Chapter 1

Introduction

1.1 Background

Kombucha is a fermented beverage from Asia that has gained popularity in recent years due to its numerous claimed health benefits such as antimicrobial, antioxidative and anticarcinogenic properties (Chakravorty et al., 2016; Coelho et al., 2020; Sreeramulu et al., 2000). It is a non-alcoholic drink traditionally made out of sweetened black tea (*Camellia sinensis*) that has been cultured with a cellulose biofilm called tea fungus, or commonly known as SCOBY (symbiotic culture of bacteria and yeast)(Coelho et al., 2020; Villarreal-Soto et al., 2018). Due to the symbiotic relationship of the microorganisms in the SCOBY, kombucha is rich in probiotics, organic acids, polyphenols, vitamins and other micronutrients (Coelho et al., 2020). Organic acids such as gluconic, lactic, malic, citric and tartaric acids are also produced during the kombucha fermentation which prevents pathogenic and spoilage contamination (Sreeramulu et al., 2000).

Fermentation period of kombucha can range anywhere from 7 to 21 days, depending on the desired organoleptic properties. Longer fermentation generally results in a more acidic flavor because of the organic acids produced by the bacteria (Sreeramulu et al., 2000). In addition, the antioxidant activity in kombucha also escalates with time (Zubaidah et al., 2018). To minimize the acidity and market kombucha to a wider audience, fruit juices are commonly added to impart additional flavor, making it widely acceptable by consumers and can serve as a healthy substitute for carbonated soft drinks (Coelho et al., 2020).

Black tea, as a beverage on its own, is packed with bioactive compounds like catechins, theaflavins, caffeine, theobromine and other volatile compounds that contribute to the sensory and nutritional profile (Samanta, 2022). As the demand and acceptance for kombucha increases, researchers have

been experimenting with different substrates, other than tea, to create varieties of kombucha with increased health benefits (Zubaidah et al., 2018). Juices or extracts of grapes, orange, blackcurrant, cherries, eucalyptus, mentha, Jerusalem artichoke, echinacea and snake fruit have been treated to replace tea in kombucha fermentation (Ahmed et al., 2020). These studies show that the kombucha consortium is not limited to fermenting sweetened tea, but also thrives in other mediums, like fruit juices, as long as the substrate contains the necessary nutrients for the bacteria and yeast to live. In addition, these substrates on their own possess unique health benefits and physicochemical characteristics that are intensified or altered during the fermentation process to create kombucha beverages rich in nutrition that are not offered by conventional black tea kombucha. Thus, the versatility of the kombucha consortium provides new opportunities for the development of kombucha from many other fruits and can serve as an excellent pathway to introduce unpopular fruits, such as black sapote, to the public.

Diospyros digyna, also known as black sapote or black persimmon, is a fruit from the Ebenaceae family originating from South America, specifically Mexico (Jiménez-González & Guerrero-Beltrán, 2021; Merino-Sánchez et al., 2022). The black sapote fruit contains varying phenolic compounds and micronutrients, thus resulting in a high antioxidant capacity (Jiménez-González & Guerrero-Beltrán, 2021). These compounds are related to the health benefits of black sapote including reduction of blood sugar level for type 2 diabetes, decreasing fever and having laxative effects (Jiménez-González & Guerrero-Beltrán, 2021). Moreover, other parts of the *D. digyna* such as its roots, woods and leaves also possess medicinal properties to treat asthma and hypertension among others.

As a climacteric fruit, black sapote are harvested before they are ripe because they mature rapidly due to their carbon dioxide (CO_2) and ethylene production, thus making it highly perishable once ripe (Jiménez-González & Guerrero-Beltrán, 2021). Furthermore, ripe black sapote is difficult to market due to its dark skin and flesh that appears unappealing. Black sapote production in Mexico has reached

approximately 15,000 tons per year, but most of them are wasted due to lack of consumption and its short shelf-life. Since it has a creamy and soft flesh, utilization of black sapote is frequently limited to desserts such as jams, mousses and ice creams (Jiménez-González & Guerrero-Beltrán, 2021). In order to increase its consumption, innovative and novel product development is needed to introduce this fruit to the market. Black sapote has a high flesh to seed ratio, sugar and water content, making it an appropriate candidate for kombucha substrate. Additionally, fermenting black sapote juice into kombucha can extend its shelf-life, while minimizing food waste from ripe black sapote. Along with the growing interest in kombucha, the development of black sapote kombucha (BSK) provides a wide range of opportunities for educational, environmental and economic sectors.

With the availability of black sapote fruits, the minimum research as well as processing methods on this fruit, this study provides a potential product development idea to process black sapote in Indonesia. This study aims to provide an overview of the suitability of black sapote as a kombucha substrate by investigating the effect of fermentation time towards its physicochemical properties over 14 days and comparing them to conventional tea kombucha (TK) made with black tea.

1.2 Objective

The objectives of this study are listed in the following:

1. Investigate the effects of fermentation time on the physicochemical properties of TK and BSK over 14 days of fermentation
2. Compare the physicochemical properties of TK and BSK during 14 days of fermentation

1.3 Hypothesis

The specific hypotheses of the study are as follows:

1. **H_0:** The duration of fermentation does not affect the physicochemical properties of TK or BSK significantly

H_a: The duration of fermentation affects the physicochemical properties of TK or BSK significantly

2. **H_0:** The physicochemical properties of TK against BSK do not have significant differences

   H_a: The physicochemical properties of TK against BSK have significant differences

1.4 Problem Formulation

Ripe black sapote is highly perishable and has a short shelf-life. In addition to that, consumption of black sapote is low which results in a lot of wasted black sapote. Apart from that, application of black sapote as a food ingredient is limited to small-scale production. Developing a functional beverage from black sapote can offer a potential utilization of this fruit as the main ingredient and help minimize waste from ripe black sapote. There have been extensive studies on the properties of kombucha and how fruit juices can be inoculated with the kombucha consortium. However, there are no current studies that investigates the potential and properties of black sapote inoculated with SCOBY. Moreover, by comparing the characteristics of TK and BSK, we can evaluate whether this product could offer improved nutritional benefits.

1.5 Research Scope

The scope of this research includes:

1. Sample preparation of TK and BSK which included purchasing of raw materials, juice extraction process and the fermentation process.
2. Characteristics of ripe black sapote fruits used in this study were olive green/dark-brown skin with black sepal and soft, dark-colored flesh (**Appendix 1**).
3. Analyzing the physicochemical properties of both BSK and TK which included the Brix value, titratable acidity (TA), pH, radical scavenging activity (RSA), total phenolic content (TPC) and color at 5 different sampling points (days 0, 4, 7, 10 and 14) over 14 days of fermentation.

4. This study is part of a bigger research which includes analyzing the physiochemical, microbial and sensorial properties of BSK. However, this study does not discuss the sensorial and microbial analysis.
5. All SCOBY used in this experiment were cultured from the same source.

1.6 Importance of Study

The contribution of this study lies on:

1. Exploring the usage of black sapote into a functional beverage to improve its economic value and introduce its versatility to a wider audience
2. The utilization of SCOBY as the main inoculum to ferment black sapote juice
3. Analyzing the physicochemical properties of BSK in relevance to the health benefits of black sapote kombucha