CHAPTER I
INTRODUCTION

1.1. Background

Folic acid (B9 vitamin) is one of the important vitamins, especially for expecting mothers and child development. Folate itself is an umbrella term comprising compounds related to the pteroylglutamic acid (folic acid) and its derivatives (Mann and Truswell, 2012). Naturally, folate in food is in reduced form called tetrahydrofolate (THF) and may contain multiple glutamic acid units (polyglutamic acid) (Mann and Truswell, 2012). On the other hand, folic acid is the most oxidized form of folate and is made synthetically by virtue of its stability. Humans cannot self-synthesize folate; thus, folate needs must be fulfilled by dietary intake of food or supplementation. Folic acid and folate serve a paramount function as co-enzyme in DNA synthesis (Ross et al., 2012; Mann and Truswell, 2012). Deficiency in folic acid can lead to megaloblastic anemia and neural tube defect (NTD).

Corn is one of the commodities in Indonesia that is not only consumed for its high carbohydrate contents as staple food, but it also provides numbers of important nutrients, such as dietary fiber, essential fatty acids, carotenoids, and vitamins, including cobalamin, niacin, and folic acid (Koswara, 2019). In terms of folate, corn has 42 µg folate per 100 g of corn kernel, which is considered high compared to other cereals such as rice, sorghum, and barley which has 7 µg, 20 µg, 23 µg per 100 g (USDA, 2020). In addition to that, dent corn, alongside flint corn, sweet corn and popcorn, is one of the most produced varieties of corn in Indonesia yet having an affordable price. USDA even estimated that in the market year of 2021/2022, Indonesia will produce up to 12.7 million tonnes of corn (USDA, 2022).

Folic acid fortification has been proposed to increase folic acid intake, and has been made mandatory to certain food groups in certain countries (Jägerstad et al., 2005). For example, in Indonesia folic acid fortification has been put mandatory for wheat flour. Even though folic acid fortification was proven to reduce NTDs, countries like the Netherlands still prohibit folic acid fortification due to some concerns (Varela-Moreiras, Murphy, & Scott, 2009). The two main concerns are the long-term high dose of folic acid intake that could promote gastrointestinal cancer (e.g., colorectal cancer) and the masking of B12 deficiency (Naderi & House, 2018). It is due to limited conversion of folic acid into 5-methyltetrahydrofolate, thus the unmetabolized folic acid will remain in the circulation. Natural folate does not need such conversion since most of them are already metabolically active, thus does not pose the aforementioned concerns (Jägerstad et al., 2005). Therefore new means to increase folate intake by using natural folate are of interest in the research.

One method that can be used to boost natural folate content is bioprocessing, including fermentation (Jägerstad et al., 2005). Tempeh is a well known fermented food from Indonesia. In tempeh production, *Rhizopus oligosporus* is a common mold to produce tempeh, either in a single species of *R. oligosporus* or in a mixture with other species of *Rhizopus*. Tempeh fermentation was shown to increase the folic acid content of the substrate significantly (Asmoro, 2016). Even though the increases in folic acid content were inseparable with the activity of lactic acid bacteria, the fact that different species of *Rhizopus* could give significant differences, it indicated that the inoculation of *Rhizopus* itself could indeed increase the folic acid (Asmoro, 2016). The problem arises where soybean production in Indonesia is still far below the consumption, thus in 2020 as much as 81% of soybean needs were imported (Harsono et al., 2021). On the other hand, corn offers high production when it also has relatively high folic acid and protein content, making it suitable for tempeh substrate substitution. Prior to tempeh production, nixtamalization, i.e. a steeping process of the corn in lime solution, has been reported to soften its pericarp and increase the nutritional quality of certain nutrients, including folic acid (Maryati et al., 2017; Kusbardini, 2017).

Membrane separation technology has been implemented in various industries, including in food, where one of its functions is for concentrating (Marson et al., 2020). This membrane technology has been used in several previous studies in concentrating folic acid and the concentration of folic acid was obtained in the permeate (Susilowati et al., 2018; Aspiyanto & Susilowati, 2010; Aspiyanto et al., 2018; Kusbardini, 2018). However, none of those studies used corn tempeh as the feed. Given that different characteristics of feeds will exhibit different results of membrane filtration, therefore it is important to understand the effect of using corn tempeh. Microfiltration is chosen in this study mainly due to the possibility of having a bigger particle size as well as higher capacity that the module has. Another gap is that only lab-scale production was used.

Therefore, this study is aiming to find out the increase of folic acid content in corn after undergoing solid fermentation and microfiltration in a bigger, bench-scale production. The findings of this study hopefully can be used to develop functional food rich in natural folic acid for making complementary baby food, and food for expecting as well as lactating mother, since the global demand for natural food additives is steadily increasing (Sun et al., 2021).

1.2. Objectives

The objectives of this study are as followings. This study was further divided into preliminary and primary study. Hence, objective (a) and (b) is for preliminary study while objective (c) is for primary study.

a. To prove that tempeh (solid) fermentation by *Rhizopus oligosporus* C1 can increase folic acid content in corn.

b. To understand the effect of corn types, nixtamalization, form of nixtamal, and inoculation types towards the chemical composition, especially folic acid, of nixtamalized corn tempeh.

c. To understand the effect of inoculum types, filtration time, and stream types toward the chemical composition, especially folic acid content, of microfiltered nixtamalized corn tempeh.

1.3. Research Questions

Based on the objectives above, the research questions that the author tried to answer are as followings. Research question (a) and (b) would be answered by the primary study while research question (c) would be answered by the primary study.

a. Can the tempeh (solid) fermentation by *Rhizopus oligosporus* C1 increase folic acid content in corn?

b. How do corn types, nixtamalization, form of nixtamal, and inoculation types affect the chemical composition, especially folic acid content, of the nixtamalized corn tempeh?

c. How do inoculum types, filtration time, and stream types affect the chemical composition, especially folic acid content, of microfiltered nixtamalized corn tempeh?

1.4. Hypotheses

Based on the research questions, the hypothesis for each questions are as followings.

a. Tempeh (solid) fermentation by using *Rhizopus oligosporus* strain C1 can increase the folic acid content in corn.

b. Corn types, nixtamalization, form of tempeh, and inoculation types will affect the chemical composition, especially folic acid content, of the nixtamalized corn tempeh.

c. Inoculum types, filtration time, and stream types will affect the chemical composition, especially folic acid content, of microfiltered nixtamalized corn tempeh.