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Nutritive improvement of indigenous *Mangifera odorata* (Kuini) beverage with *Lactobacillus acidophilus* DDS1-NRRL-B-3208 supplementation

[Penambahbaikan nutrisi minuman *Mangifera odorata* (kuini) dengan penambahan *Lactobacillus acidophilus* DDSI-NRRL-B-3208]

Nur Diyana Alyas[1*], Aimi Athirah Ahmad[2], Muhammad Anas Othaman[3], Sukirah Abdul Rahman[4], Musaalbakri Abdul Manan[1] and Mohd Shukri Mat Ali[5]

[1]Food Science and Technology Research Centre, MARDI Headquarters, Persiaran MARDI-UPM, 43400 Serdang, Selangor
[2]Socio Economic, Market Intelligence and Agribusiness Research Centre, MARDI Headquarters, Persiaran MARDI-UPM, 43400 Serdang, Selangor
[3]Transfer Technology and Entrepreneur Development Centre, MARDI Headquarters, Persiaran MARDI-UPM, 43400 Serdang, Selangor
[4]Human Resource Management, MARDI Headquarters, Persiaran MARDI-UPM, 43400 Serdang, Selangor
[5]Horticulture Research Centre, MARDI Headquarters, Persiaran MARDI-UPM, 43400 Serdang, Selangor

Abstract

As part of an effort to explore indigenous fruits potential in Malaysia, underutilised *Mangifera odorata,* locally known as kuini, was considered to be nutritively preserved. Lactic acid fermentation was carried out using *M. odorata* as a substrate media. Six strains of lactic acid bacteria were screened, namely, *Lactobacillus paracasei* SD5275, *L. acidophilus* DDS1-NRRL-B-3208, *L. plantarum* SD5209, *L. casei* 431, Bifidobacteria bb-12 and *L. lactis*. *Lactobacillus acidophilus* DDS1-NRRL-B-3208 showed the highest viability compared to the others. Improvement of fermentation profile revealed that *Lacidophilus* DDS1-NRRL-B-3208 population showed the highest viability at 48 h incubation with a colony-forming unit count at Log_{10} 9.48. The proximate and vitamin analysis were carried out to differentiate the contents between fresh and fermented *M. odorata* pulp. Most of the proximate compositions of *M. odorata* were reduced after fermentation. Surprisingly, fermented beverages energy was 15 kcal/100 mg. Meanwhile, vitamin B1 and C were increased after fermentation process. The findings suggest that underutilised fruits may serve as a rich source of nutrients and vitamin C to significantly impact the health of consumers. *Mangifera odorata* has a nutritional value which has yet to be exploited and potentially a good alternative substrate for probiotic growth.

Keywords: lactic acid bacteria, submerged fermentation (SmF), probiotic beverages

Introduction

Underutilised fruits in Malaysia are wild plants that grow naturally in one place but rarely grown or cultivated as commercial crops. In this study, an indigenous and exotic mango species, *Mangifera odorata,* was used to further distinguish its hidden potential as a potential probiotic substrate.

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Authors' full names: Nur Diyana Alyas, Aimi Athirah Ahmad, Muhammad Anas Othaman, Sukirah Abdul Rahman, Musaalbakri Abdul Manan and Mohd Shukri Mat Ali
E-mail: nurdiyana@mardi.gov.my

Mangifera odorata is one of the mango species from the Anacardiaceae family and natively grown in Philippines, Thailand, Vietnam, Indonesia, Malaysia and Singapore. In Malaysia, it is known as "kuini" by the local community, specifically in West Malaysia. *Mangifera odorata* is a cross hybrid between *M. indica*, commonly known as mango, and *M. foetida* locally known as bachang or macang (Kiew 2002). The fruit is an obliquely ellipsoid-oblong, hardly flattened drupe with green to yellowish-green skin. The flesh is orange-yellow in colour, firm and fibrous with a sweet turpentine taste and a strong aroma (Brooke and Lau 2011). The usage of fresh *M. odorata* is limited due to its fibrous flesh which causes acrid on the throat (Wijaya et al. 1996) compared to mango. Little information is known about the nutritional value of *M. odorata*. However, there is a potential demand for this underutilised fruit due to its uniqueness.

Probiotics are living microorganisms that can promote health benefits in the host if they are consumed in sufficient quantities (Mahmoudi et al. 2012). They have shown a beneficial effect on the growth, composition and metabolic activity of the human gut microbiota (Reddy et al. 2015). Unbalanced microbes can cause dysbiosis that leads to several diseases. Probiotics contribute various health benefits including preventive treatments of infectious diarrhea, irritable bowel syndrome and allergen (Butel 2014). Probiotics have been reported to play a role in helping to combat diseases related to *Helicobacter pylori* such as gastric ulcers and some gastric cancers in conjunction with antibiotic combination treatment (Malfertheiner et al. 2012; Szajewska et al. 2010). The development of probiotic products from fruity food matrices has been studied extensively due to their therapeutic benefits and can be considered as consumer's predilection (do Espirito Santo et al. 2011). To date, there is a lack of information about the incorporation of probiotic bacteria and *M. odorata* as a substrate. The aim of this study was to improve the nutritional values of *M. odorata* using probiotic bacteria strain via submerged fermentation.

Materials and methods

Sample collection

Local elite accessions of *M. odorata* samples were collected from MARDI Sintok, Kedah. The fruits were transported to MARDI Headquarters for laboratory storage at 15 °C.

Sample preparation

Matured and ripe *M. odorata* fruits were thoroughly washed in tap water to remove the gummy sap from the peel. The pulp was then removed from the fruit after removing the fruit peel and seed. The pulp was homogenised and kept at – 80 °C for further analysis. *Mangifera odorata* peels were dried in a ventilated oven at 50 °C before homogenised and kept at 30 °C.

Determination of proximate composition and vitamins analysis

The proximate parameters fat, protein, moisture, ash, carbohydrate and energy of the *M. odorata* samples were determined using the standard analytical method of Association of Official Analytical Chemists (AOAC 2000).

Fermentation preparation (SmF)

Effect of different probiotic strains using *M. odorata* pulp as a substrate in submerged fermentation on bacterial growth and final pH value

The effect of different probiotic strains using 50% *M. odorata* pulp as a substrate in submerged fermentation was investigated using *L. paracasei* SD 5275, *L. acidophilus* DDS1-NRRL-B-3208, *L. plantarum* SD 5209, *L. casei* 431, Bifidobacteria bb-12 and *L. lactis*. The bacterial inoculums were added after the mixture was pasteurised at 95 °C for 10 min. The mixture was incubated at 30 °C for 48 h at 150 rpm. Bacterial growth and final pH value were then determined at every 24 h interval time

for 48 h of incubation. All experiments were carried out in triplicate.

Effect of different concentrations of *M. odorata* pulp as a substrate in submerged fermentation of *L. acidophilus* DDS1-NRRL-B-3208 on bacterial growth and final pH value

Different concentrations of *M. odorata* pulp as a substrate in submerged fermentation of *L. acidophilus* DDS1-NRRL-B-3208 was measured at 1%, 5%, 10%, 20% and 30% (W/V) using *L. acidophilus* DDS1-NRRL-B-3208. The bacterial inoculum was added after the mixture was pasteurised at 95 °C for 10 min. The mixture was then incubated at 30 °C for 48 h at 150 rpm. Bacterial growth and final pH value were then determined at every 24 h interval time for 48 h of incubation. All experiments were carried out in triplicate.

Improvement profile of *L. acidophilus* DDS1-NRRL-B-3208 using *M. odorata* pulp as a substrate via submerged fermentation

A time profile of bacterial growth and final pH value were conducted from 0 to 48 h of fermentation at an interval of 24 h continuously after incorporating all the improved condition parameters. A 1% of *L. acidophilus* DDS1-NRRL-B-3208 with 30% of *M. odorata* pulp concentration was selected prior to the previous experiment done. The mixture was incubated at 30 °C for 48 h at 150 rpm. The bacterial inoculum was added after the mixture was pasteurised at 95 °C for 10 min. Bacterial growth, final pH value, reducing sugar and lactic acid concentration were then determined. All experiments were carried out in triplicate.

Statistical analysis

ANOVA was carried out to analyse the difference among sets of treatment means with respect to the response variables. It also provided information that was capable of producing a meaningful result on the importance of the factors studied. To analyse the pattern of difference between means, the ANOVA is often followed by specific comparisons, and the most commonly used involved mean comparisons. The mean comparisons were carried out when ANOVA concluded that there were significant differences between treatment means. In this paper, the mean comparisons used was Fisher's Least Significant Difference (LSD) method. All of the analysis were performed using Minitab 18 Software. In addition, Spearman Rho Correlation Coefficient was used in this study to analyse the relationship among variables such as reducing sugar, growth, acidity and pH. The Spearman Rho Correlation Coefficient was used because it can produce consistent results regardless of the sample size (Minitab 18 Software).

Results and discussion

Proximate composition and vitamins analysis.

Results of the proximate composition of fresh and fermented *M. odorata* pulp are presented in *Table 1*. Fat is essential for physiological function, growth and development (Liu et al. 2017). Fat produced from fresh *M. odorata* was 0.36 ± 0.07 g/100g. However, fermented *M. odorata* using SmF approach interestingly showed no fat content. A study by Lauricella et al. (2017) also showed that *M. indica* L. flesh contained 0.38% fat which suggested that the genus Mangifera had more or less the same fat content. *Mangifera odorata* is also particularly rich in carbohydrates (83.45 ± 1.67 g/100g) compared to the common mango *M. pajang* (77.9 – 80.7%) (Umi Kalsum and Mirfat 2014) but contains less carbohydrates compared to *M. indica* (14.98 g/100g) (Lauricella et al. 2017). However, after fermentation the carbohydrate content was reduced to 3.22 g/100g due to the carbohydrate uptake by the lactic acid for their metabolism. Lauricella et al. (2017) also reported that mango flesh contained small amounts of protein (0.82%) in the sample studied suggesting that Mangifera fruits commonly

contain small amounts of protein. Moisture was very high (96.14 ± 0.04g/100g) in fermented *M. odorata* flesh as it was submerged in 70% of additional water and during the fermentation process, the flesh also degraded. Our results also showed a low ash content (6.4 – 9.81%) compared to studies by Mohammed and Yakubu (2013). The differences in nutritional composition between *M. odorata* and *M. Indica* could have emerged from environmental factors, species and cultural practices (Ishu 2013).

Table 2 shows vitamins availability in fresh and fermented *M. odorata* pulp. Lauricella et al. (2017) reported that vitamins A, B1, B2 and C content of mango pulp were 1,082 IU, 28 ug, 38 ug and 36.4 mg/100g. Vitamin C in *M. odorata* pulp met the minimum requirements recommended by EU/WHO (15 mg/100 g) for fruit groups and is beneficial for human health and wellness.

There was a decrease in the nutrient value (fat, ash carbohydrate, energy), and vitamin A (*Tables 1* and *2*). Moisture content was very high in *M. odorata* submerged fermentation due to the additional water added during the fermentation process. Moreover, there was a 2% increment in vitamin B1 and a 4% increment in vitamin C compared to fresh pulp. Vitamin B was not detected in either *M. odorata* fresh or fermented pulp. There has been no report to date on vitamins in fermented Mangifera genera.

Effect of different probiotic strains

In this study, six different probiotic strains, *L. paracasei* SD5275, *L. acidophilus* DDS-1, *L. plantarum* SD5209, *L. casei* 431, Bifidobacteria bb-12 and *L. lactis* were screened for their growth and final pH values using *M. odorata* as a substrate for 48 h of fermentation as shown in *Figure 1*. All strains demonstrated the capability to utilise *M. odorata* as a substrate without additional nutrient supplementation. Nevertheless, *L. acidophilus* DDS1-NRRL-B-3208 showed highest ability to employ the substrate, expressed as Log_{10} 9.23 of colony forming units (CFU), followed by *L. lactis*, *L. casei* 431, *L.*

Table 1. Proximate composition of fresh *M. odorata* pulp and fermented *M. odorata*

Proximate composition	*M. odorata* pulp (fresh) (g/100g)	*M. odorata* SmF (g/100g)
Fat	0.36 ± 0.07*	–
Protein	0.51 ± 0.34	0.50 ± 0.04
Moisture	7.91 ± 0.31	96.14 ± 0.04
Ash	3.18 ± 0.09	0.15 ± 0.01
Carbohydrate	83.45 ± 1.67	3.22 ± 0.00
Energy (kcal/100 g)	357.00 ± 0.07	15.00 ± 0.00

*Values are mean ± SD, n = 3

Table 2. Vitamins analysis of fresh *M. odorata* pulp and fermented *M. odorata*

Vitamins	*M. odorata* pulp (fresh) (mg/100g)	*M. odorata* SmF (mg/100g)
Vitamin A (beta carotene)	3.17 ± 0.12*	–
Vitamin B1 (thiamine)	–	2.37 ± 0.57
Vitamin B2 (riboflavin)	–	–
Vitamin C (ascorbic acid)	23.69 ± 0.85	27.7 ± 0.33

*Values are mean ± SD, n = 3

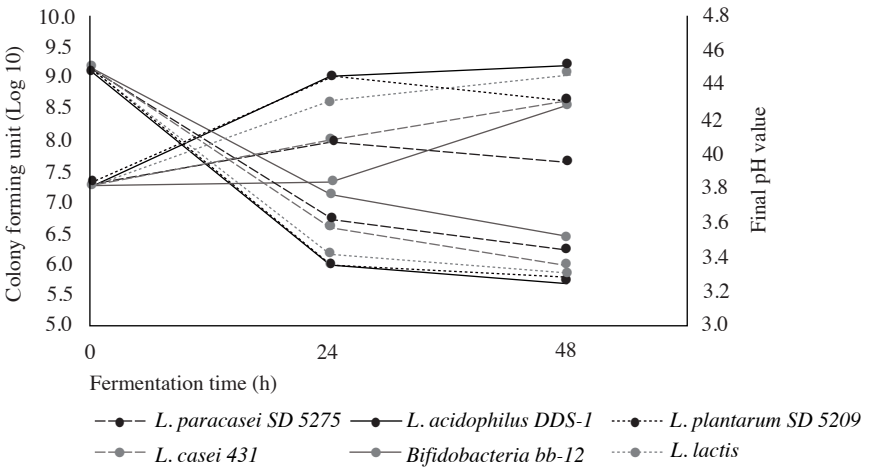

Figure 1. Effect of different probiotic strains using M. odorata pulp as substrate in submerged fermentation on bacterial growth and final pH value

plantarum SD5209, Bifidobacteria bb-12 and *L. paracasei* SD5275 during the 48 h fermentation period.

Two-way ANOVA revealed that there was a significant difference among lactic acid bacteria ($F = 9.80$, $p < 0.05$) and fermentation time ($F = 29.16$, $p < 0.05$) on bacterial growth. These results were further justified using Fisher LSD method and concluded that *L. acidophilus* DDS-1 was chosen based on a higher mean for CFUs. Similarly, the difference among bacteria ($F = 261.90$, $p < 0.05$) and fermentation time ($F = 262353$, $p < 0.05$) also significantly affected pH values. Based on Fisher LSD, *L. acidophilus* was favoured because of its lowest pH value compared to others (*Figure 1*). Lactic acid bacteria showed a decrease in the final pH value from 0 – 48 h of fermentation incubation. The pH range showed almost the same trend starting from 4.5 at 0 h until it reached an extremely acidic environment that varied according to strains. *Lactobacillus acidophilus* DDS1-NRRL-B-3208 showed the highest reduction from pH 4.5 – 3.26. Similar observations were reported by Reddy et al. (2015) and Lu et al. (2018) during mango juice fermentation by lactic acid bacteria. This was due to the colonisation of the lactic acid bacteria itself to create a suitable and favourable environment probably because of metabolic activities such as lactic acid accumulation. However, the mixture of the fermented *M. odorata* at 50% produced a thick slurry and made it difficult to shake.

Effect of different concentrations of *M. odorata* pulp on bacterial growth

In the consequence parameter, a study on the effect of different concentrations of *M. odorata* pulp as substrate was evaluated at 1%, 5%, 10%, 20% and 30% (w/v). As the proportion of *M. odorata* substrate was increased, the viability of microorganisms increased (*Figure 2*). The addition of 30% substrate showed the highest CFU for *L. acidophilus* DDS1-NRRL-B-3208 at Log_{10} 8.63. This result reflected that the addition of substrate helped in microbial growth by providing enough nutrient sources.

The analysis was further carried out to determine the effect of different concentrations of *M. odorata* pulp as substrate in submerged fermentation of *L. acidophilus* DDS1-NRRL-B-3208 and fermentation time on bacterial growth using ANOVA and Fisher LSD test. ANOVA showed that different concentrations ($F = 14.51$, $p < 0.05$) and fermentation time ($F = 36.07$, $p < 0.05$) gave significant effect

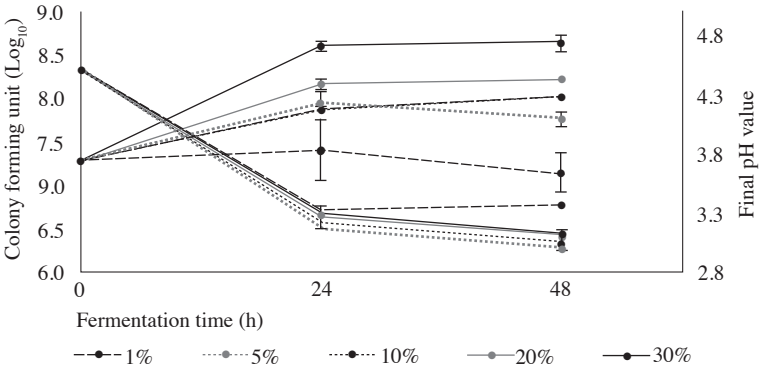

Figure 2. Effect of different concentrations of M. odorata pulp as substrate in submerged fermentation of L. acidophilus DDS1-NRRL-B-3208 on bacterial growth and final pH value

on the CFU. Similar results were obtained using the same set of concentrations ($F = 2,682.58$, $p < 0.05$) on final pH values. Fisher LSD test suggested that the best combination of concentration and fermentation time was 30% within 24 h. This combination was chosen because of the higher CFU value with a lower pH value (*Figure 2*).

Effect of different concentrations of *M. odorata* pulp on final pH value

Correlation analysis was done to quantify the linear relationship between variables as shown in *Table 3*. The variables were reducing sugar, *L. acidophillus* growth, lactic acid concentration and pH value. The Spearman Rho Correlation Coefficient analysis was used to estimate the relationship between the selected parameters in the improvement study. Results showed that all pairs of variables exhibited a significantly strong linear correlation with a different direction. A negative correlation means that as one variable increases, the other variable decreases while positive correlation means the variables move in the same direction. This means that as one variable increases, so does the other one. This statistical statement is illustrated in *Figure 3* where reducing sugar and pH showed a decreasing amount from 7.42 mg/ml to 1.83 mg/ml and from pH 4.49 to pH 3.40 as the time of incubation increased due to the sugar uptake during fermentation. This was supported by *L. acidophilus* DDS1-NRRL-B-3208 growth which showed an increasing trend due to fermentation time and reached its highest at 48 h with Log_{10} 9.48. Microbial digestion of the substrate which occurred during fermentation contributed to the sugar reduction. This finding was in agreement with a study carried out by Reddy et al. (2015) which showed that substrate consumption during mango juice fermentation by *L. acidophilus* caused a reduction of glucose concentration from 12% to 7%. This study showed that there were positive and negative correlations among all the parameters tested.

Conclusion

Lactobacillus acidophilus DDS1-NRRL-B-3208 can be used in *M. odorata* pulp fermentation conditions to improve the nutritional quality of underutilised fruits. Generally, probiotics products contain a minimum concentration of 10^6 CFU/mL or gram viability. Overall, the consumer should consume 10^8 to 10^9 probiotic microorganisms daily for effective probiotic effect (Kechagia et al. 2013). *M. odorata* also showed a potential characteristic as an alternative to the probiotic substrate for lactic acid bacteria growth. The information obtained from these

Table 3. Spearman Rho Correlation Coefficient among parameters involved in improvement of *L. acidophilus* DDS1-NRRL-B-3208 using *M. odorata* pulp as substrate via submerged fermentation

Variables	*Spearman Rho Correlation Coefficient*	*p value*
Reducing sugar vs growth	– 0.966*	0.000
Reducing sugar vs lactic acid concentration	– 0.867*	0.002
Reducing sugar vs pH	0.900*	0.001
Growth vs pH	– 0.865*	0.003
Growth vs lactic acid concentration	0.915*	0.001
Lactic acid concentration vs pH	– 0.883*	0.002

*Significantly correlated at $p < 0.05$

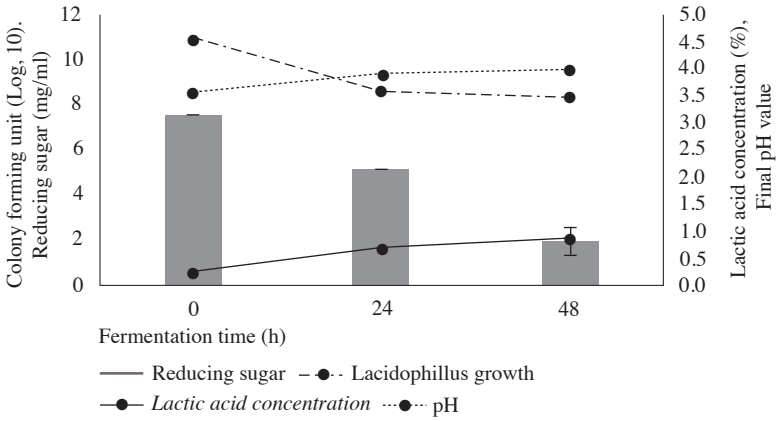

Figure 3. Improvement of L. acidophilus DDS1-NRRL-B-3208 using M. odorata pulp as substrate via submerged fermentation

proximate composition and vitamins analysis could serve as a guide for further potential utilisation of *M. odorata*.

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Abstrak

Kajian ini telah dijalankan untuk menilai potensi buah nadir *Mangifera odorata* yang dikenali dengan nama tempatan sebagai buah kuini. Penapaian asid laktik telah dijalankan menggunakan *M. odarata* yang telah dijadikan sebagai media substrat. Enam strain bakteria asid laktik yang telah disaring semasa proses penapaian ialah *Lactobacillus paracasei* SD5275, *L. acidophilus* DDS1-NRRL-B-3208, *L. plantarum* SD 5209, *L. casei* 431, Bifidobacteria bb-12 dan *L. lactis*. Peningkatan profil penapaian menunjukkan populasi *L. acidophilus* DDS1-NRRL-B-3208 adalah tertinggi berbanding strain bakteria lain dalam masa 48 jam dengan kiraan unit koloni pada Log_{10} sebanyak 9.48. Analisis proksimat dan vitamin dijalankan untuk membezakan di antara pulpa segar dan pulpa *M. odorata* yang telah difermentasi. Komposisi proksimat *M. odorata* telah berkurang selepas proses penapaian. Tenaga minuman probiotik yang ditapai hanyalah 15 kcal/100 mg. Sementara itu, vitamin B1 dan C telah meningkat selepas proses fermentasi. Hasil penemuan ini mencadangkan bahawa buah yang kurang digunakan mungkin boleh dijadikan sumber yang kaya dengan nutrien dan vitamin C yang memberi kesan yang signifikan kepada kesihatan pengguna. *Mangifera odorata* mempunyai nutrisi pemakanan yang masih belum dieksploitasi. Justeru, ia berpotensi menjadi substrat alternatif yang baik kepada pertumbuhan probiotik.