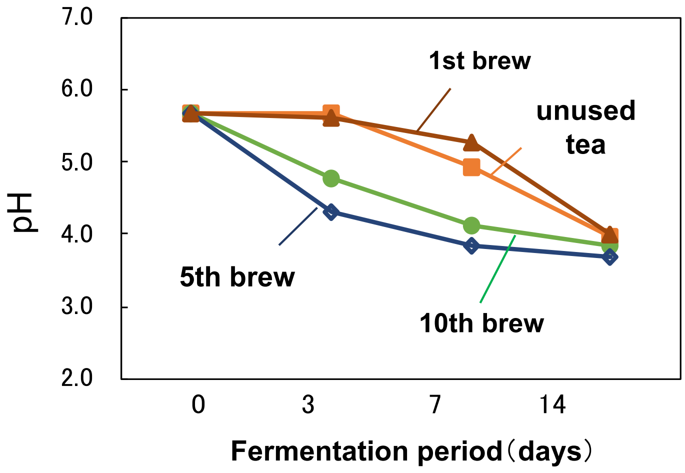

Fig. 1. Changes in pH in infusions of tea waste and unused tea leaves (control) during fermentation

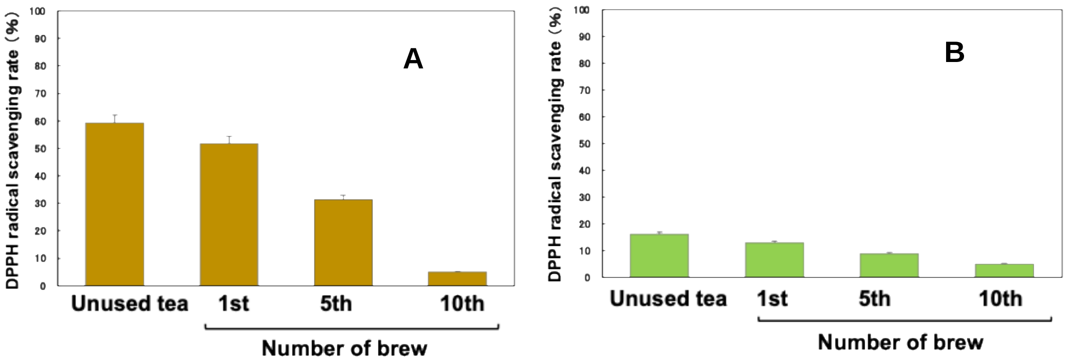

Fig. 2. Antioxidative activity of tea waste after one week of fermentation
A. Infusions of fermented tea waste. B. Fermented tea waste (leaves)

Recent studies have revealed that metabolites produced during lactic acid fermentation have physiologically active effects, and the health benefits of LAB are attracting increasing attention [18]. It is highly likely that these effects are not solely due to existing components, but also to new components generated through lactic acid fermentation. Thus, the physiological activity of infusions may result from the interaction of multiple components during fermentation. These results indicate that both the fermented tea waste leaves and their infusions possess antioxidative activity, an important characteristic of functional food.

4. CONCLUSION

We developed a novel functional food using green tea waste subjected to lactic acid fermentation. The results showed that the tea waste was effective in promoting lactic acid fermentation, and that the fermented tea waste infusions, including those from multiple brews, exhibited high antioxidant activity. This lactic acid fermented tea waste and its infusions may therefore offer health benefits. Further research is required to explore their physiological effects.

DISCLAIMER (ARTIFICIAL INTELLIGENCE)

Author(s) hereby declare that NO generative AI technologies such as Large Language Models (ChatGPT, COPILOT, etc.) and text-to-image generators have been used during the writing or editing of this manuscript.

CONSENT AND ETHICAL APPROVAL

It is not applicable.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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