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THE EFFECT OF DIFFERENT PINEAPPLE RIPENESS ON THE PHYSICOCHEMICAL, MICROBIOLOGICAL PROPERTIES OF FERMENTED KEFIR DRINK

by

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DECLARATION BY AUTHOR

This dissertation is composed of my original work, and contains no material previously published or written by another person except where due reference has been made in the text. The content of my dissertation is the result of work I have carried out since the commencement of my research project and does not include a substantial part of work that has been submitted to qualify for the award of any other degree or diploma in any university or other tertiary institution.

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LIST OF ABBREVIATIONS

Abbreviation	**Caption**
R	Fermented ripe MD2 pineapple kefir at 25ºC
UR	Fermented unripe MD2 pineapple kefir at 25ºC
R25	Fermented ripe diluted MD2 pineapple kefir at 25ºC
UR25	Fermented unripe diluted MD2 pineapple kefir at 25ºC
CR	Unfermented unripe MD2 pineapple juice at 25ºC
CUR	Unfermented unripe MD2 pineapple juice at 25ºC
CR25	Unfermented ripe diluted MD2 pineapple juice at 25ºC
CUR 25	Unfermented unripe diluted MD2pineapple juice at 25ºC
ºC	Degree Celcius
α	alpha
β	beta
L	liter
mL	milliliter
mL/min	milliliter per minute
g	gram
kg	kilogram
mg	milligrams
v/v	volume/volume
w/w	mass/mass
kcal	kilocalorie
rpm	revolutions per minute
µm	micrometer
µl	microliter

mm	millimeters
mmHg	millimeters of mercury
mm/s	millimeter per second
cm	centimeter
M	Molarity
AOAC	Association of Official Analytical Chemists
DM-E	Dichloromethane-ethanol
HCl	Hydrochloric acid
$CuSO_4$	Copper sulphate
H_2SO_4	Sulphuric acid
H_3BO_3	Boric acid
HPLC	High-performance liquid chromatography
CaEDTA	Calcium disodium edetate
TPA	Texture Profile Analysis
PCA	Plate Count Agar
PDA	Potato Dextrose Agar
MRS	De man, Rogosa, Sharpe Agar
TPC	Total Plate Count
CFU	Colony Forming Unit
CFU/mL	Colony Forming Units per milliliter
ANOVA	Analysis of Variance

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KESAN PERBEZAAN KEMASAKAN NANAS TERHADAP FIZIKOKIMIA DAN MIKROBIOLOGIKAL KEFIR YANG DITAPAI

ABSTRAK

Nanas MD2 mempunyai enam indeks kematangan berdasarkan piawaian dari Malaysia Pineapple Industry Board (2018) tetapi hanya dua peringkat kemasakan telah digunakan dalam eksperimen ini iaitu Indeks 2 yang mewakili kategori tidak masak manakala Indeks 4 mewakili kategori masak. Nanas MD2 didapati boleh menjadi substrat yang baik untuk membuat minuman probiotik bukan tenusu seperti minuman kefir dengan penapaian jus nanas MD2 bersama-sama dengan bijiran air kefir. Walau bagaimanapun, kematangan nanas yang berbeza boleh menghasilkan sifat yang berbeza daripada ciri-ciri minuman kefir, oleh itu kategori mentah (Indeks 2) dan kategori matang (Indeks 4) telah dipilih untuk menilai dan mengkaji kesan kematangan nanas MD2 yang berbeza terhadap sifat fizikokimia dan mikrobiologi minuman kefir dalam eksperimen ini supaya minuman probiotik bukan tenusu yang alternatif boleh dihasilkan untuk menggantikan minuman probiotik ternusu untuk vegetarian dan pengguna yang mempunyai alahan terhadap laktosa. Kefir nanas dihasilkan dengan menambah 5 g daripada kefir (5%) dan 100 ml jus nanas. Kemudian, penapaian telah dijalankan pada suhu 25 ° C selama 24 jam. Dari eksperimen, minuman kefir yang masak mempunyai fenolik tertinggi (0.09 ± 0.00) berbanding dengan minuman kefir nenas mentah dan kawalannya. Hal ini disebabkan nanas itu sendiri kaya dengan sebatian polyfenol seperti asid p-coumaric, asid ferulic, asid kafeik, asid sinapik, p-coumaroyl asid, dan sebatian polyfenols yang kompleks telah ditukarkan kepada polyfenols yang kecil oleh mikroorganisma dari kefir semasa penapaian. Analisis kandungan antioksidan telah dijalankan, dan ia menunjukkan bahawa minuman kefir nanas mentah (UR) (26.88 ± 9.55) adalah jauh lebih tinggi daripada kawalannya (CUR)

(1.30 ± 0.11). Ini mungkin disebabkan oleh proses oksidasi mencetuskan mekanisme pertahanan buah yang disebabkan oleh tekanan oksidatif dan membawa kepada antioksidan yang dikeluarkan ke dalam kefir semasa penapaian. Tambahan pula, jumlah gula dalam sampel masak (8.26 ± 1.53) dan sampel yang tidak masak (4.50 ± 0.78) adalah jauh lebih rendah daripada kawalan selepas penapaian kerana perpecahan daripada jus nanas dipecahkan kepada gula yang dikurangkan untuk digunakan sebagai substrat untuk penapaian. Kemudian, jumlah pepejal larut berkurangan selepas penapaian, sampel berpigmen (R) (6.85 ± 0.07) dan sampel kefir nanas mentah (5.15 ± 0.07) adalah jauh lebih rendah daripada kawalan kerana gula dalam jus nenas telah digunakan untuk penapaian untuk menghasilkan metabolit lain oleh mikroorganisma. Dalam ujian pH, ia didapati mengawal sampel masak (CR) (4.05 ± 0.01) adalah jauh lebih tinggi daripada sampel masak (R) (3.99 ± 0.01). Ini mungkin disebabkan oleh pengeluaran asid laktik yang menyebabkan pH kefir untuk mengurangkan selepas penapaian. Dalam ujian keasidan, semua sample minuman kefir tidak mempunyai perbezaan dalam kandungan asid laktik, oleh disebabkan penapaian memerlukan masa yang panjang untuk melihat perbezaan yang lebih besar. Dalam ujian microbiologi, kefir nanas yang masak dan kefir nanas mentah telah mencapai standard Codex minimum kefir untuk kiraan bakteria total ($1x10^7$ cfu/ml), dan kiraan yis ($1x10^4$ cfu/ml). Walau bagaimanapun, kefir nanas yang matang mempunyai sifat yang baik dan sesuai menjadi minuman probiotik alternatif kepada pengguna vegetarian dan pengguna yang mempunyai alahan terhadap laktosa.

THE EFFECT OF DIFFERENT PINEAPPLE RIPENESS ON THE PHYSICOCHEMICAL AND MICROBIOLOGICAL PROPERTIES OF FERMENTED KEFIR DRINK

ABSTRACT

MD2 pineapple consists of 6 indices of ripeness according to Malaysian Standards (2018) but only two ripeness stage was used in this experiment which were Index 2 that represented unripe while Index 4 represents ripe stage. It was found that pineapple can make a good substrate for the making of non-dairy probiotic drink such as kefir drink by fermenting MD2 pineapple juice together with water kefir grains. However, different ripeness of pineapple could yield different properties of the fermented kefir drink, therefore unripe stage (Index 2) and ripe stage (Index 4) were selected to evaluate and to study the effect of different ripeness of the MD2 pineapple on the physicochemical and microbiological properties of the fermented kefir drink in this experiment so that an alternative non-dairy probiotic drink can be produced to replace dairy probiotic drink for vegetarians and lactose intolerance consumers. The pineapple kefir was made by adding 5 g of kefir (5%) and 100 ml of the pineapple juice. Then, the fermentation was conducted at a temperature of 25 ºC for 24 hours. From the experiment, it was found that riper pineapples have a higher phenolic content than unripe pineapples. Ripe pineapple kefir drink has the highest phenolic (0.09 ± 0.00) compared to unripe pineapple kefir drink and its control. This was because pineapple itself were rich in polyphenol compounds such as p-coumaric acid, ferulic acid, caffeic acid, sinapic acid, p-coumaroyl quinic acid as it ripened, and the complex polyphenols compounds were hydrolyzed by the microorganisms from the kefir during

fermentation. Antioxidant content analysis was conducted, and it showed that unripe pineapple kefir sample (UR) (26.88 ± 9.55) was significantly higher than its control (CUR) (1.30 ± 0.11). This could be due to the ripening process triggered the defense mechanism of the fruit due to oxidative stress and lead to antioxidants being released into the kefir during fermentation. Furthermore, it was found that the total sugars in ripe sample (8.26 ± 1.53) and unripe sample (4.50 ± 0.78) were significantly lower than its controls after fermentation because of disaccharides from the pineapple juice being broken down to its reducing sugar to be used as substrate for fermentation. Then, the total soluble solids decreased after fermentation, fermented ripe sample (R) (6.85 ± 0.07) and unripe sample (5.15 ± 0.07) were significantly lower than its controls because the sugars in the pineapple juice were used for fermentation to produce other metabolites by microorganisms. In pH test, it was found control ripe sample (CR) (4.05 ± 0.01) was significantly higher than ripe sample (R) (3.99 ± 0.01). This could be due to the production of lactic acid which cause the pH of the kefir to decrease after fermentation. In titratable acidity, there was no significant difference in all the samples, it could be due to fermentation time, longer fermentation time was needed. In microbiological test, ripe pineapple kefir and unripe pineapple kefir had achieved the minimum codex standard of kefir for total bacterial count, and yeast count. However, ripe pineapple kefir drink overall was seen to exhibit good nutritional properties and suitable to be an alternative for vegetarians and lactose intolerance consumers