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Bread fortified with anthocyanin-rich extract from black rice as nutraceutical sources: Its quality attributes and *in vitro* digestibility

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ABSTRACT

Anthocyanin-rich black rice extract powder (ABREP) as a nutraceutical source was fortified into bread. The quality and digestibility behaviors of bread with ABREP were evaluated through instrumental and *in vitro* digestion studies. The quality of bread with 2% of ABREP was not significantly ($p > 0.05$) different from the control bread; however, increasing the ABREP level to 4% caused less elasticity and higher density of bread. A mathematical model was further developed to systemically describe the trajectory of bread digestion. The digestion rates of bread with ABREP were found to be reduced by 12.8%, 14.1%, and 20.5% for bread with 1%, 2%, and 4% of ABREP, respectively. Results of the study suggest that the fortification of anthocyanins into bread could be an alternative way to produce functional bread with a lower digestion rate and extra health benefits.

1. Introduction

As a staple food for many people, bread is popular across the world. Bread is a carbohydrate-rich product, which contains a high amount of rapidly digestible starch, and therefore many of them have a high glycemic index (GI). Due to the rapid digestion of bread, people likely consume excessive bread more than their body requires to make up the hungry feel (Therdthai & Zhou, 2014). The excessive consumption of bread could increase the risk of overweight and obesity, and therefore their associated diseases, such as Type II diabetes (Bautista-Castaño & Serra-Majem, 2012).

Among the diverse pharmacological strategies for the treatment of Type II diabetes, the inhibition of digestive enzymes by plant-extracted enzyme inhibitors is one of the most promising methods. As such, the significance of plant-based enzyme inhibitors for the modulation of diabetes has been studied for many years (McCue, Kwon, & Shetty, 2005). Among the different plant extracts, anthocyanins are recently attracted a substantial amount of interests due to their health-promoting potency. Anthocyanins belonging to the group of flavonoids are naturally occurring pigments in fruits and vegetables, which are responsible for the orange, red, violet, and blue colors observed in nature (Manach, Scalbert, Morand, Remesy, & Jimenez, 2004). Fruits and vegetables, such as blueberries, grapes, blackberries, purple cabbage, black rice, and purple potatoes, are naturally rich in anthocyanins. The well-known healthy property of anthocyanins is their antioxidant capacity due to their peculiar chemical structure that can react with reactive oxygen species (ROS), such as superoxide, singlet oxygen, peroxide, hydrogen peroxide, and hydroxyl radical (Bueno et al., 2012). Apart from this, recent studies reported that anthocyanins also had an inhibitory activity against digestive enzymes. McDougall et al. (2005) tested the inhibitory activity of several extracts from soft fruits against α-glucosidase, and they observed that the anthocyanins-rich fraction effectively inhibited α-glucosidase. Matsui et al. (2001) found that anthocyanins from various plants had a potential function to inhibit α-amylase, and to suppress the increase in postprandial glucose level from starch.

However, knowledge of using anthocyanins as active ingredients in real food systems is very limited. Hence, in this study, we aimed to develop a real food product fortified with anthocyanins. Bread was selected as the real food system and the carrier of anthocyanins, because of its popularity around the world. Meanwhile, the current approaches for developing health-promoting bread are dominated by adding whole grains and fibers in bread, partly aiming to slow down its digestion among several health benefits. The feasibility of fortifying anthocyanins into bread to achieve a slow digestion property as well as the quality attributes of such

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bread were scientifically evaluated. The anthocyanins-fortified bread may bring health benefits to consumers who seek for a healthier alternative to normal type of bread. Results of this study might serve as a guideline for bread manufacturers with regards to the level of anthocyanins in bread to achieve a desired digestibility without compromising its quality.

2. Materials and methods

2.1. Experimental materials

Anthocyanin-rich black rice extract powder (ABREP) was purchased from Shaanxi Taiji Huaqing Technology Co., Ltd., China. Bread flour containing 13% protein was purchased from Prima Ltd., Singapore. Pure cane sugar (fine grain, NTUC Fairprice Cooperative Ltd., Singapore), fine salt (NTUC Fairprice Cooperative Ltd., Singapore), vegetable shortening (Bake King, Gim Hin Lee Ltd., Singapore), and instant dry active yeast (*Saccharomyces cerevisiae*, S.I. Lesaffre, France) were purchased from a local supermarket. Pepsin (from porcine gastric mucosa, product number P6887), α-amylase (type VI-B, from porcine pancreas, product number A3176), 3,5-dinitrosalicylic acid reagent (DNS) were purchased from Sigma–Aldrich (Sigma–Aldrich, MO, USA). Cyanidin-3-glucoside standard was purchased from Polyphenols Laboratories (Sandnes, Norway).

2.2. Farinograph test

The farinograph test is commonly applied in estimating the amount of water required to make dough of good quality, and evaluating the effects of ingredients on flour mixing properties (Center, 2004). Bread flour fortified with different levels of ABREP was separately loaded on a Farinograph-E equipped with a S50 mixer and sigma blades (Brabender, Duisburg, Germany). Farinograph test was conducted according to the constant flour weight procedure of the AACC Method 54-21 (AACC, 2000). The farinograph indices of water absorption of flour with and without ABREP, dough development time, and dough stability were determined.

2.3. Extensograph test

The extensograph test is often used in determining the resistance and extensibility of dough as well as the effect of additives on dough performance (Center, 2004). Freshly prepared bread dough pieces containing 1.2% salt and different levels of ABREP were tested using an Extensograph-E (Brabender, Duisburg, Germany) according to the AACC Method 54-10 (AACC, 2000). The following extensograph indices were determined: energy, resistance to extension (at 5 cm), extensibility, and ratio number.

2.4. The preparation of bread

Black rice was reported to contain a large amount of anthocyanins in its aleurone layer (Lee, 2010). Commercially purchased anthocyanin-rich black rice extract powder (ABREP) was used as the source of anthocyanins. The type and content of anthocyanins in the ABREP have already been identified and quantified in our previous study (Sui & Zhou, 2014). ABREP was added to 100 g of plain bread flour at the levels of 0%, 1%, 2%, and 4% (weight of ABREP/weight of plain bread flour). Bread dough was prepared using the procedure reported in our previous study (Sui, Yap, & Zhou, 2015). Briefly, bread flour with added ABREP was mixed with water (water amount was adjusted according to the farinograph results as shown in Table 1), 4 g sugar, 3 g shortening, 1.2 g salt, and 1 g instant dry active yeast at a slow speed for 1 min followed by an intensive mixing for 5 min in a mixer (WAG-RN20, Varimixer, Globe, US). The dough was then divided and molded to 50 g each using an electronic molder (DR Robot, Daub Bakery Machinery B.V., Holland), followed by 70 min of proofing at 40 °C and 85% relative humidity in a proofer (Climatic chamber-KBF, Binder, Germany) before being baked in an oven (Eurofours, France). The baking temperature was set to 200 °C and the baking duration was 8 min. Despite of the severe baking condition (i.e. 200 °C for 8 min), a large amount of anthocyanins, circa 79% of cyanidin-3-glucoside, was retained in bread crumb after the baking according to our previous study (Sui et al., 2015).

2.5. Specific volume test

Bread volume is an important indicator for evaluating the quality attributes of bread (Ananingsih, Gao, & Zhou, 2013). The volumes of bread dough and freshly baked bread fortified with 0%, 1%, 2%, and 4% of ABREP were measured using a Volscan Profiler (VSP 600; Stable Micro System Ltd., Surrey, UK). The specific volume was then derived from dividing bread volume (cm^3) by its weight (g).

2.6. Texture attributes study

The texture attributes (hardness, springiness, cohesiveness, chewiness, and resilience) of bread crumb with and without ABREP were evaluated using a texture analyzer (TA.XT*plus*; Stable Micro System Ltd., Godalming, UK) according to the AIB standard procedure AIB CAKE2/P1 (AIB Standard Procedure, 2011). Since gumminess is not an appropriate measurement for baked foods, and also gumminess is mutually exclusive with chewiness, results of gumminess were not included in this study.

2.7. In vitro digestibility study

The *in vitro* digestibility study was conducted according to the study of Wolter, Hager, Zannini, and Arendt (2014) with some modifications. The dosage of pepsin and α-amylase was adopted according to the recommended amount from a recently published review (Minekus et al., 2014). After baking, freshly baked bread was placed on metal plates for cooling down to room temperature. As bread crumb was the focus of this study, bread crust was manually removed from the bread. The bread crumb fortified with 0%, 1%, 2%, and 4% of ABREP was respectively freeze-dried and then ground into powder. To keep the same amount of starch in each test, 500 mg of control bread crumb powder, 504.6, 509.2, and 518.3 mg of bread crumb powder fortified with 1%, 2%, and 4% of ABREP, respectively, were placed in 5 mL of phosphate buffer containing 40 ppm of $CaCl_2$, followed by pH adjustment (pH 1.5) using HCl (1 M). Pepsin (3.5 mg, 3200–4500 units/mg) was subsequently added into the mixture to start hydrolysis. The mixture was incubated in a shaking water bath at 37 °C for 1 h. At the end of 1 h, the pH of the mixture was adjusted to 6.9 using K_2CO_3 (0.1 g/mL) to stop the reaction and achieve the pH condition for the following enzymatic reaction. The simulation of small intestine digestion was carried out by adding 55.5 mg of α-amylase (21.6 units/mg) into the mixture followed by transferring the mixture into a dialysis tubing (cut-off size 7000 kDa). The dialysis tubing was then placed into a blue cap bottle containing 100 mL of phosphate buffer with $CaCl_2$ (40 ppm). The reaction was started by incubating the blue cap bottle in the shaking water bath at 37 °C. Although a digestion time of 2–3 h was normally recommended in the literature (Minekus et al., 2014), to thoroughly evaluate the trajectory of inhibitory activity of anthocyanins on α-amylase so that mathematical modeling could be conducted subsequently, the duration of digestion in our study had been extended to 24 h. An aliquot of dialysate was withdrawn at 0, 5, 10, 15, 20, 30, 40, 50, 60, 90,