Chapter 1

Introduction

1.1 Background

Pak-sian dong, or pickled weeds, is a traditional Thai fermented food made from *Cleome gynandra* (L.) leaves and stems, typically preserved in a rice water brine solution. It is often consumed as a sour, pickled vegetable accompaniment in Thai delicacies and is valued for its nutritional and medicinal properties, owing to the bioactive compounds present in *Cleome gynandra* (L.) (Mashamaite et al., 2022). This fermentation commonly relies on spontaneous fermentation, which is also widely used in other vegetable fermentations, such as kimchi and pickled cucumbers (Song et al., 2021). Spontaneous fermentation is driven by the sequential activity of indigenous microbes, particularly lactic acid bacteria (LAB), with the most adapted strains eventually dominating the process (Knez et al., 2023).

The presence of salt in the brine facilitates the extraction of water and carbohydrates from plant tissues, thereby creating an anaerobic environment that promotes the proliferation of lactic acid bacteria (Henney et al., 2010). Salt also favors the growth of halotolerant microorganisms while inhibiting spoilage organisms through its antimicrobial properties and its influence on pH and nutrient availability (Abriouel et al., 2019; Yalçınkaya & Kılıç, 2019). The optimization of salt concentration is therefore critical in selectively enhancing the growth and metabolic activity of desirable microbes, particularly lactic acid bacteria (LAB), which thrive under high water activity and saline conditions (Muhialdin et al., 2018). In contrast, excessive salt concentration may also decrease LAB growth due to hyperosmotic shock, which potentially causes cell death. High dietary salt (NaCl) intake may also induce hypertension, which increases the risk of other non-communicable diseases, such as cardiovascular diseases, osteoporosis, Meniere's disease, and kidney disease (Hunter et al., 2022; World Health Organization, 2025). As a result, the World Health Organization (WHO) global action plan for the prevention of non-communicable diseases aims to attain a 30% reduction in the mean population intake of salt (Hoppu et al., 2017). Nevertheless, spontaneous fermentation is often time-consuming and climate-dependent, which can result in inconsistent outcomes and increased risk of contamination by undesirable or pathogenic microorganisms due to inadequate hygiene (Capozzi et al., 2017; Grujović et al., 2022; Tang et al., 2024).

Another type of fermentation, controlled fermentation, is being explored to create functional foods with health benefits, particularly on an industrial scale. This process utilizes specific microbial cultures to target specific fermentation processes (Szutowska et al., 2021). Controlled fermentation allows for the inoculation of selected probiotic strains, enabling precise identification of the dominant microorganisms and ensuring a consistent probiotic count, unlike spontaneous fermentation, where microbial populations are undefined and variable. This is essential for developing probiotic functional products, as probiotics must remain viable in sufficient numbers to confer health benefits (Behera & Panda, 2020). To be effective, the final product should contain at least 10^6–10^7 CFU/g or CFU/mL at the time of consumption, with a daily intake of approximately 100 g required to deliver around 10^9 viable cells to the intestine (Parvarei et al., 2021; Terpou et al., 2019).

Food-grade microorganisms, particularly lactic acid bacteria (LAB), enhance foods' and beverages' nutritional value, flavor, texture, and shelf life stability throughout the fermentation period (Harper et al., 2022; Leeuwendaal et al., 2022). A strain of gram-positive lactic acid bacteria, namely *Lactiplantibacillus pentosus*, formerly known as *Lactobacillus pentosus*, is found to act as a prominent strain that can produce high levels of gamma-aminobutyric acid (GABA). As cited by Cui et al. (2020) in Kittibunchakul et al. (2021), GABA from lactic acid bacteria has received extensive attention from both academia and industry in recent years because of the “GRAS” status of the lactic acid bacteria and their crucial roles in the food industry. *Lactiplantibacillus pentosus* 9D3 strain isolated from pickled spider plant (*Pak-sian dong*) was successfully incorporated as a probiotic with a high number of live bacteria (8.6 log CFU/ml) in a plant-based GABA drink using brown rice as a raw material (Kittibunchakul et al., 2021; Raethong et al., 2022). Thus, reintroducing this GABA-producing strain into fermented *Cleome gynandra* (L.) helps with consistency, safety, and quality control of the end product.

Based on the aforementioned theories, controlled fermentation by utilizing *Lactiplantibacillus pentosus* 9D3 has the potential to increase the probiotic count, exert high GABA levels, and enhance the properties of *Cleome gynandra* (L.) compared to spontaneous fermentation. This study further investigates the proper salt concentration for *Cleome gynandra* (L.) fermentation, utilization of *Lactiplantibacillus pentosus* 9D3 as a probiotic strain to improve the fermentation, and analyzes changes in physicochemical properties along with microbial activity compared to spontaneous fermentation. This study will also assess the antioxidant activity, total phenolic content (TPC), and GABA content of the fermented products using both spontaneous and controlled fermentation. The findings will contribute to the development of a functional food product with potential health benefits for consumers.

1.2 Objective

The study will focus on the following specific objectives:

1. To determine the optimal salt concentration in rice-washed brine solution for fermenting *Cleome gynandra* (L.) under spontaneous and controlled fermentation with *Lactiplantibacillus pentosus* 9D3
2. To investigate the physicochemical parameters and microbial enumeration of *Cleome gynandra* (L.) pre- and post-fermentation under spontaneous and controlled conditions, and quantify antioxidant activity, total phenolic content (TPC), and gamma-aminobutyric acid (GABA) levels following fermentation.

1.3 Hypothesis

Along with the objectives mentioned above, several hypotheses were made for the effect of the spontaneous fermentation of *Cleome gynandra* (L.):

H_0 : No significant differences in the pH, total soluble solids, total acidity, and microbial growth occurred between day 0 and day 3 of spontaneous fermentation of *Cleome gynandra* (L.)

H_1 : Significant differences in the pH, total soluble solids, total acidity, and microbial growth occurred between day 0 and day 3 of spontaneous fermentation of *Cleome gynandra* (L.)

Several hypotheses were also proposed for the different brine solution concentrations:

H_0 : There were no significant differences in different salt concentrations in the brine solution towards the pH, total soluble solids, total acidity, and microbial growth.

H_1 : Different salt concentrations in the brine solution significantly affected the pH, total soluble solids, total acidity, and microbial growth.

The hypotheses for the effect of fermentation time of spontaneous and controlled fermentation include:

H_0 : The duration of fermentation significantly affects the physicochemical properties and microbial count of fermented *Cleome gynandra* (L.)

H_1 : There was no significant difference in the physicochemical properties and microbial counts of fermented *Cleome gynandra* (L.) across different fermentation times

Meanwhile, the hypotheses for the effect of different fermentation types of *Cleome gynandra* (L.) were:

H_0 : There were no significant differences between spontaneous and controlled fermentation using *Lactiplantibacillus pentosus* 9D3 towards the physicochemical, microbial count, antioxidant, total phenolic content (TPC), and GABA content.

H_1 : There were significant differences between spontaneous and controlled fermentation using *Lactiplantibacillus pentosus* 9D3 towards the physicochemical, microbial count, antioxidant, total phenolic content (TPC), and GABA content.