

Antioxidant Properties of *Mandai* Prepared at Different Fermentation Time

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Abstract

Mandai is a fermented product made from the inner skin of *chempedak* (*Artocarpus integer*). The influence of traditional fermented food as functional food has significantly increased due to its health-promoting properties. However, there is still a lack of studies on the antioxidant properties of *Mandai* prepared at different fermentation times. This study is significant as fermentation time is a critical factor in determining the antioxidant properties of *Mandai*. This study investigates the antioxidant properties of *Mandai*, a traditional fermented food prepared at different fermentation times. The primary objective is to evaluate the antioxidant properties of *Mandai* prepared at different fermentation times and identify the optimal fermentation time for maximizing the antioxidant properties of *Mandai*. The samples of *Mandai* were prepared in the laboratory by using the inner skin of *Artocarpus integer* (*chempedak*). The samples were then fermented for 0, 7, 14, 21, and 28 days. The antioxidant properties of *Mandai* were measured using the 2,2-diphenyl-1-picrylhydrazyl (DPPH) assay and Ferric Reducing Antioxidant Power (FRAP) assay. The DPPH assay showed that the antioxidant activity was highest at 58.63±5.29% after 7 days of fermentation, compared to 52.06±0.88% at day 0. The activity then decreased to 46.71±0.14% on day 14, 32.91±1.53% on day 21, and 34.64±6.74% on day 28. In contrast, the FRAP assay demonstrated the highest antioxidant activity (62.13±1.59%) at 14 days of fermentation, starting from 29.74±1.52% at day 0, and followed by a reduction to 48.51±2.60% at day 21 and 32.67±3.42% at day 28. These findings suggest that 7 days of *Mandai* fermentation is recommended as the optimal fermentation time as it demonstrated positive effects from both DPPH and FRAP assays. In conclusion, the result showed that *Mandai* prepared at different fermentation times highlight the valuable source of antioxidants. Future studies should standardize other fermentation condition, investigate the effect of temperature and pH and identify specific bioactive compounds contributing to antioxidant activity as well as the biochemical changes during fermentation.

1. Introduction

The utilization of natural antioxidants found in fruits and vegetables [1] has gained significant attention due to their health-promoting benefits and application in food preservation. *Artocarpus* is a large genus of the Moraceae family that comprises over 50 species [1] with a high prevalence in Southeast Asia, particularly Malaysia, Thailand,

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Vietnam, and Indonesia [2]. Previous studies mentioned that utilizing the inner peel of *chempedak* in nutritional food innovation can help reduce agricultural waste [3]. According to [4], *Mandai* is a fermented product made from the inner skin of *chempedak* (*Artocarpus integer*). *Mandai* has been traditionally consumed in South and East Kalimantan, Indonesia, and Banjar community in Malaysia [3, 5]. Typically, it is used as a seasoning and consumed as a supplementary dish with rice [5], serving as a reliable food source due to its high salt content from fermentation [6].

The inner skin of unfermented *chempedak* contains bioactive components like carotenoids, phenolics, and flavonoids which provide higher antioxidant activity than the flesh and seeds [3]. Despite abundant antioxidant-rich food, continuous research on antioxidants is still significant. For example, *Mandai* contains phenolics, flavonoids, tannins, and antioxidant activity that may function as probiotic food [3]. While there is a growing interest in the health benefits of traditional fermented foods, there is a lack of comprehensive studies specifically focusing on the antioxidant properties of *Mandai* prepared at different fermentation times. Previous research has highlighted the presence of bioactive compounds in *Mandai* [3], but the optimal fermentation time to maximize its antioxidant properties remains unclear. Thus, this study aims to evaluate the antioxidant properties of *Mandai* prepared at different fermentation times (0, 7, 14, 21, and 28 days) and to determine the optimal fermentation time for maximizing the antioxidant properties of *Mandai*.

Fermentation, an anaerobic process where carbohydrates are converted into alcohol or organic acids by bacterial enzymes, enhances the bioactive compounds and functional properties of plant-based food materials [5], [7]. *Mandai* fermentation typically occurs at 23 to 25°C for 7 to 14 days [5] and can be stored for 1-2 years [6]. Fermentation time is critical as it can affect the number of beneficial bacteria and their health benefits. The fermentation time of yogurt that is too short results in low probiotic bacteria, while too long fermentation can lead to the overgrowth of harmful bacteria [8]. In addition, [9] stated that fermentation increases antioxidant activity by releasing more phenol compounds through sugar hydrolysis by lactic acid bacteria.

Previous studies highlighted that biochemical changes during fermentation can alter the composition of plant secondary metabolites, affecting properties such as antioxidant activity [10]. Research on the antioxidant properties of *Mandai* contributes to Sustainable Development Goal (SDG) 2: Zero Hunger, promoting food security and sustainable agriculture [11]. Besides, *Mandai* can serve as a meat substitute due to its similar texture [4]. In food processing industries, such as manufacturing dried fruit, jams, and juices that use fruits as raw materials, fruit peel is the primary waste product [12]. The utilization of agricultural food wastes and byproducts for recycling can promote sustainable food production and the circular economy [13].

2. Materials and Methods

Chempedak fruit was collected from the nearby fruit shop in Pagoh, Johor. The chemicals used were absolute ethanol, ascorbic acid, 2,2-dephenyl-1-picrylhydrazyl (DPPH), acetate buffer, Acetic Acid, Sodium acetate, 2,4,6-Tris(2-pyridyl)-s-triazine Acid (TPTZ), Hydrochloric acid (HCl), Iron (III) chloride, and Iron (II) Sulphate. The apparatus used in this study was a drying oven, UV-Vis spectrophotometer, water bath, weighing balance, pH meter, vortex mixer, and fume hood.

2.1 *Mandai* preparation

The ripe *chempedak* fruit was peeled and separated from its husk and flesh, then washed and cut into pieces [14]. The pieces of *chempedak* inner skin were boiled at 100 °C for 5 minutes to remove the sap. The sample was drained and boiled again in a sealed container at 100 °C for 5 minutes. The sample was allowed to cool until the temperature was below 40 °C. 75 g of salt and 625 ml of water were added to the inner skin of the *chempedak*, which had been removed from the sap [4]. The spontaneous *Mandai* were stored in an autoclaved screw-tight glass jar for 0 days, indicating no fermentation, 7 days, 14 days, 21 days, and 28 days at ambient temperature (27 °C) to allow slow fermentation. Traditional practices of *Mandai* fermentation typically involve a fermentation period ranging from 7 to 14 days [5]. Previous studies have shown that different fermentation times can significantly impact the bioactive compounds and antioxidant properties of fermented foods [5, 7]. Specifically, short-term fermentation (around 7 days) has been found to enhance antioxidant activity, while longer fermentation periods (up to 28 days) can lead to changes in microbial communities and biochemical properties [8]. These intervals were selected to systematically investigate the changes in antioxidant properties of *Mandai* over a range of fermentation times and to identify the optimal fermentation period for maximizing its health benefits. After the fermentation period, *Mandai* was drained and blended. The puree of *Mandai* was dried for 18 hours at 45 °C in the oven, then ground and screened with an 80-mesh sieve [3]. The drying process was repeated every 14 days, 21 days, and 28 days of fermentation times.

2.2 *Mandai* extraction

The *Mandai* samples were extracted using the method described by [3] with some modifications. The samples of *Mandai* were weighed 10 g and dissolved in 30 ml absolute ethanol, then macerated for 24 hours. The mixture

was then filtered using filter paper and the liquid extract was air dried at room temperature (27 °C) for 3 weeks in the fume hood. The crude extract was stored at 4 °C in the chiller until further analysis on the determination of antioxidant properties.

2.3 Antioxidant Evaluation

2.3.1 2,2-diphenyl-1-picrylhydrazyl (DPPH)

The DPPH assay measures the antioxidant properties of compounds by their ability to scavenge the radical anion 2,2-diphenyl-1-picrylhydrazyl (DPPH) [8, 15]. As antioxidants donate hydrogen atoms or electrons to neutralize DPPH radicals, the colour changes from purple to yellow [16-17]. 1 mM DPPH stock solution was made by dissolving 39.4 mg of DPPH in 1 L of ethanol. Then, the standard was diluted into different concentrations (1 µg/mL, 2 µg/mL, 3 µg/mL, 4 µg/mL, 5 µg/mL). 2 mL of 1 mM DPPH solution was mixed with 1 mL of *Mandai* extract in a test tube. After that, the mixtures were shaken vigorously and kept in complete darkness for 30 min. The absorbance was measured at 517 nm by using a UV-Vis spectrophotometer. Ascorbic acid was used as a standard under the same assay condition. The assay was done in triplicate. The DPPH free radical scavenging activity was calculated by using the following equation (1).

$$\text{DPPH Radical Scavenging activity (\%)} = \frac{\Delta C - \Delta S}{\Delta C} \times 100 \quad (1)$$

Where ΔC is the absorbance reading of the negative control, and ΔS is the absorbance reading of the sample.

2.3.2 Ferric Reducing Antioxidant Power (FRAP)

[8] described FRAP assay as measuring the antioxidant capacity by studying the reduction of the complex ferric tripyridyl triazine (FeIII-TPTZ) at low pH. The FRAP reagent was prepared according to the method outlined by [18]. FRAP working solution was prepared by mixing 10 volumes of acetate buffer (300 mM, pH 3.6) with 1 volume of TPTZ (40 mM dissolved with 40 mM HCl) and 1 volume of ferric chloride (20 mM in ethanol). Then, it was incubated in a water bath at 37 °C for five minutes. The standard was prepared using ferrous sulphate. 1.5 mL of FRAP reagent was added into a cuvette, and an initial blank reading was obtained using a spectrophotometer at an absorbance of 593 nm. The standard was diluted to different concentrations (1 µg/mL, 2 µg/mL, 3 µg/mL, 4 µg/mL, 5 µg/mL). Then, 50 µL of each sample extract, or standard, was mixed with 1.5 mL of FRAP reagent. After mixing the FRAP reagent with the sample extract or standard, the absorbance was measured after 30 minutes at 593 nm. A standard curve of FRAP values was plotted against the concentration of each standard. This value represents the antioxidant concentration with ferric-reducing activity [19]. Ferrous sulphate (FeSO₄) was used as a standard. The assay was done in triplicate, and the percentage of ferric-reducing antioxidant activity was calculated based on the following equation (2).

$$\text{FERIC reducing antioxidant activity (\%)} = \frac{\Delta C - \Delta S}{\Delta C} \times 100 \quad (2)$$

Where ΔC is the absorbance reading of the negative control, and ΔS is the absorbance reading of the sample.

2.4 Statistical analysis

All the experiments were performed in triplicates, and the results were expressed as means \pm standard deviation (SD). Data analysis was conducted using Microsoft Excel. The differences between the mean values were analyzed using one-way Analysis of Variance (ANOVA). The ANOVA test was used to determine if there were any statistically significant differences between the antioxidant activities of *Mandai* at different fermentation times. A *p*-value of less than 0.05 was considered statistically significant, indicating that the differences observed were unlikely to have occurred by chance.

3. Results and Discussion

3.1 Antioxidant Evaluation

3.2 2,2-diphenyl-1-picrylhydrazyl (DPPH)

Based on Table 1 and Fig. 1, the DPPH radical scavenging activity was 52.06 ± 0.88 % at the start of fermentation (day 0). This indicates moderate antioxidant activity in the fresh sample [20]. After 7 days of fermentation, the scavenging activity increased significantly to 58.63 ± 5.29 % suggesting that fermentation enhanced the

production of bioactive compounds, such as phenolics or flavonoids, which contribute to antioxidant activity [14]. In *Mandai*, phenolic compounds such as gallic acid have been identified [3]. Quercetin and catechins are both flavonoids that have been found in *Mandai* [21]. This peak activity corresponds with findings from other studies, which indicate that short-term fermentation can enhance antioxidant capacity [22]. The microbial activity and enzymatic hydrolysis that release bioactive compounds from their precursors contribute to this enhancement [1]. The scavenging activity of *Mandai* decreased gradually after 7 days, reaching 46.71 ± 0.14 % on day 14, 32.91 ± 1.53 % on day 21, and 34.64 ± 6.74 % on day 28, respectively. This decline may be due to the degradation or consumption of antioxidant compounds by microbial metabolism during extended fermentation [23]. The observed slight increase in scavenging activity at day 28, following a decrease until day 21, may be due to the complex interplay of microbial metabolism, transformation of polyphenolic compounds, and changes in microbial community dynamics during the fermentation process. *Mandai* also contains tannins [1] which are polyphenolic compounds with strong antioxidant properties that can bind to proteins and other organic molecules. According to [24], the polymerization of polyphenolic compounds may decrease the antioxidant activity, which reduces their detectable content. However, depolymerization can occur over extended fermentation periods, which breaks down these complex polyphenols into simpler forms with enhanced antioxidant properties. Research has indicated that antioxidant activity can vary throughout fermentation. For example, kombucha beverages demonstrate higher antioxidant activity during the initial days of fermentation, then decline and, in some cases, increase after prolonged fermentation [25]. Additionally, the concentration of bioactive compounds tends to increase over longer fermentation periods, which increases antioxidant activity [26]. Changes in microbial communities can also result in different metabolites that enhance antioxidant activity [25]. The fermentation times showed no significant difference ($p > 0.05$) in the antioxidant properties of *Mandai*.

This study observed the highest scavenging activity on 7 days of *Mandai* fermentation (58.63 ± 5.29 %). The DPPH radical scavenging activity decreased after 7 days of fermentation. The reduction in antioxidant activity during prolonged fermentation might be resulted from oxidative reactions, thermal degradation, or conversion of active compounds into less effective forms. Extended fermentation can lead to oxidative stress, which oxidates antioxidant compounds, thus reducing their functionality [27]. In addition, active antioxidant compounds can be converted into less effective forms through microbial and chemical changes within the fermentation medium over time [23]. The content of polyphenols (gallic acid, tannic acid, catechin) may be preserved by lowering the drying temperature to increase further the antioxidant activity [21]. The peak antioxidant activity observed at 7 and 14 days of *Mandai* fermentation aligns with findings from other fermented foods. For instance, kombucha, a fermented tea, has been shown to exhibit peak antioxidant activity around 7 to 14 days of fermentation, after which the activity may decline or stabilize [25]. Similarly, fermented soy products like miso and tempeh also demonstrate increased antioxidant activity during the initial stages of fermentation, attributed to the release of bioactive compounds by microbial activity [9, 10]. These comparisons suggest that the fermentation process enhances the antioxidant properties of various foods by increasing the availability of phenolic compounds and other antioxidants.

The results of this study are consistent with previous research on the antioxidant properties of fermented foods. For example, [3] found that the antioxidant activity of *Mandai* increased significantly during the initial stages of fermentation, with peak activity observed around 7 to 14 days [3]. This study also aligns with findings from [7], who reported that fermentation enhances the release and production of bioactive components, leading to increased antioxidant activity [5]. The observed decline in antioxidant activity after 14 days is also supported by literature, which suggests that prolonged fermentation can lead to the degradation or consumption of antioxidant compounds by microbial metabolism [23].

Table 1 Antioxidant activity of Mandai at different fermentation times

| Time (Days) | DPPH radical scavenging activity (%) |
|-------------|--------------------------------------|
| 0 | 52.06 ± 0.88 |
| 7 | 58.63 ± 5.29 |
| 14 | 46.71 ± 0.14 |
| 21 | 32.91 ± 1.53 |
| 28 | 34.64 ± 6.74 |

Values are given as mean \pm SD from triplicate

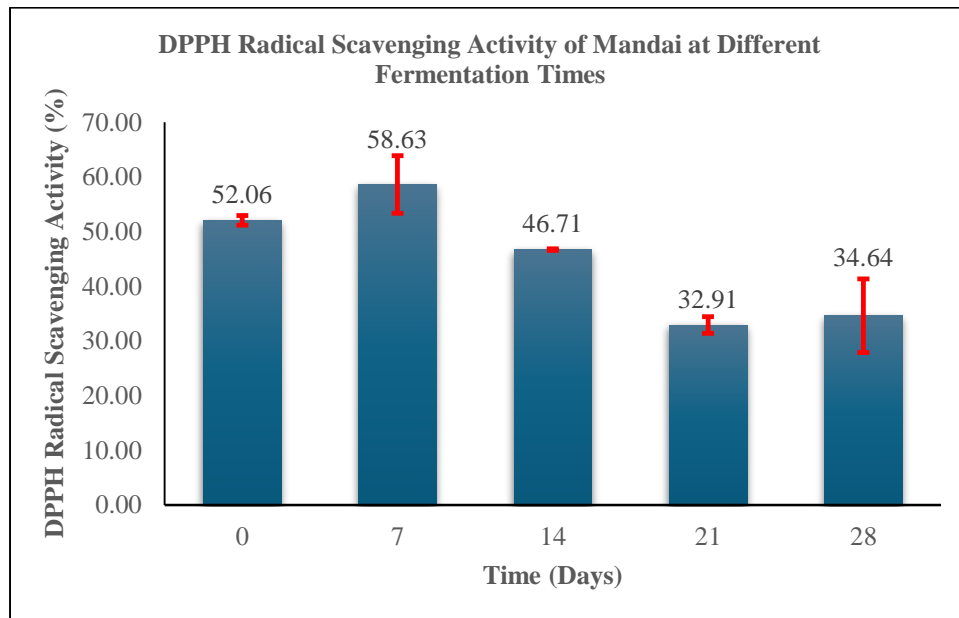


Fig. 1 DPPH radical scavenging activity of Mandai at different fermentation times

3.3 Ferric Reducing Antioxidant Power (FRAP)

Table 2 and Fig. 2 show that the highest ferric-reducing activity was observed on day 14 (62.13 ± 1.59 %). At day 0, the ferric-reducing activity starts relatively low at 29.74 ± 1.52 %, which is expected since unfermented *Mandai* may contain limited amounts of bioactive compounds or antioxidants such as carotenoids, phenolics, and flavonoids [3]. The unfermented inner skin of *chempedak* (*Artocarpus integer*) contains significant amounts of carotenoids, particularly α -carotene and β -carotene [28]. The initial fermentation process after 7 days likely facilitates the release of antioxidant compounds, leading to a marked increase in antioxidant activity (41.06 ± 1.05 %). According to [29], this increase may be due to the degradation of complex compounds into relatively smaller, bioavailable antioxidants through microbial enzymatic activities. The highest antioxidant activity was observed on day 14 (62.13 ± 1.59 %), indicating the optimal point of fermentation. This result is consistent with other studies establishing mid-stage fermentation as the most efficient stage for releasing phenolic compounds such as gallic acid, which are key contributors to antioxidant activity [30]. The increased antioxidant activity may also be related to the synthesis of new antioxidant metabolites by fermenting microbes [29]. However, this antioxidant activity reduces to 48.51 ± 2.60 % at day 21 and further decreases at day 28 (32.67 ± 3.42 %). This decrease may result from the breakdown of bioactive compounds caused by over-fermentation or the depletion of fermentable substrates. Similar patterns have been noted for other fermented foods in which extended fermentation reduces the availability of beneficial compounds [29, 31]. The results from the ANOVA analysis show that *Mandai* prepared at different fermentation times showed no significant difference in the antioxidant properties ($p > 0.05$).

Table 2 FERIC reducing activity of Mandai at different fermentation times

| Time (Days) | FERIC Reducing Activity (%) |
|-------------|-----------------------------|
| 0 | 29.74 ± 1.52 |
| 7 | 58.63 ± 5.29 |
| 14 | 46.71 ± 0.14 |
| 21 | 32.91 ± 1.53 |
| 28 | 34.64 ± 6.74 |

Values are given as mean \pm SD from triplicate

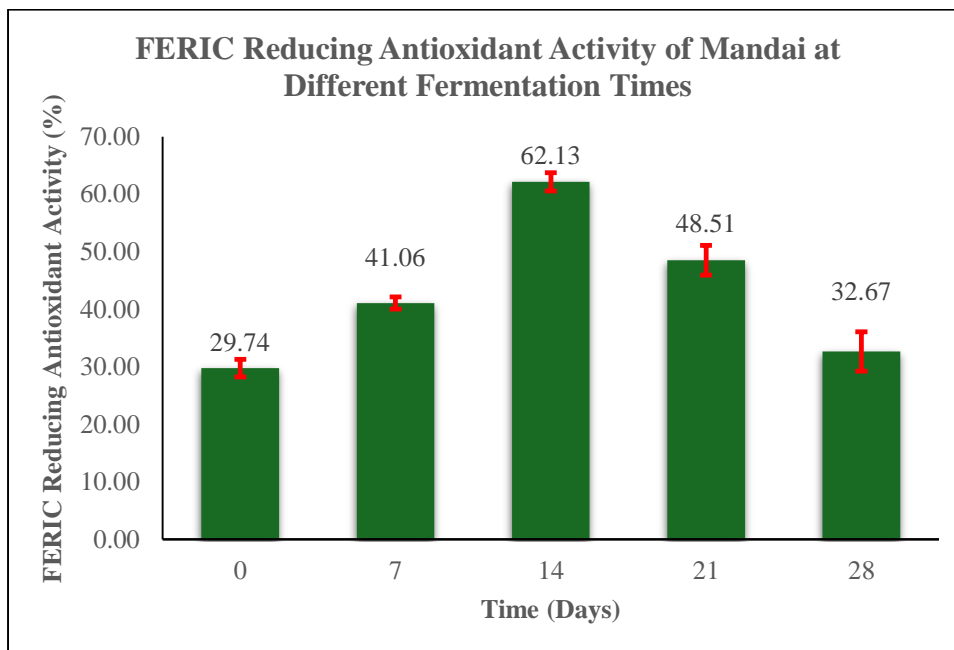


Fig. 2 FERIC reducing antioxidant activity of Mandai at different fermentation times

3.4 Implications for Food Preservation and Product Development

The findings of this study have significant implications for food preservation and product development. The highest antioxidant activity on days 7 from the DPPH assay and days 14 on the FRAP assay indicates that short-term fermentation can effectively maximize the health benefits of fermented products. This is particularly important for the food industry, as enhancing the antioxidant properties of products can improve their nutritional value and attract health-conscious consumers [5]. Additionally, maintaining antioxidant activity through controlled fermentation processes can extend product shelf life, reduce food waste, and promote sustainability [3]. For product development, the insights from this study can provide guidance on the manufacture of new fermented food products that benefit from the health benefits of *Mandai*. By optimizing fermentation conditions, manufacturers can develop products with enhanced antioxidant properties, meeting the increasing demand for functional foods. Moreover, *Mandai* can be utilized as a natural antioxidant source in various food applications, including functional beverages, snacks, and dietary supplements.

4. Conclusion

The study highlights the potential of *Mandai* prepared at different fermentation times as a valuable source of antioxidants. The antioxidant evaluation shows that the highest antioxidant properties of *Mandai* are around 7 days for the DPPH assay and 14 days for the FRAP assay. The difference between the results obtained in both assays may be influenced by the characteristics of the assay used. The best antioxidant should have demonstrated multiple mechanisms such as the ability of antioxidants to neutralize free radicals by donating electrons or hydrogen atoms (radical scavenging activity) and the ability of antioxidants to reduce ferric (Fe^{3+}) ions to ferrous (Fe^{2+}) ions (reducing power). In this study, 7 days of *Mandai* fermentation is recommended as the optimal fermentation time as it demonstrated positive effects from both DPPH and FRAP assays. These findings are useful for food industries and manufacturers aiming to maximize the health benefits of fermented products. Further research should consider identifying the specific bioactive compounds contributing to the antioxidant activity and their changes during fermentation. Methods to identify bioactive compounds include High Performance Liquid Chromatography (HPLC), Gas Chromatography (GC), and Mass Spectrometry (MS). Additionally, testing the antioxidant activities under different temperatures, pH, and light exposure can help to understand their stability and effectiveness. Specific temperatures to test could include 20°C, 25°C, and 30°C, as these ranges are commonly used in fermentation studies to observe the effects on microbial activity and bioactive compound production.

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Conflict of Interest

Authors declare that there is no conflict of interests regarding the publication of the paper.

Author Contribution

The authors confirm contribution to the paper as follows: **study conception and design:** Nurul Aina Natasha Abdul Kadir, Siti Fatimah Sabran; **data collection:** Nurul Aina Natasha Abdul Kadir; **analysis and interpretation of results:** Nurul Aina Natasha Abdul Kadir, Siti Fatimah Sabran; **draft manuscript preparation:** Nurul Aina Natasha Abdul Kadir, Siti Fatimah Sabran. All authors reviewed the results and approved the final version of the manuscript.

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