#### **CHAPTER 1**

# INTRODUCTION

### 1.1. Background

Anthocyanins are the most abundant flavonoid glycoside and they are water-soluble pigments responsible for red, purple, and blue colors in a variety of edible plant materials, including the skin of red apples, plums, grapes as well as on the pigmented rice (colored rice such as black and red rice) (Branen *et al.*, 2001; Kim *et al.*, 2008; Chung *et al.*, 2015; Wu, Guan, & Zhong, 2015). These anthocyanins are widely used in food and beverage products as natural colorants to substitute the synthetic colorants that can cause toxicity in humans (Branen *et al.*, 2001; Castañeda-Ovando *et al.*, 2009; Chung *et al.*, 2015). In addition to their application in food and beverage industries, anthocyanins also have beneficial properties for human health. Studies from Chung *et al.* (2015) and Wu, Guan, & Zhong (2015) listed several beneficial properties of anthocyanins that had been studied previously, including excellent antioxidant stability, neuroprotective, anticarcinogenic, and antidiabetic functions, visual acuity, and dermal health. However, as the critical quality parameter in some range of products, the stability of anthocyanins is relatively low, and some of the factors that affect the stability of anthocyanins are oxygen, temperature, light, enzymes, pH, and food matrix composition (Chung *et al.*, 2015). Therefore, the attempt to maintain the stability of anthocyanins in foods is greatly desirable to provide humans with their health benefits.

Being thermally unstable, thermal degradation of anthocyanins has become a significant issue for the food industry; yet heat treatment is one of the most widely used methods for preserving and extending the shelf life of foods (Wang & Xu, 2007; Hou *et al.*, 2013). The thermal stability of anthocyanins depends on thermal treatment condition (temperature and time) and environmental factors such as pH, oxygen, light, and co-solutes. Anthocyanins are thermally stable below pH 3 and unstable at neutral pH; thus improving the thermal stability of anthocyanins, specifically at neutral and weakly acidic conditions, will expand their applications (Wu, Guan, & Zhong, 2015). Co-pigmentation is a well-known strategy to improve the stability of anthocyanins. It is a phenomenon of complex formation between pigments and other compounds (co-pigments), including flavonoids, alkaloids, amino acids, organic acids, nucleotides, polysaccharides, metals or other anthocyanins which often leads to an improvement in color stability (Castañeda-Ovando *et al.*, 2009). The co-pigmentation of anthocyanins with several co-pigments has been studied, for examples with  $\beta$ -cyclodextrin and pectin.  $\beta$ -cyclodextrin was observed to be able to improve the stability of chokeberry anthocyanins at pH 2.8-3.6 and improve the thermal stability of anthocyanins at pH 4.0 while pectin was observed to stabilize the anthocyanins of blackcurrant at pH 3.0 during storage (Wu, Guan, & Zhong, 2015). The complex formation of anthocyanins with several co-pigments at pH aco-pigments that have been studied previously can be used as a consideration to utilize other compounds as the co-pigment of anthocyanins, and one of the possible compounds that has the potential to improve the stability of anthocyanins through co-pigmentation is rice bran hydrolysates (RBH) derived from rice bran.

Rice bran is one of the by-products of the milling process of paddy. It is the brown rough layer present between the endosperm and the outer husk of the paddy, and it makes up roughly 10% by weight of the paddy (Shih, 2003; Park, Lee, & Choi, 2017). In 2018, FAO reported that there are about 769.9 million tonnes of rice produced as paddy. In such a case, the estimated amount of rice bran produced globally in 2018 is about 77 million tonnes, and the high amount of rice bran waste creates more opportunities for the utilization of this waste. However, rice bran is still often underutilized and it is usually used as an ingredient for animal feed or other low-value purposes, such as fertilizer (Cheetangdee, 2014; Park, Lee, & Choi, 2017). Despite of that, rice bran still contains a number of nutrients and biologically active compounds, including several antioxidant compounds such as tocopherol, tocotrienol, gamma-oryzanol, polyphenolic compounds, flavonoids, and peptides as well as phytochemicals, dietary fibers, and also proteins (Boonla *et al.*, 2015; Supawong, Thawornchinsombut, & Park, 2018).

Proteins in rice bran are divided into four groups according to their solubility and extractability; they are albumin (water-soluble), globulin (salt-soluble), prolamin (alcohol-soluble),

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and glutelin (alkali-soluble) (Fabian & Ju, 2011). Several methods have been developed to extract bioactive peptides from rice bran with their advantages and disadvantages, for example, alkaline extraction, use of multiple solvents, enzymatic extraction, and subcritical water extraction. A more recent method, the combination of subcritical water and enzyme extraction to extract rice bran protein has also been developed to improve the extractability of the protein (Fabian & Ju, 2011).

RBH is the product of proteolysis full fat or defatted rice bran by proteases and during the past few years, there have been studies relating to the application of RBH for their potential effect to prevent certain diseases in animal studies (Boonla *et al.*, 2015; Boonloh, Kukongviriyapan, Pannangpetch, *et al.*, 2015; Boonloh, Kukongviriyapan, Kongyingyoes, *et al.*, 2015; Boonloh *et al.*, 2018; Senaphan *et al.*, 2018). Furthermore, there are also other researches that study the effect of RBH on the physicochemical stability of oil-in-water emulsion (Cheetangdee, 2014) and lipid and protein oxidation in fried fish cakes (Supawong, Thawornchinsombut, & Park, 2018). Nevertheless, the effect of RBH on the stability of natural colorants has not been reported. Therefore, the present research aimed to investigate the effect of RBH on the stability of anthocyanins from pigmented rice bran.

### 1.2. Objective

The objective of this research was to evaluate the effect of RBH on the stability of anthocyanins extracted from pigmented rice bran at pH 4 and 7 after thermal treatment at 80°C for 0, 10, 20, 30, 40, 50, and 60 minutes.

# 1.3. Hypothesis

Addition of RBH will improve the stability of anthocyanin during thermal degradation at both pHs. It is also expected that the addition of RBH to anthocyanin solution at pH 4 will result in a lower degradation rate (k) compared when RBH is added to the anthocyanin solution at pH 7.

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# 1.4. Benefits of Research

This research can be used as a consideration to utilize RBH from rice bran and use it to maintain the stability of natural colorants such as anthocyanins in food and beverage products. Furthermore, this research also serves as preliminary research for further studies concerning the utilization of RBH as the stabilizing agent for the natural colorant.