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A novel non-dairy beverage from durian pulp fermented with selected probiotics and yeast

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ABSTRACT

This study investigated the effects of sequential inoculation (Seq-I) of *Bifidobacterium animalis* subsp. *lactis* or *Lactobacillus casei* with yeast *Williopsis saturnus* on durian pulp fermentation. Seq-I of *W. saturnus* following *B. animalis* subsp. *lactis* did not bring about any significant differences compared to the *B. animalis* subsp. *lactis* monoculture due to the sharp early death of *W. saturnus* soon after inoculation. However, Seq-I of *W. saturnus* significantly enhanced the survival of *L. casei* and improved the utilization of fructose and glucose compared to *L. casei* monoculture. In addition, there were significant differences in the metabolism of organic acids especially for lactic acid and succinic acid. Furthermore, Seq-I produced significantly higher levels of volatile compounds including alcohols (ethanol and 2-phenylethyl alcohol) and acetate esters (2-phenylethyl acetate, isoamyl acetate and ethyl acetate), which would positively contribute to the flavour notes. Although the initial volatile sulphur compounds were reduced to trace levels after fermentation, but the durian odour still remained. This study suggests that the use of probiotics and *W. saturnus* to ferment durian pulp could act as a potential avenue to develop a novel non-dairy durian-based functional beverage to deliver probiotics.

1. Introduction

Probiotics including clinically proven lactobacilli and bifidobacteria are typically viable microorganisms that confer a beneficial effect on human and animals when consumed in proper amounts (Hill et al., 2014; Oliveira et al., 2012). Various studies have shown their health benefits on gastrointestinal infections, antimicrobial activity, reduction in serum cholesterol, immune system stimulation, improvement in lactose metabolism, anti-mutagenic properties, anti-carcinogenic properties, and anti-diarrheal properties (Imasse et al., 2007; Shah, 2007). In addition, Whelan and Quigley (2013) reported that probiotics played positive roles in the management of inflammatory bowel diseases and irritable bowel syndrome. Furthermore, probiotics could induce remission and maintain therapy in ulcerative colitis, Crohn's disease and pouchitis (Shen et al., 2014).

Lactobacilli are facultative or obligatory hetero-fermentative lactic acid bacteria (LAB) frequently used as food starter cultures such as cheese starter cultures (Dudley and Steele, 2005; Wyder and Puhan, 1999) and bifidobacteria have a long safe history of use and are commonly used in dairy fermented foods (Shah, 2007). Generally, probiotics are added to dairy products especially yoghurt (Bansal et al., 2016; Shah, 2011) in which they do not play a fermentation role. Nevertheless, the use of dairy products to deliver probiotics may cause some health issues for those with lactose intolerance (Whelan and Quigley, 2013; Yang et al., 2013). Therefore, there is a genuine and increasing demand for non-dairy based (e.g. cereals and fruits) probiotic products (Bansal et al., 2016; de Souza Leone et al., 2017).

Durian (*Durio zibethinus* Murr.) is a climacteric fruit that is widely grown in Southeast Asian countries such as Malaysia, Thailand and Philippines (Ho and Bhat, 2015; Lu et al., 2015, 2016; Voon et al., 2006). It is well-known in tropical countries due to its distinctive form and aroma. The consumption of durian is believed to benefit human health due to its high contents in various nutrients like carbohydrates, proteins, lipids, polyphenols and vitamins (Ho and Bhat, 2015). However, durian fruits have a short fruiting season of May to August and a limited shelf life of 3 to 5 days at room temperature; as such, large amounts of durian are spoiled and wasted if not consumed timely after harvest (Ho and Bhat, 2015; Voon et al., 2006). Therefore, the

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preservation of durian and adding value to the fruits by producing commercial durian products require urgent attention for durian farmers as well as researchers.

Traditionally, the preserving methods of durian are drying or freezing, but the products are of low value. People in Indonesia and Malaysia have a means of preserving the durian pulp through spontaneous lactic acid fermentation, resulting in a product called tempoyak (Yuliana and Dizon, 2011). Species of *Lactobacillus* have been reported as the main LAB isolated from tempoyak (Leisner et al., 2001; Yuliana and Dizon, 2011). Aside from tempoyak, there are no other known fermented durian products found in the market. In addition, there is no study related to a combination of probiotics and durian; therefore, durian offers a novel matrix of developing a probiotic functional beverage and its uniqueness provides significant opportunities for research within the field of durian probiotic fermentation. Furthermore, since most probiotics are LAB and bifidobacteria, utilization of probiotics to ferment durian pulp would not only ensure consistency and reliability in the final product quality, but also provide nutritional values and health benefits to the consumers. To the best of our knowledge, this has not been explored.

In order to deliver its therapeutic effects to the host, probiotics need to be active and present in sufficient amounts. The viability of probiotics is of prime importance because it determines their health efficacy. Therefore, a high survival rate of the probiotics during production and storage is crucial for the consumer to gain the associated health benefits so as to meet the minimum dosage required for therapeutic effects of 10^8–10^9 colony forming units (CFU) per serving. However, it has been a challenge to maintain the stability of probiotics from manufacturing to consumption of probiotic foods. However, previous studies showed that the viability of probiotics could be enhanced through co-culturing with selected yeasts (Liu and Tsao, 2009) or supplemented with metabolizable sugars (Corcoran et al., 2005). The survival of *Lactobacillus bulgaricus* was enhanced by $\sim 10^2$ and 10^5-fold when co-cultured with *Yarrowia lipolytica* B9014 and *Saccharomyces bayanus* CVC-NF74 in yoghurt, respectively (Liu and Tsao, 2009). In addition, *Geotrichum candidum* CMICC335426 and *Williopsis saturnus* var. *saturnus* CBS254 improved the survival of *L. rhamnosus* DR20 of 10^6-fold in fermented milk incubated at 30 °C (Liu and Tsao, 2009). Corcoran et al. (2005) reported that the survival of *L. rhamnosus* GG was enhanced by $\sim 10^6$ to 10^8-fold when supplemented with glucose at concentrations from 1 to 19.4 mM in simulated gastric juice at pH 2.0. The possible reason is that glucose could provide ATP to F_0F_1-ATPase via glycolysis, which enables proton exclusion and thereby enhancing the survival of *L. rhamnosus* (Corcoran et al., 2005; Cotter and Hill, 2003).

The aim of this study was for the first time to investigate the effect of sequential inoculation of *W. Saturnus* var. *saturnus* NCYC22 on the viability of *Lactobacillus casei* L26 and *Bifidobacterium animalis* subsp. *lactis* B94, utilization of substrates and formation of metabolites at 30 °C using durian pulp as the fermentable substrate so as to develop a new non-dairy functional beverage. *L. casei* L26 and *B. animalis* subsp. *lactis* B94 were selected due to their better growth and viability in durian pulp fermentation in our preliminary study (data not shown). *Williopsis* yeasts were selected because they are regarded as non-pathogenic and food associated (Liu and Tsao, 2009; Wyder and Puhan, 1999). In addition, *W. saturnus* var. *saturnus* could impart a fruity flavour to fermented products because of its relatively high production of acetate esters compared to other yeasts (Lee et al., 2012).

2. Materials and methods

2.1. Probiotics and yeast pure cultures preparation

Lactobacillus casei (now *paracasei*) L26 and *Bifidobacterium animalis* subsp. *lactis* B94 were obtained from DSM (Heerlen, Netherlands), *Williopsis saturnus* var. *saturnus* NCYC22 was purchased from National Collection of Yeast Cultures (Norwich, UK). *L. casei* L26 was propagated in sterile MRS broth (Oxoid, Basingstoke, UK) for 48 h at 37 °C, while *B. animalis* subsp. *lactis* B94 was propagated anaerobically in a sterile MRS broth added with 0.05% (w/w) L-cysteine (Sigma-Aldrich, St. Louis, USA) for 48 h at 37 °C. The active dried *W. saturnus* var. *saturnus* NCYC22 was propagated in a sterile broth (0.25% w/v yeast extract, 0.25% w/v peptone, 0.25% w/v malt extract and 2% w/v glucose) for 48 h at 25 °C. All of the microorganisms were stored at − 80 °C until use.

2.2. Durian pulp preparation and fermentations

The durian pulp (XO-D24 cultivar, Malaysia) was diluted with deionized water at a ratio of 3:7 (w/w) and homogenized to form a puree before pasteurization at 60 °C for 20 min. The effectiveness of pasteurization was verified by spread plating on potato dextrose agar (PDA) and MRS agar for yeasts and bacteria checking, respectively. The probiotic pre-cultures were prepared by inoculating 5% (v/v) of the respective pure culture in the same medium above and incubated at 37 °C for 48 h to achieve colony forming units (CFU) between 10^7 and 10^8 per mL. The yeast pre-culture was prepared by inoculating 5% (v/v) of *W. saturnus* var. *saturnus* NCYC22 ($\sim 10^5$ CFU/mL) into the pasteurized durian pulp and incubated at 25 °C for 96 h to obtain yeast counts of $\sim 10^7$ CFU/mL.

Triplicate fermentations were carried out with 300 mL of pasteurized durian pulp in sterile 500-mL conical flasks. For each pair of probiotics-yeast sequential inoculation, the fermentations were conducted by first inoculating 1% (v/v) of the respective probiotics preculture to the pasteurized durian pulp and incubated at 37 °C for 3 days to achieve a maximum cell population (statically for *L. casei* L26, and anaerobically for *B. animalis* subsp. *lactis* B94 in an anaerobic jar). After that, 1% (v/v) of *W. saturnus* var. *saturnus* NCYC22 pre-culture was inoculated on day 3, followed by incubation at 30 °C for another 32 days to simulate the ambient temperature in tropical areas. Similarly, for the control groups, the respective probiotics pre-culture (1%, v/v) was inoculated to the pasteurized durian pulp and incubated at 37 °C for 3 days and then stored at 30 °C for another 32 days. Samples were taken periodically for analyses of various parameters.

2.3. Analytical determinations

MRS agar and MRS agar added with 0.05% (w/w) L-cysteine were used to assess growth of *L. casei* L26 and *B. animalis* subsp. *lactis* B94, respectively. PDA was used to assess the growth of *W. saturnus* var. *saturnus* NCYC22. In addition, the colonies of *L. casei* L26 (smooth round and glossy) can be morphologically distinguished from those of *W. saturnus* var. *saturnus* NCYC22 (wrinkled and matt). The pH value was measured using a pH meter (Metrohm, Switzerland). The organic acids and sugars were analysed based on reported methods (Lu et al., 2016). The organic acids were performed on a Supelcoge1 C-610H column (300 × 7.8 mm, Sigma-Aldrich, Spain) connected with photodiode array (PDA) at 210 nm of a Shimadzu HPLC system. The column was eluted with 0.1% (v/v) sulphuric acid at a flow rate of 0.4 mL/min. The sugars (sucrose, fructose and glucose) were analysed on a Zorbax carbohydrate column (Agilent Technologies, USA) connected to an ELSD-LT detector eluting with a mixture of 80% (v/v) acetonitrile and deionized water at 40 °C, at a flow rate of 1.4 mL/min.

The volatile compounds were analysed according to the method of Lee et al. (2012) using headspace (HS)-solid phase microextraction (SPME) combined with gas chromatography (GC)-mass spectrometer (MS) and flame ionization detector (FID) (HS-SPME-GC–MS/FID). Samples were extracted at 60 °C with HS-SPME (CAR/PDMS fiber) for 50 min using a SPME autosampler and thermally desorbed into the injector port for 3 min at 250 °C. Separation of samples was performed with a DB-FFAP capillary column (60 m × 0.25 mm I.D. × 0.25 μm film thickness) connected to a GC (Agilent 7890A GC-Agilent 5975C) triple-axis MS and FID (Santa Clara, CA, USA) and the oven temperature