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BLACK TEA WATER KEFIR BEVERAGE

A Thesis submitted in partial fulfilment of the requirement for the degree of
Master of Food Technology

Massey University
Albany, New Zealand

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ABSTRACT

Fermented foods and beverages play an important role in the human diet as they provide essential nutrients as well as contribute towards prevention of diseases. Lactic acid bacteria and yeasts are a major group of microorganisms associated with fermented products. Some of the microorganisms, known as probiotics, confer health properties to human health. Thus, many different types of fermented foods and beverages containing probiotics are produced around the world to support wellness and health. In recent years, there has been increased interest in the development of fermented functional plant-based foods and beverages due to a surge in scientific research of the products. Further, there is evidence that probiotic microorganisms can grow well in plant-based substrates.

Water kefir is a sparkling fermented beverage with an acidic, sweet, slightly alcoholic taste, and a yeasty flavour. Water kefir fermentation can be achieved by the inoculation of water kefir grains as a starter culture into a solution containing sugar. Kefir grains consist of a symbiotic starter culture of lactic acid bacteria (LAB) and yeasts contained in a polysaccharide matrix. Microorganisms present in kefir grains are recognized as probiotics. The majority of previous studies have focused on the isolation and identification of water kefir cultures responsible for fermentation. There is, therefore, scanty information on the fermentation of plant-based water kefir beverages. The main objective of this study was to develop fermented black tea beverage using water kefir grains as a starter culture.

Fermentation of black tea infusions as single and mixed substrate with carrot juice using water kefir grains were investigated. Microflora of water kefir grains used consisted of symbiotic starter culture of lactic acid bacteria (Lactococcus spp. and Lactobacillus spp.) and a yeast (Saccharomyces cerevisiae). The study was conducted in three main phases. The first phase investigated the effect of sucrose concentration (5% and 10%) and fermentation temperature (25°C and 30°C) in black tea water kefir fermentation for 72 h. Meanwhile, the effect of added carrot juice (5%, 10%, and 15%) on kefir beverage during secondary fermentation (24 h) at 25°C was investigated in the second phase. The stability of the final black tea water kefir beverage formulation during storage (4°C) for four weeks was investigated in phase three. Samples of black tea water kefir beverages were subjected to various analyses during fermentation and storage (4°C) for 4 weeks: titratable acidity, total soluble solids (°Brix), colour, viable cell counts of constituent starter culture, sensory evaluation, sugars, organic acids, antioxidants, and pH was also measured.

Results showed that fermentation temperature, sugar concentration, and carrot juice concentration contributed to the physico-chemical and microbiological characteristic as well as sensory properties of the product. In phases one and two, pH and total soluble solids (°Brix) decreased, while titratable acidity and cell counts of LAB and yeasts increased during fermentation of the products. LAB and yeasts were able to grow in black tea and addition of carrot juice into the beverages slightly increased their growth. The best fermentation conditions based on physico-chemical and sensory properties were kefir beverage containing sugar (10%) and carrot juice (10%) fermented at 25°C for 96 h. In phase three, the growth and survival of Lactococcus spp. and Lactobacillus spp. were low during storage of the product (4°C) while Saccharomyces cerevisiae maintained high cell numbers (7.03±0.07 log cfu/ml) at the end of storage (28 days). Results showed the possibility to produce low sugar water kefir beverage containing 0.08±0.01% (w/v) sucrose, 1.55±0.04% (w/v) glucose, and 2.93±0.20% (w/v) fructose. The fermented kefir beverage also contained 0.20±0.02% (w/v) lactic acid, 0.11±0.03% (w/v) acetic acid and some antioxidants (gallic acid, ECG, EGC, EGCG, theobromine and caffeine) which may be beneficial to human health. There was significant difference (p<0.05) in the colour (L*, a*, b*) of the fermented beverages during storage (4°C).

Black tea water kefir beverage containing 10% sugar and 10% carrot juice fermented at 25°C for 96 h was well-liked by consumer sensory panellists.
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<table>
<thead>
<tr>
<th>Abbreviation</th>
<th>Meaning</th>
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<tbody>
<tr>
<td>a*</td>
<td>redness-greenness</td>
</tr>
<tr>
<td>AAB</td>
<td>Acetic acid bacteria</td>
</tr>
<tr>
<td>ACK</td>
<td>Acetate kinase</td>
</tr>
<tr>
<td>Acs</td>
<td>Acetyl-coenzyme A synthetase</td>
</tr>
<tr>
<td>Adh</td>
<td>Alcohol dehydrogenase</td>
</tr>
<tr>
<td>ADP</td>
<td>Adenosine diphosphate</td>
</tr>
<tr>
<td>Ald</td>
<td>Acetaldehyde dehydrogenase</td>
</tr>
<tr>
<td>ANOVA</td>
<td>Analysis of variance</td>
</tr>
<tr>
<td>AOAC</td>
<td>Association of Official Analytical Chemist</td>
</tr>
<tr>
<td>ATP</td>
<td>Adenosine triphosphate</td>
</tr>
<tr>
<td>B</td>
<td>Bifidobacterium</td>
</tr>
<tr>
<td>b*</td>
<td>yellowness-blueness</td>
</tr>
<tr>
<td>BOP</td>
<td>Broken orange pekoe</td>
</tr>
<tr>
<td>BT</td>
<td>Black tea</td>
</tr>
<tr>
<td>CFU</td>
<td>Colony forming per Unit</td>
</tr>
<tr>
<td>CTC</td>
<td>Crush-Tear-Curl</td>
</tr>
<tr>
<td>DPPH</td>
<td>2,2-diphenyl-1-picrylhydrazyl</td>
</tr>
<tr>
<td>EC</td>
<td>Epicatechin</td>
</tr>
<tr>
<td>ECG</td>
<td>Epicatechin gallate</td>
</tr>
<tr>
<td>ECP</td>
<td>Endless chain pressure</td>
</tr>
<tr>
<td>EGC</td>
<td>Epigallocatechin</td>
</tr>
<tr>
<td>EGCG</td>
<td>Epigallocatechin gallate</td>
</tr>
<tr>
<td>EMP</td>
<td>Emden-Meyerhoff-Parnas</td>
</tr>
<tr>
<td>EPS</td>
<td>Exopolysaccharides</td>
</tr>
<tr>
<td>F</td>
<td>Fanning</td>
</tr>
<tr>
<td>FADH</td>
<td>Flavin adenine dinucleotide</td>
</tr>
<tr>
<td>FAO</td>
<td>Food and Agriculture Organization</td>
</tr>
<tr>
<td>FBD</td>
<td>Fluid bed dried</td>
</tr>
<tr>
<td>FSANZ</td>
<td>Food Standards Australia New Zealand</td>
</tr>
<tr>
<td>g</td>
<td>Gram</td>
</tr>
<tr>
<td>GABA</td>
<td>Gamma-Amino Butyric Acid</td>
</tr>
<tr>
<td>Acronym</td>
<td>Definition</td>
</tr>
<tr>
<td>---------</td>
<td>------------</td>
</tr>
<tr>
<td>GAP</td>
<td>Glyceraldehyde-3P</td>
</tr>
<tr>
<td>GRAS</td>
<td>Generally Recognized As Safe</td>
</tr>
<tr>
<td>GC</td>
<td>Gas chromatography</td>
</tr>
<tr>
<td>GI</td>
<td>Gastrointestinal</td>
</tr>
<tr>
<td>H</td>
<td>Hour</td>
</tr>
<tr>
<td>HIV</td>
<td>Human Immunodeficiency Virus</td>
</tr>
<tr>
<td>HPLC</td>
<td>High performance liquid chromatography</td>
</tr>
<tr>
<td>L</td>
<td>Litre</td>
</tr>
<tr>
<td>L*</td>
<td>Lightness</td>
</tr>
<tr>
<td>LAB</td>
<td>Lactic acid bacteria</td>
</tr>
<tr>
<td>Lb.</td>
<td>Lactobacillus</td>
</tr>
<tr>
<td>Lc.</td>
<td>Leuconostoc</td>
</tr>
<tr>
<td>LDH</td>
<td>Lactate dehydrogenase</td>
</tr>
<tr>
<td>Min</td>
<td>Minute</td>
</tr>
<tr>
<td>ml</td>
<td>milliliter</td>
</tr>
<tr>
<td>mg</td>
<td>milligram</td>
</tr>
<tr>
<td>mm</td>
<td>millimeter</td>
</tr>
<tr>
<td>NaCl</td>
<td>Sodium chloride</td>
</tr>
<tr>
<td>NAD</td>
<td>Nicotinamide adenine dinucleotide</td>
</tr>
<tr>
<td>NADH</td>
<td>Nicotinamide adenine dinucleotide hydride</td>
</tr>
<tr>
<td>OP</td>
<td>Orange pekoe</td>
</tr>
<tr>
<td>ORS</td>
<td>Oral rehydration solution</td>
</tr>
<tr>
<td>OXPHOS</td>
<td>Oxidative phosphorylation</td>
</tr>
<tr>
<td>PCR</td>
<td>Polymerase chain reaction</td>
</tr>
<tr>
<td>Pdc</td>
<td>Pyruvate decarboxylase</td>
</tr>
<tr>
<td>Pdh</td>
<td>Pyruvate-dehydrogenase complex</td>
</tr>
<tr>
<td>PFL</td>
<td>Pyruvate formate-lyase</td>
</tr>
<tr>
<td>PKP</td>
<td>Phospho-ketolase pathway</td>
</tr>
<tr>
<td>PTA</td>
<td>Phospho-trans-acetylase</td>
</tr>
<tr>
<td>SD</td>
<td>Standard deviation</td>
</tr>
<tr>
<td>spp</td>
<td>species (plural)</td>
</tr>
<tr>
<td>TSS</td>
<td>Total soluble solids</td>
</tr>
<tr>
<td>T.A.</td>
<td>Titratable Acidity</td>
</tr>
<tr>
<td>TCA</td>
<td>Tricarboxylic acid</td>
</tr>
<tr>
<td>Acronym</td>
<td>Definition</td>
</tr>
<tr>
<td>---------</td>
<td>------------</td>
</tr>
<tr>
<td>TF</td>
<td>theaflavins</td>
</tr>
<tr>
<td>TFA</td>
<td>trifluoroacetic acid</td>
</tr>
<tr>
<td>TR</td>
<td>thearubigins</td>
</tr>
<tr>
<td>MRS</td>
<td>de Man, Rogosa and Sharpe</td>
</tr>
<tr>
<td>VOC</td>
<td>Volatile Organic Compounds</td>
</tr>
<tr>
<td>v/v</td>
<td>volume per volume</td>
</tr>
<tr>
<td>WHO</td>
<td>World Health Organization</td>
</tr>
<tr>
<td>w/v</td>
<td>weight per volume</td>
</tr>
<tr>
<td>YGC</td>
<td>Yeast Glucose Chloramphenicol</td>
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