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# Effect of blanching on enzyme activity, color changes, anthocyanin stability and extractability of mangosteen pericarp: A kinetic study

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ABSTRACT

Mangosteen pericarp is a rich source of anthocyanins. However, the high polyphenol oxidase (PPO) activity challenges potential applications of pericarp as natural colorant. The kinetics of PPO inactivation, anthocyanin loss and color changes were determined over a temperature range of 60–100 °C. First-order kinetic model provided the best prediction of the PPO inactivation ($R^2 \geq 0.977$), while anthocyanin loss was described by a Weibull kinetic model ($R^2 \geq 0.969$). The activation energies of PPO inactivation, anthocyanin loss and total color changes were 43.11, 57.66 and 18.86 kJ/mol, respectively. Anthocyanin content was the most sensitive parameter towards temperature changes, suggesting the importance of its monitoring as a quality parameter during thermal processing. Blanching enhanced the efficiency of anthocyanin extraction. Gathering the quantitative information on the changes of PPO activity as well as quality characteristics during blanching of mangosteen pericarp is important in order to design a proper pre-processing condition.



## 1. Introduction

Natural colorants are considered safer than synthetic colors. They are highly demanded in the fast-growing functional and natural markets. Anthocyanins are interesting natural red colorants because of their attractive coloring property, high antioxidant activity and potential health effects (He and Giusti, 2010). Natural colorants are more expensive than synthetic ones. Selection of a source rich in pigment is a way to improve yield and produce a cost-effective natural color. The major commercial sources of anthocyanins are grape and grape skin, with anthocyanin concentration of 30–750 mg/100 gr (Bridle and Timberlake, 1997) and 154.6 mg/100 gr (Liazid et al., 2011), respectively.

Mangosteen (*Garcinia Mangostana* L.) is widely cultivated in Southeast Asia, Central America and tropical Africa (Dembitsky et al., 2011). Fruits consist of two parts, the purplish pericarp and the white inner pulp. The pulp is processed into juice, jam and syrup. While, the pericarp composes 70% of fruit and is considered as agriculture waste (Chisté et al., 2010). It has high anthocyanin concentration of 182.4–423.5 mg/100 gr (Palapol et al., 2009). Based on availability and anthocyanin concentration, mangosteen pericarp is a promising source of natural colorant. Its major anthocyanin is cyanidin 3-sophoroside followed by cyanidin 3-glucoside (Palapol et al., 2009) and pelargonidin 3-glucoside at lower concentrations (Zarena and Udaya Sankar, 2012). Nevertheless, the rapid browning of pericarp after it is cut or crushed limits its use as a red colorant. Enzymatic browning is the main reason for this discoloration.

Polyphenol oxidase (PPO) is one of the common endogenous enzymes that contribute to anthocyanin degradation. PPO is a copper-containing enzyme, with a di-nuclear copper center (Klabunde et al., 1998). This enzyme catalyzes two different reactions in the presence of molecular oxygen: the o-hydroxylation of monophenols to o-diphenols and the oxidation of o-diphenols to o-quinones (Klabunde et al., 1998). The quinones are very reactive electrophilic molecules that can covalently modify nucleophiles such as anthocyanins, which results in formation of brown pigments called melanins (Altunkaya and Gökmen, 2012). PPO can react directly with anthocyanins, though they are weak substrates for the enzyme (Kader et al., 1997), but mostly anthocyanin

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degradation involves a co-oxidation of enzymatically generated o-quinones and/or secondary oxidation products formed from quinone (Fang et al., 2007; Kader et al., 1997; Ruenroengklin et al., 2009). According to Falguera et al. (2012), mangosteen has one of the highest PPO activity compared to other tropical fruits and the enzymatic browning of fruit is strongly related to its PPO activity.

Blanching is a thermal pretreatment to inactivate oxidase enzymes via denaturation of enzyme proteins. This unit operation has dual effect on quality characteristics of product. On the one hand, the inhibition of enzyme activity stabilizes bioactive compounds during processing and storage (Brownmiller et al., 2008; Rossi et al., 2003); furthermore, blanching causes structural changes in plant tissue, which leads to increase in the cell wall porosity and consequently enhances mass transfer and extraction yield of phenolic compounds (Cipriano et al., 2015; Lin et al., 2012; Stamatopoulos et al., 2012). On the other hand, losses of phenolic compounds occur due to thermal degradation and/or leaching during blanching (Volden et al., 2008). Through applying optimal blanching conditions, it is possible to achieve an acceptable inactivation of enzymes while minimizing food quality degradations. Proper design of blanching processes requires the knowledge of thermal properties of product and quantitative changes of its enzyme activity as well as quality attributes during thermal processes (Ling et al., 2014). This information is specific for each fruit depending on its species, cultivar and environmental conditions (Gonçalves et al., 2010).

Kinetic modeling is a useful approach for designing and optimizing thermal processes in order to maximize quality (Anthon and Barrett, 2002; Shivhare et al., 2009). The first step is to choose a good kinetic model. Depending on the sample, different mathematical models may be employed for prediction of degradation reaction rates in foods. Generally, kinetic models can be classified into fundamental and empirical models (Van Boekel, 2008). Arrhenius model is a semi-empirical model that commonly used for modeling. Since food reactions are complex in nature, it is recommended to use a pure empirical model along with the Arrhenius model and choose the model with better fit (Van Boekel, 2008). Weibull model is a pure empirical model and has a great flexibility due to its non-linear nature (Corradini and Peleg, 2004). It was used to model enzyme inactivation (Shivhare et al., 2009) and anthocyanin degradation (Odriozola-Serrano et al., 2009).

This research is the first attempt to stabilize anthocyanins of mangosteen pericarp. PPO inhibition is a crucial step in this regard. Considering the necessity of blanching mangosteen pericarp and the intrinsic susceptibility of anthocyanins to heat, the aim of this study was to analyze and quantify the effect of thermal processing on inactivation of PPO, stability and extractability of anthocyanins as well as color changes, by applying a kinetic approach, in order to select a proper pretreatment condition.

## 2. Materials and methods

### 2.1. Plant materials

Fresh mangosteens (*Garcinia Mangostana* L.), 35 kg, of commercial maturity stage (a fully purple color), were obtained from a local market in Serdang, Selangor, Malaysia, in Jun 2012.

### 2.2. Physicochemical characteristics of mangosteen

Soluble solids content (SSC) and titratable acidity (TA) of aril juice were determined as indicator of fruit maturity stage. To extract the juice, the white flesh with the enclosed seeds was wrapped in cheesecloth and squeezed by hand. SSC was measured using a digital portable refractometer (Atago-Master-20 M, Japan). TA (% citric acid) was determined by titration of 5 mL of the juice with 0.1 M NaOH. The maturity index was calculated as SSC/TA ratio (Palapol et al., 2009). Moisture content was determined by oven drying at 105 °C overnight. To measure the pH of pericarp, 15 g of pericarp was blended with 45 mL of distilled water for 2 min and filtered through Whatman No.1 filter paper. The pH of filtrate was measured using a pH-meter (pH-Meter Mettler-S20 SevenEasy, USA). All assays were performed in triplicate. Fruit firmness was determined on whole fruit using a texture analyzer (TA-XT2, Stable Micro System Ltd. Surrey, England, UK) equipped with a 2 mm round flat probe. Color was determined at the surface of fruits using a Minolta CR-300 portable colorimeter (illuminant D 65). The $a^*/b^*$ ratio was calculated as color index (Palapol et al., 2009). Three measurements were done at three different locations of five fruits.

### 2.3. Preparation of crude enzyme extract

The enzyme extracts were obtained by blending of mangosteen pericarp with 0.1 M potassium phosphate buffer (pH 7) (1:6 v/w). The buffer contained 4% (w/v) polyvinilpolypyrrolidone (PVPP) (Sigma–Aldrich, Germany) and 1% (v/v) Triton X-100 (Sigma–Aldrich, Germany) (Garcia-Palazon et al., 2004). PVPP is a not-specific phenolic absorbent and Triton X-100 is a surfactant. The homogenate was passed through cheesecloth followed by centrifugation at 4 °C (4323× *g*, 15 min), using Kubota centrifuge, model 5800 (Kubota Corp., Tokyo, Japan). The supernatant was collected as crude enzyme extract.

### 2.4. Enzyme activity assay

PPO activity was assayed by measuring the initial rate of increase in absorbance at 410 nm (Arnnok et al., 2010) using a Thermo Scientific GENESYS 10S UV–Vis spectrophotometer. The substrate solution was composed of 1.95 mL phosphate buffer (0.1 M, pH 7.5) and 1 mL of 0.1 M catechol. The reaction was initiated by the addition of 50 μL enzyme extract and monitored for 5 min at 5-s intervals. The data represent the average of triplicate assays.

One unit of PPO activity (U) was defined as a change in absorbance of 0.001 in 1 min. The specific activity was determined as U/mg protein. Protein content was determined by the Lowry method (Peterson, 1977), using bovine serum albumin (0.1–1.0 mg/mL) as the standard. Samples were diluted so their protein content fell within the linear range. The average protein content of samples was 12.05 ± 1.98 mg/mL.

Residual enzyme activity in heat-treated samples is expressed as a fraction of initial activity ($C_0$).

$$\text{Residual enzyme activity } (\%) = C_t/C_0 \times 100 \quad (1)$$

Where $C_t$ and $C_0$ were the specific enzyme activities of treated and untreated samples, respectively.

### 2.5. Preparation of anthocyanin extracts

The extraction was performed under equal conditions for all samples following the method described by (Chisté et al., 2010) with a slight modification. Mangosteen pericarp was made into paste using blender. 4 g of paste was macerated with 40 mL ethanol 95%:HCl 1.5 M (75:15, v/ v) in a 250 mL beaker under stirring (300 rpm) for 12 h at 4 °C under light-free conditions. The extract was filtered through Whatman No.1 filter paper and concentrated at 40 °C under vacuum using a rotary evaporator (Eyela, Tokyo, Japan) to remove ethanol. The concentrated extract was made up to 10 mL with distilled water and centrifuged (Kubota 5800; Kubota,