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Physio-chemical, microbiological properties of tempoyak and molecular characterisation of lactic acid bacteria isolated from tempoyak

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ABSTRACT

This study aims to determine physio-chemical properties of tempoyak, characterise the various indigenous species of lactic acid bacteria (LAB) present at different stages of fermentation and also to determine the survival of selected foodborne pathogens in tempoyak. The predominant microorganisms present in tempoyak were LAB (8.88–10.42 log CFU/g). *Fructobacillus durionis* and *Lactobacillus plantarum* were the dominant members of LAB. Other LAB species detected for the first time in tempoyak were a fructophilic strain of *Lactobacillus fructivorans*, *Leuconostoc dextranicum*, *Lactobacillus collinoides* and *Lactobacillus paracasei*. Heterofermentative *Leuconostoc mesenteroides* and *F. durionis* were predominant in the initial stage of fermentation, and as fermentation proceeded, *F. durionis* remained predominant, but towards the end of fermentation, homofermentative *Lb. plantarum* became the predominant species. Lactic, acetic and propionic acids were present in concentrations ranging from 0.30 to 9.65, 0.51 to 7.14 and 3.90 to 7.31 mg/g, respectively. Genotyping showed a high degree of diversity among *F. durionis* and *Lb. plantarum* isolates, suggesting different sources of LAB. All tested *Lb. plantarum* and *F. durionis* (except for one isolate) isolates were multidrug resistant. *Salmonella* spp., *Listeria monocytogenes* and *Staphylococcus aureus* were not detected. However, survival study showed that these pathogens could survive up to 8–12 days. The results aiming at improving the quality and safety of tempoyak.

1. Introduction

Durian (*Durio zibethinus*) fruit is one of the most important seasonal fruits cultivated in tropical Asian countries for its high price, exotic aromas and flavours. It is regarded as “King of fruits” in South East Asia. In most Asian countries, except for Thailand, durians are not harvested but allow to drop to the ground when it ripens. Durian has a short shelf-life ranging from two to three days post-harvest. Following harvesting, during retailing or storage, the durian undergoes rapid physio-chemical changes leading to dehiscence and softening of the pulp or the pulp becomes soggy or watery which leads to rapid decline in value (Paul and Ketsa, 2014). Durian pulp from low-quality durians is frozen and used as a flavouring ingredient in many desserts such as durian cake, ice cream and candies (Ho and Bhat, 2015).

Tempoyak is a popular acid-fermented condiment used with certain fish and vegetable dishes in ASEAN countries. The pH of tempoyak ranges from 3.96 to 4.08 (Amin et al., 2004; Wasnin et al., 2014). Tempoyak is usually produced using low-quality durian pulp obtained from crack, poor quality or over-ripen durian. Production of tempoyak allows for the salvage of durians which would be otherwise be discarded (Gandjar, 2000). Thus, fermentation of the over-ripen and poor quality fruit pulp is a cheap and efficient mean of preserving highly perishable climacteric fruits especially in developing countries where proper refrigeration or post-harvest handling facilities are lacking. Durian pulp is traditionally fermented through spontaneous and uncontrolled processes. Like other naturally fermented fruits, tempoyak produced is of variable quality as the indigenous microflora is not consistent (Chen et al., 2013; Nyanga et al., 2008). The fermentation relies on the

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microflora already present in the pulp. The microorganisms present in the pulp might originate from the environment, particularly the soil, rotting vegetation in the orchards, from the hands of vendors or customers, knives, retailing environment, baskets and cross contamination from other crack durians (Papalexandratou et al., 2011). Pulp from different durians is mixed with or without an addition of salt and allowed to ferment in a tightly sealed container (some cottage producers use earthen pots) at ambient temperature for a minimum of seven days for the development of acidity and flavour (Merican, 1977). From our observation while visiting small scale producers and households, we observed that tempoyak is allowed to ferment for months. Tempoyak is produced from one durian fruiting season to another and domestically produced for self-consumption.

Leisner et al. (2001) reported that LAB are the predominant microorganisms present in tempoyak, ranging from 8.4 to 9.2 log CFU/g of tempoyak. They had reported that *Lactobacillus plantarum* were the predominant members of the LAB flora in tempoyak. Other species of LAB present in tempoyak as reported by other researchers are *Lactobacillus brevis*, *Leuconostoc mesenteroides*, *Lactobacillus mali*, *Lactobacillus fermentum*, *Lactobacillus durianis* (Leisner et al., 2002), *Lactobacillus corynebacterium* (Wirawati, 2000), *Lactobacillus fersantum* (Ekowati, 1998), *Lactobacillus casei* (Mohd Adnan and Tan, 2007), *Fructobacillus durionis* (Endo and Okada, 2008; Leisner et al., 2005), *Weissella paramesenteroides and Pediococcus acidilactici* (Yuliani and Dixon, 2011). Among the species reported, *F. durionis* and *Lb. brevis* were reported to exhibit fructophilic characteristics. The term "fructophilic LAB" (FLAB) refers to a specific group of LAB that prefers fructose and sucrose as carbohydrate substrate. FLAB has been isolated from fructose-rich environments such as fruits, flowers, honey, and fermented foods and beverages as well flower- and fructose-related insects (Endo et al., 2009; Endo and Salminen, 2013; Neveling et al., 2012). They are characterised by comparatively rapid growth on fructose than glucose, requires external electron acceptors for glucose metabolism, and a limited number of carbohydrates (Endo et al., 2009).

Like many other fermented or acidified vegetable and fruit products which are consumed without heat treatment, tempoyak being a fermented product is also regarded as microbiologically safe. However, it is not unprecedented that foodborne pathogens were detected in fermented foods and beverages (Marty et al., 2012). Akaki et al. (2011) reported on the occurrence of various potential pathogenic bacteria (*Bacillus* spp., *Klebsiella pneumoniae* spp. *pneumoniae* and staphylococci) in traditional millet-based fermented gruels and *Bacillus* spp. were present at the end of the cooking (82–85 °C). They attributed the incidence to unhygienic practices and environmental conditions, as well as a possible adaptation of the bacteria to the new conditions. Outbreaks traced to contamination in fruits and products of fruit have also been reported (Laidler et al., 2013; Senkel et al., 2003). These researchers were of the opinion soil contaminated with faeces or use of fallen fruits for production as possible routes of contamination. In view of this, microbial safety of tempoyak which is mainly produced using fallen or cracked durian is of great concern.

Previous studies on tempoyak mainly focused on the isolation and identification of LAB present in tempoyak (Ekowati, 1998; Leisner et al., 2001, 2005; Yuliani and Dixon, 2011) as well as characteristics of tempoyak (Neti et al., 2011; Wasnin et al., 2014). The microbial changes of LAB during tempoyak fermentation and the safety aspects of tempoyak, however, have not been reported. As fermented foods possess diverse microflora, the presence of multidrug-resistant LAB is a concern as they pose a threat to food safety and human health (Hummel et al., 2007). The aims of this study were to study the biodiversity of LAB and microbial changes taking place during natural fermentation of tempoyak, as well as highlighting the presence of multidrug-resistant LAB isolates involved in the fermentation of tempoyak. In addition, the microbial safety of the naturally fermented tempoyak was evaluated by determining the survival of foodborne pathogens such as *Salmonella enterica* subsp. *enterica* serovar Enteritidis, *Listeria monocytogenes* and *Staphylococcus aureus* in naturally fermented tempoyak.

2. Materials and methods

2.1. Physio-chemical and microbiological properties of naturally fermented tempoyak

2.1.1. Preparation of tempoyak

The pulp of overripe, crack, unripe durians or durians of inferior quality is pooled by the farmers and usually purchased by individuals for tempoyak and durian candies production. We purchased durian pulp from a durian orchard in Balik Pulau, Penang. According to our observations, the orchard owner removes the pulp of unsold durians which are of inferior quality, over ripen, undergone dehiscence, crack or infested with larvae of fruit flies. The pulp from different durians is pooled and frozen and kept in a −18 °C freezer. The durian pulp used is obtained from different cultivars in the same orchard. The pulp was transferred to food processing laboratory within 1–2 h acquisition but the storage time in the orchard was unknown. The durian pulp was mixed well with a food mixer and separated into aliquots of 200 g each in tightly sealed glass jars. Three individual replicates of tempoyak were prepared. Tempoyak was produced by naturally fermenting (allowing the already present microorganism to grow) the durian pulp for 24 days at 30 ± 1 °C. No starter culture and preservative was added. The durian pulp was subjected to physio-chemical and microbiological analysis.

2.1.2. Physio-chemical analysis of naturally fermented tempoyak

Determination of pH, titratable acidity (TA), total sugar content and organic acid content of the naturally fermented tempoyak were carried out as described by Voon et al. (2006), with minor modifications. Measurements were performed in duplicate with three individual replicates of tempoyak on day 0, 1, 2, 4, 6, 8, 12, 16, 20 and 24.

Briefly, durian pulp (10 g) was homogenized in 90 mL of distilled water for 1 min using a Stomacher 400 Circulator (Seward, West Sussex, United Kingdom) and pH of the slurry was measured. Subsequently, TA of the slurry was determined by titration using 0.1 N NaOH to pH 8.1 and the results were expressed as the percentage of lactic acid.

$$\%\ lactic\ acid\ = \frac{mL\ of\ NaOH\ used \times Normality\ of\ NaOH \times 9}{Weight\ of\ sample}$$

Sugar extraction was performed as previously described (Hunt et al., 1977), with minor modifications. Briefly, 10 g of tempoyak was mixed with 85% methanol and allowed to stand for 30 min in a water bath (60 °C). The sample was centrifuged and the pellet was re-extracted twice with 75% methanol. The supernatant was evaporated using a rotary evaporator. Sample was diluted 100 folds with deionised water and cation exchange resin was added before filtration using muslin cloth. The same procedure was repeated using anion exchange resin. The sample was passed through a pre-activated Sep-Pak C_{18} disposable cartridge (Waters Corporation, Milford, USA) and subsequently filtered using a 0.45 μm membrane filter (Milipore, USA). Twenty microlitres of the extracted sample were injected to a Waters 2414 series HPLC system equipped with a