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Acetic Acid Fermentation of Kuini (*Mangifera odorata*) and Its Potential Substrate for Human Health

(Fermentasi Asid Asetik bagi Kuini (*Mangifera odorata*) dan Potensi Substratnya untuk Kesihatan Manusia)

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ABSTRACT

The kuini (Mangifera odorata) fruit has a strong scent, attractive orange-yellow colour of flesh and sweet-sour taste. Since ages, kuini parts have been used in folk medicine such as fever indicated that kuini contains prophylactic measures against certain illness and rich of beneficial constituents such as phenolics. In this study kuini underwent fermentation using inoculum (GAB) containing acetic acid bacteria of Gluconacetobacter sp. and the changes during fermentation were then observed. The objectives were (1) to identify the potential of kuini flesh as substrate to GAB inoculum, and (2) to evaluate the physicochemical and nutritional changes during fermentation process. Fermentation of kuini flesh had changed the acidity and affects the growth of inoculum and its biocellulose yield. Results indicated that fermentation using 3% of kuini substrate (KF3) exhibited slightly lower pH compared to 5% of kuini substrate (KF5) while the acetic acid production in KF3 was found higher than KF5. This finding indicated that only small amount of kuini at 3% (w/v) of substrate was able to change the physicochemical property in kuini fermentation thus create a favourable environment for the growth of GAB inoculum and production of its biocellulose. Effect of sucrose in the fermentation also showed that acidity and growth of inoculum were increased with increase of sucrose concentration however depressed the yield of biocellulose. Sucrose at 5 to 20% (w/v) in kuini fermentation had given no distinct effect on the pH and acetic acid content. During fermentation the GAB inoculum had exhibited a poor growth in control, a high growth at 5% (w/v) sucrose and moderate growth between 10-20 % (w/v) sucrose in kuini substrates. The control and kuini substrate at 5% (w/v) sucrose also exhibited a high production of biocellulose (13.7 %) whereas high sucrose content in kuini substrate had exhibited low production of biocellulose (5.1%). The increase in sucrose concentration was found concurrently enhanced the total phenolic content and antioxidant activity during the fermentation. High total phenolic content (200 mg/mL GAE) was obtained from 15-20% (w/v) sucrose with control exhibited the least with less than 100 mg/mL GAE. All samples had exhibited high antioxidant activity at which the addition of sucrose into kuini substrate had increased nearly double the antioxidant activity. In conclusion, acetic acid fermentation able to change the physicochemical and nutritional properties of kuini flesh into a health beneficial fermented kuini produced with high antioxidant activity. The fermented kuini produce also contains prophylactic property and therefore potential to be studied for human health application. In near future the antimicrobial activity of kuini substrate with bioactive property against certain bacteria causing health-illness is also interested to be studied.

Keywords: Gluconacetobacter sp.; Sucrose; Physicochemical; Total phenolic; Antioxidant

ABSTRAK

Buah kuini (Mangifera odorata) mempunyai bau yang kuat, isi warna jingga-kuning yang menarik dan rasa manis masam. Sejak dahulu, bahagian kuini telah digunakan dalam perubatan manusia, menunjukkan kuini mengandungi nilai profilaktik ke atas penyakit tertentu dan kaya sebatian bermanafaat. Dalam kajian ini kuini melalui fermentasi asid asetik menggunakan inoculum (GAB) yang mengandungi bakteria asid laktik Gluconacetobacter sp. dan perubahan semasa fermentasi dipantau. Objektif kajian (1) mengenalpasti potensi isi kuini sebagai substrat kepada inokulum GAB, dan (2) menilai perubahan fisikokimia dan nutrisi semasa proses fermentasi. Fermentasi isi kuini telah mengubah keasidan dan memberi kesan kepada pertumbuhan inokulum dan hasil bioselulos. Keputusan menunjukkan fermentasi menggunakan 3 % substrat kuini (KF3) memberikan pH yang sedikit rendah berbanding 5 % substrat kuini (KF5) manakala penghasilan asid asetik dalam KF3 juga didapati tinggi berbanding KF5. Penemuan ini menunjukkan hanya dengan kuantiti yang

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sedikit kuini berupaya untuk mengubah nilai fisikokimia dalam fermentasi kuini seterusnya menyediakan persekitaran yang disukai untuk pertumbuhan inokulum GAB dan penghasilan bioselulusnya. Kesan sukros di dalam fermentasi juga menunjukkan keasidan dan pertumbuhan inokulum meningkat dengan peningkatan kandungan sukros tetapi merencatkan hasilan bioselulos. Sukros pada 5 – 20% (b/i) dalam fermentasi kuini tidak memberi kesan ke atas pH dan kandungan asid asetik. Semasa fermentasi inokulum GAB telah menunjukkan pertumbuhan yang lemah di dalam kontrol, pertumbuhan tinggi pada 5% (b/i) sukros dan pertumbuhan pertengahan diantara 10-20% (b/i) sukros dalam substrat kuini. Kontrol dan substrat kuini pada 5% (b/i) sukros memberikan penghasilan tinggi bioselulus (13.7%) manakala kandungan sukros yang tinggi telah memberikan penghasilan bioselulus yang rendah (5.1%). Peningkatan kepekatan sukros didapati telah meningkatkan kandungan jumlah fenolik dan aktiviti antioksidan semasa fermentasi. Kandungan jumlah fenolik yang tinggi (200 mg/mL GAE) telah didapati daripada 15-20% (b/i) sukros dengan control menyumbangkan nilai terendah dengan kurang daripada 100 mg/mL GAE. Kesemua sampel telah mempamerkan aktiviti antioksidan yang tinggi dengan menunjukkan penambahan sukros ke dalam substrat kuini telah meningkatkan hampir dua kali aktiviti antioksidan. Kesimpulan, fermentasi asid asetik berupaya mengubah nilai fisikokimia dan nutrisi isi kuini kepada hasilan kuini difermentasi dengan aktiviti antioksidan. Kuini difermentasi yang dihasilkan juga mengandungi nilai profillaktik dan berpotensi dikaji untuk penggunaan kesihatan manusia. Dalam masa akan datang aktiviti antimicrobial keatas substrat kuini dengan nilai bioaktif terhadap bakteria tertentu penyebab penyakit juga menarik untuk dikaji.

Kata kunci: Gluconacetobacter sp.; Sukros; Fisikokimia; Jumlah fenolik; Antioksidan

INTRODUCTION

Kuini (*Mangifera odorata*) is mentioned as a hybrid between *M. indica* and *M. foetida* (Orwa et al. 2009). The flesh is less fibrous, juicy and much appreciated as table fruit. Approximately 70% of kuini is edible and consists of carbohydrate, fibre, protein, vitamins and small amount of fat in the peel (Nur Diyana et al. 2017).

In this study, the nutritional constituents of kuini as fresh and fermented produce are important to be studied. To date this is the first paper discussed on fermentation of kuini. Thus, the aims are to identify the physicochemical characteristics and biochemical properties of kuini during the fermentation. Acetic acid fermentation was previously developed as a selective bioprocessing method for kuini (Adnan et al. 2017). The acetic acid fermentation was done using an inoculum namely GAB as a separate process which involved the (1) basic fermentation, and (2) enrich fermentation with an addition of sucrose as growth substance.

In the basic fermentation, kuini substrate was inoculated with the GAB starter culture and then underwent fermentation. Whereas, in the enrich fermentation an amount of sucrose was added into kuini substrate and then inoculated with the GAB starter culture. The solution was mixed well before underwent fermentation. Sucrose acts as a carbon source for the growth of the starter culture (Chen and Liu, 2000).

Fermentation using suitable amount of GAB starter culture and specific growth condition would affect the physicochemical property of fermented kuini. During fermentation, starter cultures could extract the kuini substrate into small components and transformed the components into beneficial fermented kuini substrate. The bioactivity exhibited by the fermented kuini offer a potential fermented substrate containing of bioactive compounds that could beneficial to human health.

METHODOLOGY

PREPARATION OF SAMPLES

Matured kuini (*Mangifera odorata*) fruit was obtained from MARDI Bukit Tangga, Kedah. The accession used in this study was Acc.103 (Kijal) and verified based on the herbarium voucher specimen No. 12838 (Herbarium MDI/MARDI), collected from Kijal, Terengganu in 2002.

In the sample preparation, the fruits were washed, air dried and peeled off. The flesh was sliced and blends as puree and then dried in a dryer at 40ºC for 2 days with approximately 10% (w/v) moisture content. Dried kuini flesh was blend as fine powder and sample was packed in a plastic bag, sealed and labelled.

FERMENTATION PROCESS

The fermentation process for kuini was conducted as mentioned below. The inoculum (GAB) containing a mixed culture of mainly *Gluconacetobacter sp.* and a small amount of yeast was obtained from Collection of Functional Food Culture (CFFC) MARDI. In the preparation of starter culture, the GAB inoculum was revived on a media alcohol (MA) agar and incubated for four days at 30°C and then subculture in a MA broth. Fresh grown of GAB starter culture was used in the fermentation process.

Briefly, in basic fermentation process using samples at 3 and 5% (w/v) were poured into a separate conical flask and dissolved in distilled water. Both solutions (KF3 and KF5) were pasteurised and let to cool at room temperature. The GAB starter culture at 2% (w/v) was inoculated in each solution and the solution was mixed well by slowly shake the flask. The flask was covered using sterilised Aluminium foil and ferment at 30°C for 14 days. The physicochemical properties and growth profile were then observed for both basic fermentations.

In enriched fermentation process, sample at 3% (KF3) was dissolved in distilled water and separately each solution was added with sucrose at 0, 5, 10, 15 and 20% (w/v) before underwent fermentation. The changes on the physicochemical profile, growth and biochemical properties in the enrich fermentation were then observed.

PHYSICOCHEMICAL ANALYSES AND GROWTH STUDY

Sample was analysed at interval days during fermentation period. The pH was determined using a calibrated pH meter (Mettler Toledo, Switzerland). The acetic acid content was estimated using titration method against 0.1N of sodium hydroxide (NaOH) solution (AOAC 2000) and expressed as percentage of anhydrous of acetic acid. The growth profile of GAB inoculum was measured using cell density meter (WPA Biowave, UK) which is a rapid and automated method of optical density (OD) observed at 600nm absorbance. The total growth (%, w/w) of GAB known as a layer of biocellulose was weighed at the final day of fermentation.

TOTAL PHENOLIC CONTENT AND ANTIOXIDANT ACTIVITY

The biochemical properties as such total phenolic content and antioxidant scavenging activity were determined on the fermented kuini at the end of enrich fermentation. Total phenolic content was quantified as mentioned by Singleton & Rossi (1965) using Folin-Ciocalteu method with gallic acid as standard (Nazish et al. 2017). The antioxidant scavenging activity was measured using modified method of Thaipong et al. (2006) using DPPH (1,2-diphenyl-2-picrylhydrazyl) radical with ascorbic acid as standard and expressed as percentage (%) of inhibition.

RESULTS AND DISCUSSION

Physicochemical characteristics of kuini and inoculum growth Figure 1 (a-b) show the changes in pH and acetic acid content during fermentation of kuini at 3 and 5% (w/v) of substrate. Fermentation of 3% of kuini substrate (KF3) exhibited slightly lower pH compared to fermentation for 5% of kuini substrate (KF5). The acetic acid production of KF3 was found higher than KF5. Results indicated correlation between pH and acetic acid production at which reduced of pH had gradually increased the production of acetic acid during the fermentation process.

The decreases in pH value during fermentation of kuini was probably due to the production of acetic acid and other organic acids (Dufresne & Farnworth, 2000). This paper showed that GAB inoculum was responsible to the biochemical changes of organic acids and successfully produced a mild acidic environment that suitable for kuini fermentation.

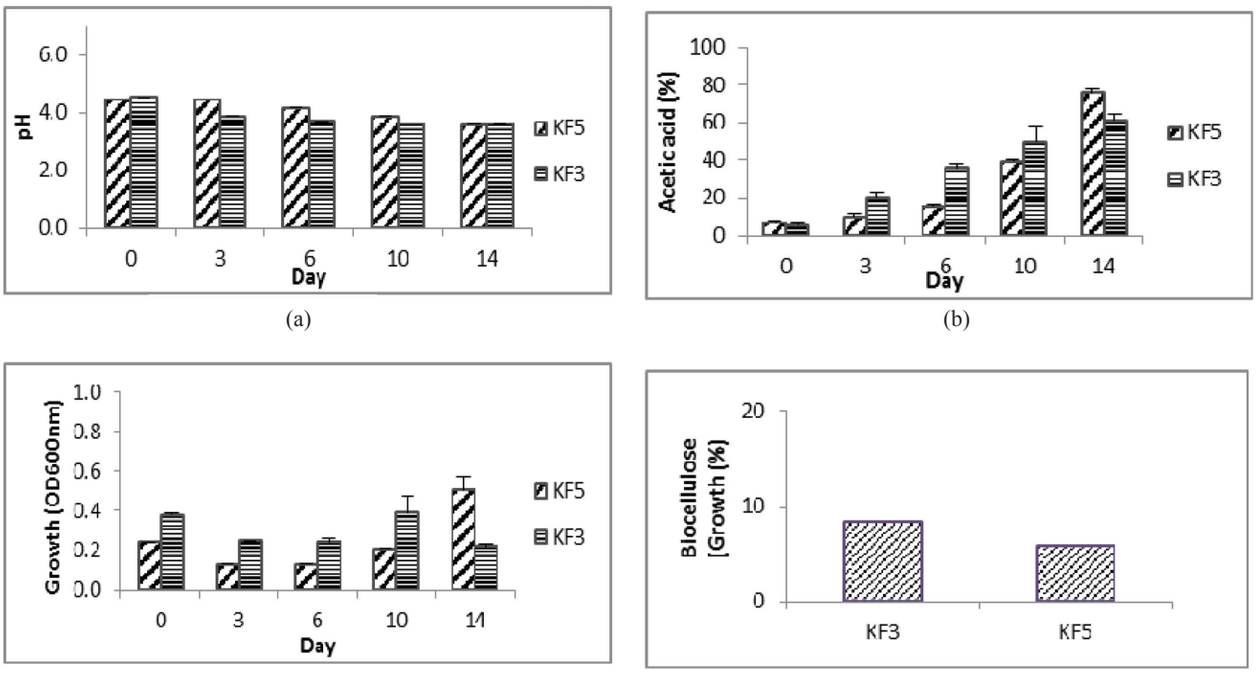


FIGURE 1. Physicochemical changes (a-b) and growth profiles of Gluconacetobacter sp. (c-d) during fermentation of kuini at 3 and 5 % (w/v) substrates.

Figure 1(c) shows the growth of GAB in kuini fermentation and total growth of GAB inoculum in the form of biocellulose (Figure 1d). Results in Figure 1(c) indicated that KF3 was able to enhance the growth of GAB in comparison to KF5. The amount of 3 % (w/v) kuini substrate was sufficient to increase the growth of GAB in comparison to 5 % kuini substrate and revealed that KF3 was the optimum value for kuini fermentation. Results in Figure 1 (d) indicated that KF3 fermentation had produced higher amount of GAB biocellulose compare to KF5 at the end of fermentation.

These findings revealed that KF3 had created a favourable environment for GAB fermentation that initiated by the growth, physicochemical changes and production of GAB biocellulose. This paper has indicated that KF3 was the best substrate for basic kuini fermentation. The fermentation study was continued with the application of sucrose as carbon source for enrich fermentation of kuini.

EFFECT OF SUCROSE ON PHYSICOCHEMICAL CHARACTERISTICS AND INOCULUM GROWTH

Figure 2(a) shows changes of pH profile on the effect of different sucrose concentrations in kuini fermentation. Results indicated that pH was gradually reduced with fermentation day in all samples. All samples had showed a similar declined pattern of pH excluding control (G0) which exhibited much higher pH values than other samples.

The changes in acetic acid profile were shown in Figure 2(b). The acetic acid content increased gradually with fermentation day. Results showed that all samples had produced high acetic acid content except control during kuini fermentation. All samples had enhanced the production of acetic acid and exhibited a similar increment pattern, except for control.

This finding indicated that addition of sucrose at 5 to 20% (w/v) in kuini fermentation gave no distinct effect on the pH and acetic acid content. Similar pattern profiles of pH and acetic acid among all samples indicated different concentration of sucrose did not affect the fermentation process. This finding had revealed that GAB inoculum was probably consumed only at a certain amount of sucrose in kuini fermentation.

During kuini fermentation, GAB inoculum could degrade sucrose into glucose and fructose and metabolised sucrose into a number of organic acids as such acetic and glucuronic acids (Yassine et al. 2016). During metabolism, GAB used glucose to produce gluconic acid and ethanol to produce acetic acid. The increased concentration of organic acid during fermentation therefore decreases pH values lower than 3.5 at the end of fermentation day (Zeng et al. 2011).

Figure 2(c) shows the growth of GAB inoculum was affected by sucrose concentration. In comparison to control, the increased of growth for GAB in all samples was parallel with sucrose concentration added into kuini substrate. Results showed that GAB exhibited poor growth in control, high growth at 5% (w/v) sucrose and moderate growth between 10-20% (w/v) sucrose in kuini substrates. This finding indicated that catabolism of sucrose by GAB could release energy that required for cell growth.

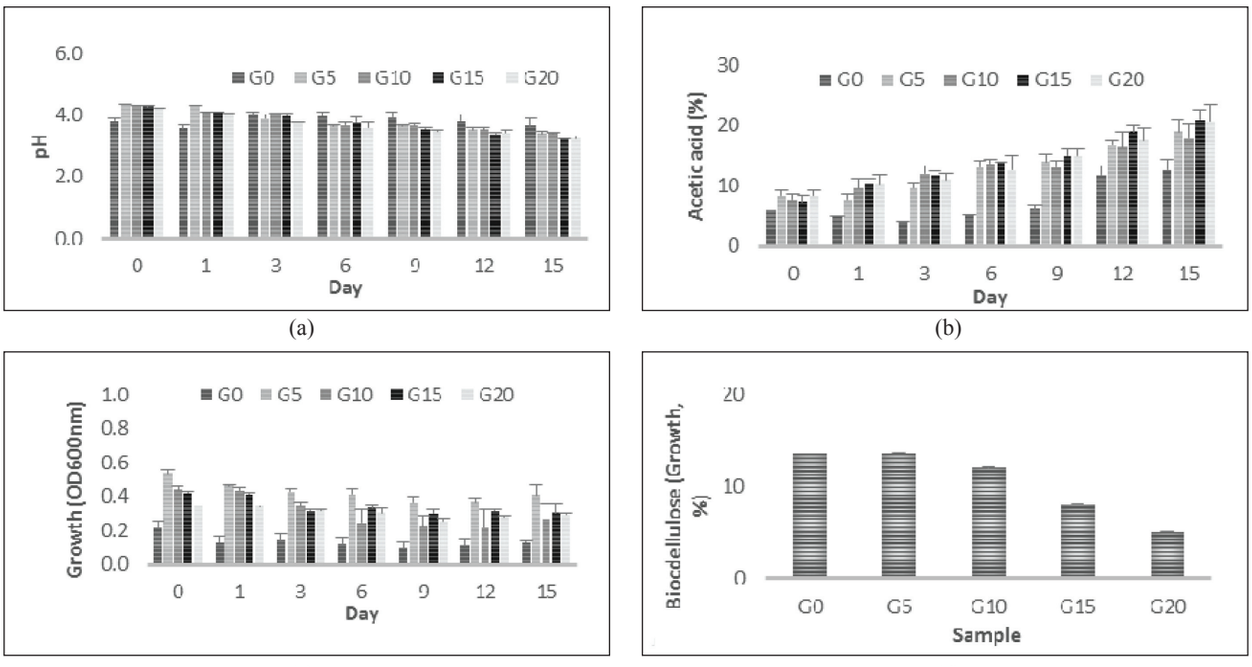


FIGURE 2: Effect of sucrose (G) concentrations (%, w/v) on the physicochemical (a-b) and the growth profiles (c-d) of Gluconacetobacter sp. (GAB) during fermentation of kuini at 3% (w/v) of substrate.

The final growth profile of GAB in the form of biocellulose against sucrose concentration was shown as in Figure 2(d). Sucrose at different concentration had effect on the production of GAB biocellulose. Results indicated that control and kuini substrate at 5% (w/v) sucrose exhibited high production of biocellulose, while high sucrose content in kuini substrate had exhibited low production of biocellulose. This finding indicated that production of GAB biocellulose can be enhanced by addition of sucrose, however exceeding at certain concentration of sucrose could depressed the production of GAB biocellulose.

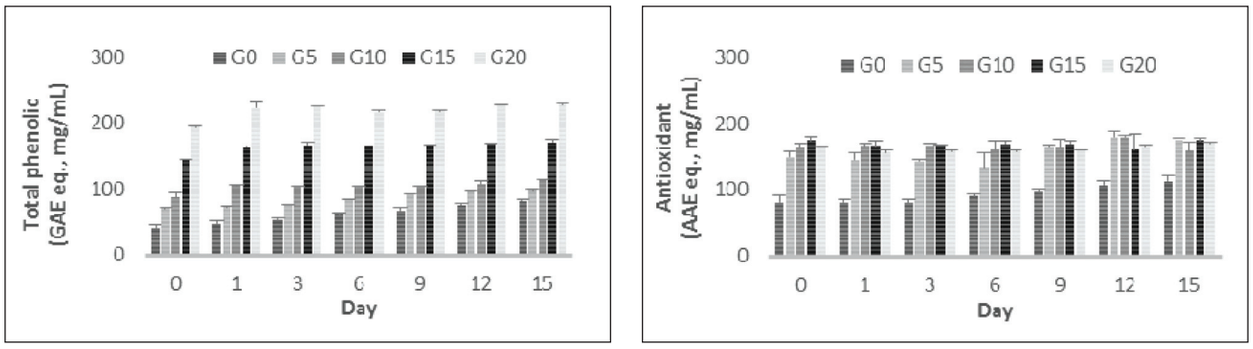
This finding showed that the effect of GAB growth and biocellulose production were probably due to buffering capacity by acetic acid content and antimicrobial activity (Cheung et al. 2010). During kuini fermentation, high content of acetic acid produced from sucrose metabolism could disturb ionic equilibrium across the bacterial cell membrane as such inhibit the cell growth, attacks cell components and later causes lethal effects on bacteria.

TOTAL PHENOLIC AND ANTIOXIDANT CONTENTS

Figure 3(a) shows the total phenolic contents in kuini fermentation at different concentration of sucrose. Results indicated that total phenolic contents increased parallel with the increased of sucrose concentration in kuini fermentation. High total phenolic content was obtained from kuini substrate with 15-20% (w/v) sucrose with more than 200 mg/mL GAE, while control exhibited the least with less than 100 mg/mL GAE of total phenolic content. This finding also showed that sucrose had exhibited a slow and gradual increment of total phenolic content during the fermentation period.

Figure 3(b) shows the antioxidant activity in kuini substrate with different concentration of sucrose. In comparison to control, all samples had exhibited high antioxidant activity as exhibited by the scavenging activity on DPPH radical. The addition of sucrose into kuini substrate had increased nearly double the antioxidant activity. The antioxidant content however exhibited similar profiles with no distinct difference among each sample.

This finding indicated that addition of sucrose into kuini substrate could therefore enhance the total phenolic contents and increased the antioxidant activity in the kuini fermentation. High total phenolic content indicated that fermented kuini had possessed antioxidant property. This finding showed that fermented kuini produced in this study contained good level of phenolic content and antioxidant activity that could be beneficial to human health.



(a) (b)

FIGURE 3: Effect of sucrose (G) during fermentation of kuini on the total phenolics content (a) measured as gallic acid equivalent (GAE eq.) and the antioxidant content (b) measured as ascorbic acid equivalent (AAE eq.)

CONCLUSION

Kuini is a potential fermentation substrate to GAB inoculum consists mainly of *Gluconacetobacter sp*. and produced beneficial phytochemicals in its fermented produce. Kuini flesh had successfully offered the best environment for the growth of GAB and yield of biocellulose. Addition of sucrose in kuini fermentataion had tremendously enhanced the total phenolic and antioxidant contents. Fermented kuini may contain bioactive compounds that beneficial to human health and therefore should be further identified in the near future.

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