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Bioconversion of Garcinia mangostana rind extract juice with yeast fermentation: Impact on its key bioactivity

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Abstract - Mangosteen (*Garcinia mangostana* L.) peel, a by-product of fruit processing, contains bioactive polyphenols with potential health benefits. However, the consumption of mangosteen peel as a supplement is generally limited due to its astringent taste. This study proposed the process for adding value the *Garcinia mangostana* rind extract juice (GREj) by bioconversion with yeast fermentation. The GREj was extracted by hydraulic pressing machine. The GREj medium was formulated by diluting with water to contain 20% GREj adjusted total soluble solids (TSS) with sugar to 12.00 °Brix and pH to 4.00 with lactic acid. After fermentation 3L of GREj medium with *Saccharomyces cerevisiae* in 5L bioreactor, the composition of GREj evaluated through HPLC (High Performance Liquid Chromatography) and TLC (thin layer chromatography) were significantly changed. The main acid of GREj, acetic acid was found greater than citric acid, the migration pattern on TLC with retention factor (Rf) was changed from 0.24 to 0.26. The antioxidant; DPPH (2,2-diphenyl-1-picrylhydrazyl) and TPC (total phenolic content) demonstrated the significant improvement of the GREj antioxidant capacity. The fermented GREj had intense red-purple color with fermented aroma and sour taste. This fermented juice could be further developed to be a functional drink as well as processed to be a powder form to use as functional

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ingredient and/or supplement. This demonstrated that yeast fermentation could be a potential mean in value adding of mangosteen peel from being agricultural waste to functional beverages and meals.

Keywords: *Saccharomyces cerevisiae*, yeast, mangosteen peel, antioxidant

1. Introduction

Mangosteen, a fruit cultivated primarily in Southeast Asia and Indonesia, is predominantly produced in Thailand, making it the world's leading mangosteen producer with an annual output of 240,000 trees. The mangosteen tree yield of nearly 500 per harvest, which can escalate to 1,000-3,000 fruit per tree at maturity. The fruit is characterized by the presence of an average percentage of about 27-32% that is edible and can be consumed, while the 62% residues derived from peel (inner pericarp tissue and bark) and seed (Iswari, 2011). Within the fruit, the edible part (aril), also known as the pulp, comprises 5-7 soft, intensely flavored segments enclosing 2-3 seeds. The inedible outer and inner part of the mangosteen, known as the peel (pericarp), constitutes approximately 60% of the fruit's total weight and contains significant amounts of tannins and anthocyanins. While the aril contains bioactive polyphenols, they are present in significantly lower concentrations compared to the pericarp (Palakawong & Delaquis, 2018). Mangosteen peel can be classified as an agricultural waste that has a very low economic value.

Several studies have indicated that the Mangosteen peel (rind), which is generated as a by-product during the processing of mangosteen, is abundant in xanthones and anthocyanins. These compounds exhibit essential biological activities, including anti-cancer properties. Additionally, research has demonstrated that *Garcinia mangostana* rind extract juice (GREj) possesses a range of diverse biological activities (Widowati et al., 2014). The presence of phytochemicals, which are natural chemicals such as favoniods and/or polyphenols in the mangosteen peel has been attributed to these activities. Through physical and chemical characterization, it has been revealed that the peel contains various xanthones, such as alpha-mangostin, gamma-mangostin, garcinone C, garcinone D, garcinone E, gartanin, and smeathxanthone-A (Gondokesumo, 2019).

The *Garcinia mangostana* rind extract (GRE) has been found to possess a flavonoid content of approximately 17.66 µg QE/mg extract. Notably, the extract has demonstrated potent antioxidant properties, as well as anti-collagenase and anti-elastase activities. Furthermore, gamma-mangostin has exhibited strong anti-hyaluronidase and anti-tyrosinase effects. These findings suggest that GRE, along with its compounds, could serve as promising anti-aging agents (Widowati et al., 2020). Currently, mangosteen peel, particularly the pericarp or peel, is consumed in various forms such as juices, syrup, tea, capsules, and candy. However, the consumption of mangosteen peel as a supplement is generally limited due to its astringent taste (Gondokesumo, 2019).

The fermentation process stands out as a promising alternative for value adding mangosteen peel. Specifically, yeast fermentation emerges as a potential strategy to convert the astringent mangosteen peel into a more flavorful and functionally

enriched meal or beverage. Extract compounds within the peel, could serve as carbon and nitrogen sources, can effectively support yeast fermentation. Additionally, many evidences from various reports highlights the capacity of yeast fermentation to enhance the bioactive profile of juices (Chanprasartsuk et al., 2010; Adiningsih et al., 2022). Thus, this study is aimed to (i) develop the fermentation process for bioconversion of Garcinia mangostana rind extract juice (GREj) (ii) to determine the alterations of composition and bioactive attributes GREj after fermentation.

2. Materials and methods

2.1 GREj preparation

Mangosteen peel was collected from the fruit at the commercial harvest stage with an average weight of 41.67 g per one mangosteen fruit. Mangosteen peel juice was directly extracted from the fresh pericarp (rind) using hydraulic press machine without adding water. The juice was centrifuged prior to use as concentrated Garcinia mangostana rind extract juice (GREj) in preparation of fermentation medium.

2.2 Yeast cultivation condition assessment

Fresh culture of commercial *Saccharomyces cerevisiae* (Bioflor, BIOCODEX) was prepared by cultivation in Yeast Malt Broth (YMB, HiMedia, India), 150 rpm, 30 °C for 24-48 hours. Fresh yeast culture was adjusted with OD_{600} value more than 1.0, washing cells with 0.85% saline solution, and adding with 10% of juice for use as seeding culture in fermentation process.

2.3 Fermentation profile of yeast in GREj medium and changes of GREj composition

The GREj medium was prepared by diluting GREj at concentrations of 20% (v/v) with water, supplemented with di ammonium phosphate $(NH_4)_2(HPO_4)$. at concentrations of 0.2% (w/v). Following adjustments to pH to 4.00 with lactic acid (Loba chemie, USA), and total soluble solids (TSS) to 12.00 °Brix with sugar syrup (Mitrphol, Thailand), the medium was sterilized. Seeding culture of yeast was further inoculated in GREj medium at 3 liters scale conducted in 5 liters bioreactor with agitation 100-120 rpm, 30 °C for 72 hours. Fermented juice was collected at 0, 14, 22, 36, and 62 hours for yeast population count to investigate specific growth rate and fermented juice test to determine physicochemical properties, some bioactivities, sugars, and organic acids, and GREj compositions (modified from Buranavanitvong & Prakitchaiwattana (2022).

2.4 Analytical techniques

The viability of yeast during cultivation was determined by cultural plating methods. The inoculum (0.1 mL) was spread onto Yeast Malt Agar (YMA, HiMedia, India) and incubated under 30°C for 48 hours.

Total soluble solid (TSS) was determined using a refractometer (Atago, Japan), pH was determined using a pH meter (Horiba, Japan) and finally Total Titratable acidity (TTA) was determined and reported as percentage of lactic acid.

Antioxidant activity was determined using the DPPH inhibition method and

the GREj was measured based on the inhibition of the DPPH from 1 mL of GREj that was centrifuged at 4000 ×g for 10 minutes. 1 mL suspension of GREj was transferred into a new tube mixed with 3 mL of DPPH solution (1 mM of 2,2-diphenyl-1-picrylhydrazyl in Methanol) and incubated at room temperature for 30 minutes in a dark place. Then, absorbance of juice samples was measured at 517 nm by spectrophotometer using methanol as a blank. The percent of DPPH inhibition was calculated by the equation modified from Shimada et al. (1992). Where initial was the absorbance of 1 mL of methanol mixed with 3 mL of DPPH solution and a sample was the absorbance of GREj.

The total phenolic content (TPC) in GREj was determined using the Folin-Ciocalteu method. 0.5 mL of juices was mixed with 10 mL of distilled water and added to 0.5 mL of Folin-Ciocalteu reagent. Solutions were left to react for 5 minutes at room temperature in the dark; to which 2 mL of sodium carbonate (10% (w/v)) was added. After 10 minutes, the absorbance was measured at 760 nm using a spectrophotometer (Eppendorf, Germany). The result was showed in equivalents of gallic acid (mg GAL/L (Buranavanitvong & Prakitchaiwattana, 2022).

HPLC: sugars and organic acids in GREj was analyzed in duplicate by HPLC (Agilent HPLC 1100 series, USA). Sugars were separated using a Zorbax NH_2 column of 4.6 x 150 mm. The mobile phase was Acetronitril: DI water in a ratio 80:20, for 5 µL. The organics acids were separated using the Mightysil RP18 column, for a size 4.6 x 150 mm. The mobile phase was 5 µL Sodium Dihydrogen phosphate (NaH_2PO_4) at pH 2.0; the identification and quantification of them were carried out using retention times and standard curves of pure sugars and acids (Detudom et al., 2023).

Thin-layer chromatography (TLC) was conducted following Larasati et al. (2020).

2.5 Statistical analysis

IBM-SPSS statistics software version 22 was used to conduct an analysis of variance (ANOVA), The significance between samples and multiple comparisons using T-test and Duncan test (SPSS Inc., Chicago, IL, USA), respectively. A probability was considered statistically significant when it was at $p < 0.05$.

3. Result and discussion

To facilitate the conversion of *Garcinia mangostana* rind extract juice (GREj) with yeast, the GREj was adjusted to standardize composition with a targeted 12 °Brix and pH of 4. This adjustment aimed to align the properties of the GREj with typical fruit juices, optimizing conditions for yeast fermentation (Buranavanitvong & Prakitchaiwattana, 2022). In evaluation of fermentation properties of yeast in GREj, the results as shown in Figure 1. The highest specific growth rate (0.015 log µ/h) of GREj in the fermenter at 14 hours was observed and after that it decreased at 22 hours, increased again at 36 hours, and decreased again at 62 hours. The final population at 48 hours was 9.00 ± 0.24 log CFU/mL.

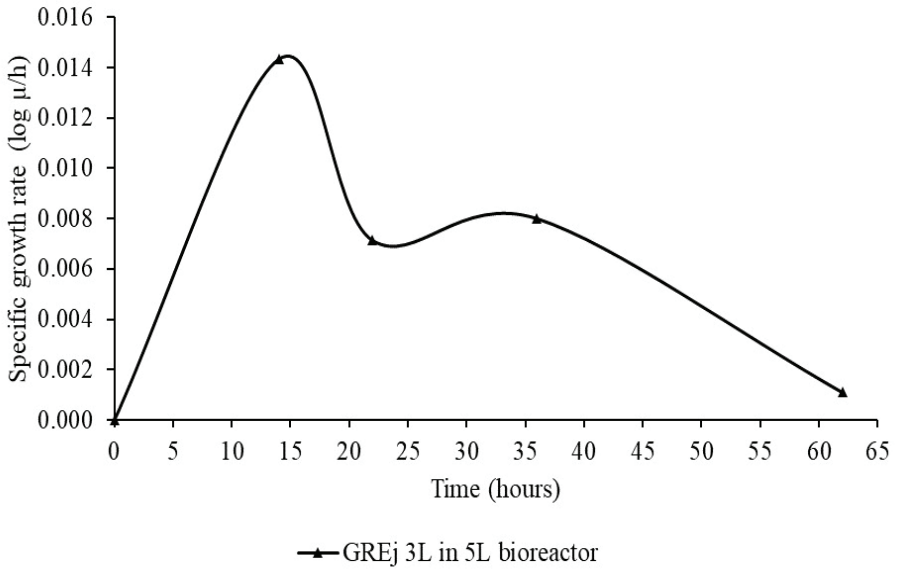


Figure 1. Specific growth rate (log μ/h) of *S. cerevisiae* from GREj in bioreactor during fermentation at 30°C for 62 hours

Figure 2 shows the physicochemical results, which revealed a change in the values of TSS, TTA, and pH of the GREj during fermentation. The reference compound used for the TTA value was lactic acid as the major acid because it was added to acidify GREj. The trend of the parameters shows a decrease of TSS and pH of juice and an increase of TTA during fermentation that related to the change profile of yeast (figure 2). At 62 hours, TSS dropped from 14.00 ± 0.00 °Brix to 4.00 ± 0.00 °Brix; pH dropped from 4.09 ± 0.01 to 3.93 ± 0.00; TTA increased from 19.82 ± 0.00 to 23.87 ± 0.64 g lactic acid/L. These variations were attributed to the fermentative process of yeast, which involved a decrease in sugar content and the production of acidic compounds by lowering the pH (Niamah, 2017). HPLC results revealed that acid profiles changed after fermentation. Citric acid was the main acid in GREj followed by, malic acid and succinic acid, respectively. After fermentation, citric acid was disappeared and acetic acid became a dominant acid followed by, malic acid, and succinic acid, respectively. Moreover, all sugar content decreased and their profiles also changed after fermentation. Glucose was the main sugar in GREj follow by, fructose, sucrose, and maltose, respectively. However, sucrose was not found after fermentation, and fructose became main sugar follow by, maltose, and glucose, respectively. The changes of physicochemical properties were in line with the acid profiles from the HPLC analysis (Table 1), and can be attributed to the high content of malic, acetic, and succinic acid produced during fermentation by the action of *S. cerevisiae*. As mentioned before, the increase in acetic acid content was related to the synthesis of it through the combination of alcohol and oxygen during fermentation (Rantsiou et al., 2012); the increase in succinic acid was because yeasts produce it as a by-product of the fermentation process (Jayaram et al., 2014), and the increase in malic acid was related to the natural metabolism of yeast during the fermentation process (Fu et al., 2023)

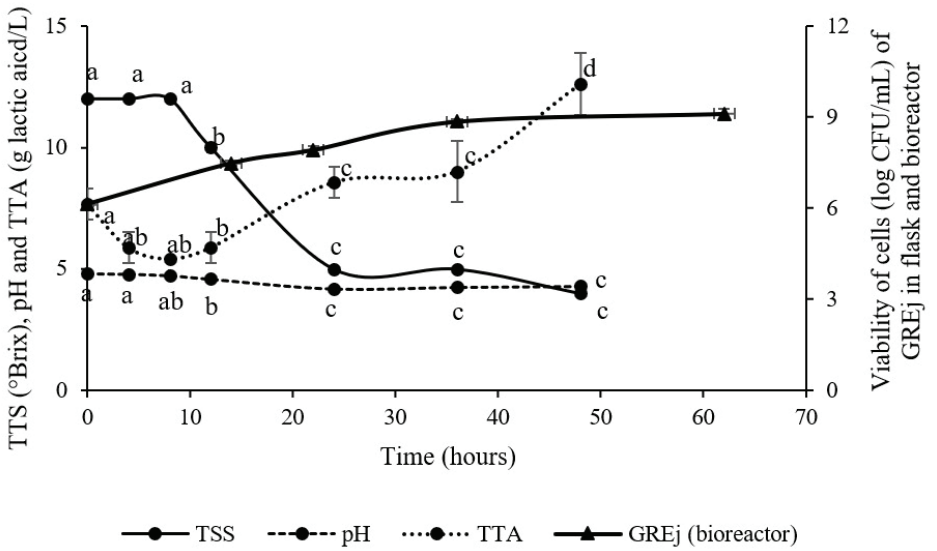


Figure 2. Changes of physicochemical properties of GREj (total soluble solids (TSS, °Brix), pH, total titratable acidity (TTA, g lactic acid/L)), and viability of *S. cerevisiae* (log CFU/mL) during fermentation in 3L bioreactor. a-d values with different superscript letters in the same row indicate significant differences (p<0.05)

Table 1. The organic acids and sugar profiles using HPLC analysis and migration properties using TLC analysis of GREj before and after fermentation in 3L bioreactor.

Properties		0 hour	62 hours
Organic acids (mg/L)	Citric acid	0.09 ± 0.03	0.00± 0.00
	Acetic acid	0.00± 0.00	0.33± 0.01
	Malic acid	0.05 ± 0.01	0.29 ± 0.01
	Succinic acid	0.04 ± 0.00	0.08 ± 0.00
Sugar (g/100 mL)	Sucrose	0.77 ± 0.19	0.00± 0.00
	Fructose	3.34 ± 0.00	0.05 ± 0.00
	Maltose	0.21 ± 0.16	0.04 ± 0.02
	Glucose	3.47 ± 0.14	0.02 ± 0.00
TLC	Distance (cm)	3.90	4.10
	Rf	0.24	0.26

TLC analysis was carried out to monitor the changes in the GREj compositions after fermentation. In TLC, a stationary phase and a mobile phase are employed. As the mobile phase moves over the stationary phase, compounds in the mixture separate based on their affinity for the stationary phase. The separation results in distinct spots or bands on the TLC plate, allowing for qualitative analysis

and comparison of different substances in the mixture (Santiago & Strobel, 2013). Table 1 shows the Rf value of GREj before and after fermentation; an increase in the Rf value was observed after 62 hours of fermentation, compared to GREj before fermentation. This variation was related to the biotransformation activity of the yeast employed in the fermentation of GREj, which converted complex polyphenolic compounds into simpler ones, resulting in a greater migration of GREj on the plate. Another variation was related to the conversion of complex sugars, such as sucrose, into simpler sugars, like glucose and fructose. This has led to a greater movement of the sample in the mobile phase and consequently an increase in the Rf value (Sun et al., 2015). The pattern was like the first TLC analysis but with a higher movement maybe to the longer fermentation period that had a higher impact on the disintegration of larger molecules.

To evaluate the antioxidant properties of the GREj before and after fermentation, the DPPH analysis was performed. The purple color of DPPH changes when combined with substances possessing antioxidant properties, resulting in a lighter yellow color. The change in color corresponds to the quantity of these compounds, allowing for the quantitative measurement of antioxidant properties by comparing light absorption to standard substances. Figure 3 shows that the results before and after fermentation are significantly different, starting with a value of 47.66 ± 0.24% DPPH inhibition and resulting in an increase, after fermentation, of 60.97 ± 0.08% DPPH inhibition. It is well known that the organic acids produced during fermentation can have potential antioxidant effects as they can donate electrons and neutralize free radicals, and this easily explain how the % of DPPH inhibition is increased (Wei et al., 2023) as well as the other transformed/converted substances that presented in juices after fermentation as observed from TLC analysis.

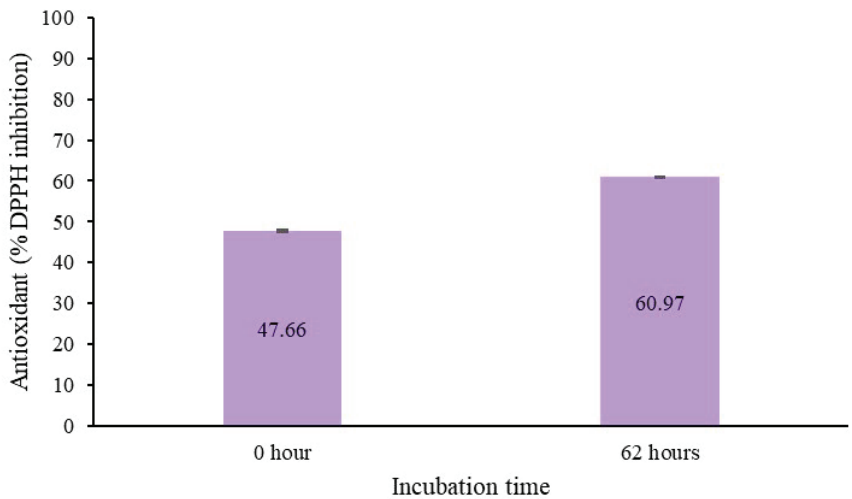


Figure 3. The percentage of inhibition DPPH of GREj before and after fermentation in 3 L bioreactor at 30 °C for 62 hours.

For the analysis of the bioactive compounds, particularly the phenolic compounds, of the GREj before and after fermentation, the TPC analysis was performed. This chemical compound turns from yellow to blue as it accepts electrons from phenolic compounds in the sample. Figure 4 indicates that the results before and after fermentation were significantly different, starting with a value of 1,578 ± 0.02 mg GAL/L and resulting in a decrease, after fermentation, of 1,271.30 ± 0.16 mg GAL/L. During fermentation, the involved yeasts can metabolize or modify polyphenolic compounds. For instance, they can hydrolyze or transform polyphenols into different compounds, and this can explain why there was a reduction in the total amount of them (Suazo et al., 2014). However, several studies demonstrated that small molecules of phenolic compounds hydrolyzed from yeast could be better absorbed in human small intestine than natural plant phenolic compound (Chanprasartsuk et al., 2010).

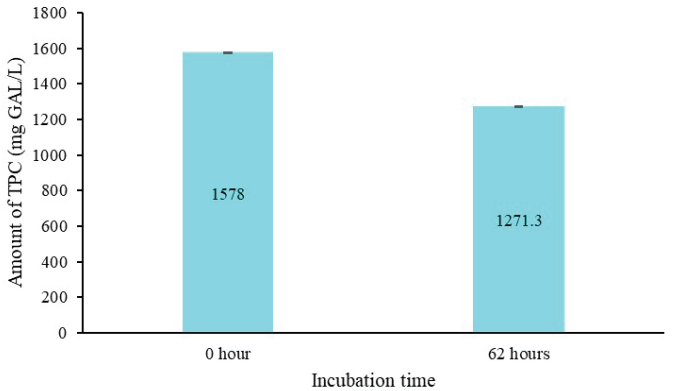
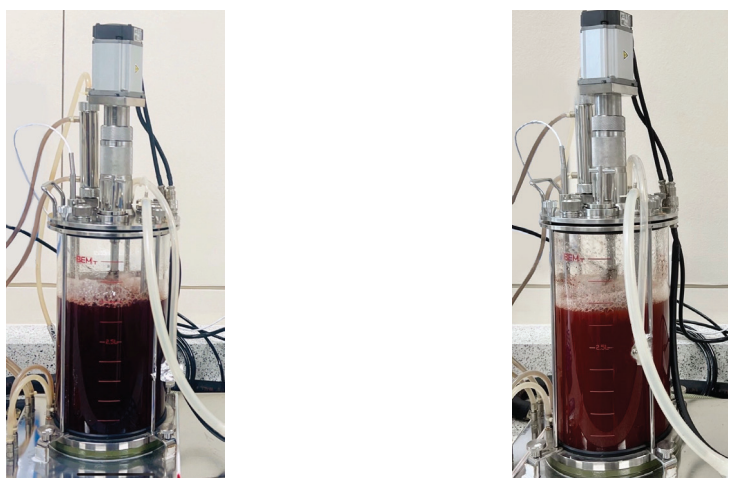


Figure 4. The amount of total phenolic compound (TPC) of GREj before and after fermentation in 3 L bioreactor at 30 °C for 62 hours.



(a) (b)

Figure 5. *Garcinia mangostana* rind extract juice (GREj) in 3 L bioreactor (a) before and (b) after fermentations at 30 °C for 62 hours.

The results confirmed that yeast fermentation significantly enhanced the antioxidant properties of the GREj due to yeast activity as previously described. The fermented GREj, as prepared in this study, displayed a vibrant red-purple hue (Figure 5), accompanied by a fermented aroma and a pleasantly sour taste profile. Based on partial sensory evaluation, yeast fermentation likely boosted the juice's aroma and taste by adding complexity and acidity while reducing its astringency. These distinct qualities position the fermented juice for further refinement into a functional beverage. Moreover, there is potential to process it into a powdered form, expanding its versatility as a functional ingredient or dietary supplement.

This research highlights the promising role of yeast fermentation in adding value to mangosteen peel. By utilizing this biotechnological approach, mangosteen waste can be transformed into a valuable resource for creating functional beverages and nutritious meal components. This not only helps reduce waste but also promotes the development of innovative and health-enhancing products, aligning with sustainable food production principles.

4. Conclusion

The study results point towards a potential strategy: optimizing yeast fermentation to convert Garcinia mangostana peel extract juice (GREj), thereby improving its aroma and taste. Furthermore, this fermentation process has the capacity to elevate GREj's antioxidant qualities, potentially leading to the development of a novel health beverage or functional ingredient. This innovative approach may offer a sustainable solution for valorizing agricultural waste, contributing positively to economic and environmental considerations.

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