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Role of quercetin in the physicochemical properties, antioxidant and antiglycation activities of bread

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ABSTRACT

As a natural glycation inhibitor, quercetin was incorporated into bread to develop antiglycative functional food. Quercetin added in the wheat bread flour at 0.05, 0.1, and 0.2% caused a loss of dough elasticity with lower resistance and higher extensibility. It further altered the quality of bread in terms of deceasing bread volume and increasing bread hardness. The antioxidant potential of the bread with quercetin was enhanced in a dose-dependent manner. The antiglycation capacity was assessed according to the ability of the bread to inhibit the formation of advanced glycation endproducts (AGEs) *in vitro*. Results showed that bread with 0.2% quercetin addition was able to inhibit 46–52% of total AGEs formed during protein glycation. Overall, the results support quercetin as a functional food ingredient in bread system, offering consumers a higher intake of antioxidant and a lower load of AGEs.

1. Introduction

Using natural compounds as glycation inhibitors to reduce the intake of advanced glycation end-products (AGEs) from diet is a promising way to decrease the risk of diabetic complications. Among these compounds, quercetin is one of the most efficient antiglycative agents (Wu, Hsieh, Wang, & Chen, 2009). Quercetin is a flavonol comprising of 15 carbon atoms, with two aromatic rings connected by a three-carbon bridge. The main structure of benzopyran-4-one makes it a hydrophobic compound. The rich food sources of quercetin are onions, berries, and apples. It exhibits multiple biological activities such as antioxidative, cardioprotective, and hypoglycemic effects that are related to the protein glycation process (Erlund, 2004; Wu & Yen, 2005).

Protein glycation starts from the reactions between reducing sugars and amino groups in proteins. Diverse intermediates are produced during this process, including AGEs. The accumulation of AGEs in the body contributes to the development of many chronic diseases, especially diabetic complications (Peng, Ma, Chen, & Wang, 2011). Besides, reactive carbonyl species such as methylglyoxal (MGO) are also generated along the same process. MGO can bind to the amino groups of proteins and further produce AGEs. Such reactions can result in the undesired modification of proteins and cause protein dysfunction.

The formation of AGEs occurs both *in vivo* through normal metabolism and *in vitro* from food sources. Dietary AGEs (dAGEs) produced by Maillard reaction is the major external source of exposure to human bodies. These compounds can enter the *in vivo* circulation of AGEs binding to the cellular receptors and cause abnormal metabolism such as the production of inflammatory cytokines, further contributing to the diabetic complications (Chinchansure, Korwar, Kulkarni, & Joshi, 2015). Meanwhile, reactive oxygen species (ROS) are largely generated during glycation, causing high oxidative stress *in vivo*. Different mechanisms have been reported to inhibit the glycation process (Peng et al., 2011). As for quercetin, it has been shown to inhibit protein glycation by its free radical scavenging activity to combat the oxidative stress and its ability to trap MGO (Li, Zheng, Sang, & Lv, 2014; Zhang, Chen, & Wang, 2014).

Despite the well-known antiglycation activity of quercetin, most of the previous investigations were conducted with plant extract rich in quercetin or with quercetin standard (Kim, Lee, Yokozawa, Sakata, & Lee, 2011; Li et al., 2014; Wu et al., 2009). Only few studies have tried to explore the *in vitro* antiglycation behavior of quercetin in fortified food systems (Szawara-Nowak, Koutsidis, Wiczkowski, & Zieliński, 2014; Zhang et al., 2014). Still, these researches did not focus on just quercetin but also other polyphenols. For example, Szawara-Nowak et al. (2014) reported that buckwheat enhanced wheat bread rich in both rutin and quercetin reduced *in vitro* AGEs generation, and a high correlation between AGEs inhibition and the quercetin content ($r = 0.92$) as well as the rutin content ($r = 0.86$) was found. As there were more than one effective ingredient, it's hard to distinguish the AGEs inhibitory effect of quercetin itself. It is thus necessary to

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elucidate the antiglycation function of quercetin in practical food products in more detail and specifically.

In this research, we aimed to develop quercetin-fortified baked bread as a functional food that helps consumers to lower the risk of diabetic complications. We used bread as a carrier for quercetin fortification because bread is a popular staple food widely consumed around the world. The tasty bread crust formed through Maillard reaction between proteins and sugars during baking process is a key attraction to consumers; however, it is also a rich source of dietary AGEs, posing a potential risk to human health, especially to the diabetic patients. Quercetin may exert its anti-glycation effect on both the AGEs in the body and the dAGEs in bread matrix. On the other hand, the quality of quercetin-fortified bread would largely affect the consumer's acceptance. Therefore, it is also of great interest to examine the effect of quercetin fortification on dough development and the final bread quality attributes.

In this study, the rheological properties of quercetin-fortified baked bread dough and the bread quality were evaluated. The antioxidant capacity of the bread with quercetin was examined. Bovine serum albumin (BSA) and glucose (GLU) *in vitro* model system was employed to investigate the potent therapeutic values of this functional bread. The knowledge gained from this research would provide some useful insights to the potentials of quercetin-containing functional food.

2. Materials and methods

2.1. Chemicals

Quercetin (food grade) was obtained from Xi'an Dowell Bio-tech Co. Ltd., China with 98% of purity. Wheat bread flour (Prima brand, protein content 13.1%), instant dry yeast (*Saccharomyces cerevisiae*, S.I. Lesaffre, France), salt (Fairprice Cooperative Ltd., Singapore), sugar (Fairprice Cooperative Ltd., Singapore), and vegetable shortening (Bake King, Gim Hin Lee Ltd., Singapore) were purchased from a local supermarket. DPPH (2,2-diphenyl-1- picrylhydrazyl), ABTS (2,2′-azinobis (3-ethylbenzothiazo- line-6-sulphonic acid)), potassium persulfate, trolox (6-hydroxy-2,5,7,8-tetramethylchloromane-2-carboxylic acid), gallic acid (3,4,5-Trihydroxybenzoic acid), Folin-Ciocalteu reagent, GLU, BSA, MGO (40% aqueous solution), and aminoguanidine (AG), were purchased from Sigma–Aldrich (Sigma–Aldrich, St Louis, MO, USA).

2.2. Farinograph and extensograph tests

Wheat bread flour fortified with various levels of quercetin (0, 0.05, 0.10, and 0.20%) was 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 AACC Method 54–21 (AACC, 2000). In the extensograph test, freshly prepared quercetin-fortified dough samples were examined using an Extensograph-E (Brabender, Duisburg, Germany) following the AACC Method 54–10 (AACC, 2000).

2.3. Bread sample preparation

Bread making process was modified following the study of Sui, Yap, and Zhou (2015). Quercetin was firstly mixed with 1 kg wheat bread flour at the addition levels of 0, 0.05, 0.10, and 0.20%, which was further mixed with 10 g instant dry yeast, 12 g salt, 30 g shortening, 40 g sugar, and 600 g water for 5 min to form bread dough in a mixer. The dough was rested for 10 min at 20 °C followed by molding into small pieces (50 g each) and proofing in a proofer (Climatic chamber-KBF, Binder, Germany) at 40 °C and 85% relative humidity condition. The baking process was conducted at 200 °C in an oven (Eurofours, France) for 8 min. The bread samples are cooled down to room temperature for 1 h after baking.

2.4. Measurement of bread quality attributes

Specific volume, texture, colour, moisture content, and pH value were measured, as they are important indicators to assess the overall quality of quercetin-fortified bread. Bread volume was measured using a Volscan profiler (VSP 600, Stable Micro System Ltd., Surrey, U.K.). The specific volume was obtained through dividing bread volume (cm^3) by bread weight (g). Texture analysis of the bread was performed using a texture analyser (TAXT2i, Stable Micro System, Surry, U.K.) with a 20 mm diameter cylindrical probe. A bread slice of 2 cm thick cut from the central part of the bread was compressed up to 40% of its original thickness using a double compression cycle at a cross head speed of 2 mm/s. Hardness, springiness, cohesiveness, chewiness and resilience of the bread crumb were quantified. The measurements of three chromatic coordinates L^*, a^*, and b^* of bread crust and crumb individually were performed using a colourimeter (CM-3500d Spectrophotometer, Konica Minolta, Japan). The coordinate L^* represents the lightness of colour ($L^* = 0$ points to black and $L^* = 100$ points to white); a^* indicates the colour between red and green (negative values yield green, and positive values yield red); b^* expresses the colour between yellow and blue (negative values suggest blue, and positive values suggest yellow). The moisture content was evaluated according to the study of Sui et al. (2015). Bread samples (2 g) were heated at 100 °C in on oven until constant weight, and the moisture content was expressed as the percentage change in the weight of the sample. As for the pH value measurement, 5 g of bread sample was agitated in 50 ml of deionized water for 30 min and the supernatant layer obtained was tested by a pH meter (Metrohm 744 pH meter, Switzerland).

2.5. Extraction of quercetin

Bread crumb and crust were separated, frozen for 24 h, and freeze-dried. The dry maters were ground into fine bread powders. Bread powder samples (2 g) were extracted with methanol (10 ml) under 30 min shaking at 300 rpm using an orbital shaker (IKA VXR basic Vibrax, Staufen, Germany). Liquid extracts were obtained after centrifugation at 3233*g* for 5 min and combined together for concentration at 40 °C by a vacuum rotary evaporator after 3 rounds of extraction where the maximum extractability of quercetin was achieved at around 92% and 77% for bread crumb and crust respectively. The same extraction process was conducted on the control bread powder spiked with quercetin to examine the recovery rate of the extraction process, which was found to be above 96%.

2.6. Quantification of quercetin using HPLC/DAD

Quercetin was quantified by a Shimadzu High Performance Liquid Chromatography (HPLC) system equipped with a diode array detector (DAD) (Shimadzu, Kyoto, Japan) using a C18 reserved-phase column (250 × 4.6 mm, Sunfire, Waters, Wexford, Ireland). The flow rate was 1 ml/min and the oven temperature was 30 °C. Mobile phase A (1% acetic acid in DI water) and B (100% acetonitrile) were applied according to the ratio of 6:4. Detection was performed at 257 nm. Quantification of quercetin was accomplished by the external calibration ranging from 0.025 to 0.250 mg/ml.

2.7. Antioxidant capacity analysis: ABTS assay

The ABTS assay was performed according the method of Sui, Dong, and Zhou (2014) with some modifications. ABTS radical cation ($ABTS^+$) was prepared by mixing 7 mM ABTS stock solution with 2.45 mM potassium persulfate and being left in the dark for 12–16 h before use. The $ABTS^+$ solution was diluted with methanol to an absorbance of 0.70 ± 0.05 at 734 nm. Bread extract or trolox (0.1 ml) was mixed with the diluted $ABTS^+$ solution (1 ml) for 7 min. The absorbance at 734 nm was then measured using a UV–vis