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Physical quality and *in vitro* starch digestibility of bread as affected by addition of extracted malva nut gum

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ABSTRACT

Malva nut gum (MNG) was extracted by alkaline solution. The previous studies showed that the extract had viscosity and gelling properties as well as inhibited α-amylase activity in starch solution 1.5–2.0 folds higher than that of original malva nut gum. This research was aimed to investigate the α-amylase inhibitory effect of alkaline-extracted MNG in solid food and to determine physical properties of MNG-containing bread. The scanning electron microscopy of *in vitro* digestibility with α-amylase of MNG-containing breads showed less porosity and more undigested starch granules remained intact with the matrix compared to control. This finding was consistent with the reduction of glucose (33–40%) and maltose (23–39%) levels compared to that of control after α-amylase digestion for 180 min in a dialysis system. The results showed that extracted MNG significantly ($p < 0.05$) increased loaf volume, and moisture content by 1.5–12%, and 8.2–12.8%, respectively compared to that of control. Addition of extracted MNG in bread formulation significantly reduced moisture loss and firmness of the bread crumb after storage for three days.

1. Introduction

Increased postprandial plasma glucose during 2 h after meal of type 2 diabetic subjects (T2D) is associated with the incidence of cardiovascular disease (Ceriello, 2005; Heine & Dekker, 2002). A clinical goal of treating diabetic subjects is to decrease postprandial hyperglycemia and cardiovascular risk factors. Diets play a role in preventing the rapid rise of plasma glucose levels in the post-prandial state. Viscous fibers have been well-known to reduce postprandial blood glucose concentrations in humans and animals (Anderson, Akanji, & Randles, 2001). It was proposed that the fully hydrated chains of soluble fibers diminish the contact between glucose molecules and small intestinal mucosal cells therefore reduce the rate of digestion and absorption of carbohydrate (Blackburn, Redfern, & Jarjis, 1984; Rainbird, Low, & Zebrowska, 1984; Torsdottir, Alpsten, Anderson, & Einansson, 1989).

Addition of hydrocolloid to enhance functional properties of bread has been investigated by increasing number of researchers (Woolnough, Monro, Brennan, & Bird, 2008). These authors reported a lower rate of *in vitro* starch digestibility of bread containing galactomannan compared to wheat bread without galactomannan (Brennan, Blake, Ellis, & Schofield, 1996; Slaughter, Ellis, Jackson, & Butterworth, 2002). Other studies used guar gum, locust bean gum, and xanthan gum reported increasing the weight of baked products, improving dough development (Rosell, Rojas, & Benedito, 2001), increasing gas retention of dough, improving texture of crumb and crust by controlling moisture retention (Huttner & Arendt, 2010). However, these results are not consistent in some studies. Addition of β-glucan in bread formulation contributed to a reduced loaf volume and increased crumb firmness compared to control wheat bread (Gaosong & Vasanthan, 2000; Gill, Vasanthan, Ooraikul, & Rossnagel, 2002; Symons & Brennan, 2004). Addition of hydroxypropylmethylcellulose, a hydrocolloid that forms thermoreversible gel networks in baked products, increased crumb firmness when added at levels higher than 1.5 kg/100 kg starch–flour blend basis (Sabanis & Tzia, 2011).

Malva nut fruit (*Scaphium scaphigerum* (G. Don) Guib. et Planch) is native to the South East Asia and China. In Thailand, people have used the mucilaginous substance of the seeds as traditional medicine for laxative benefits. My laboratory has succeeded in producing dietary fibers extracted from malva nut seeds. The extract contains total dietary fiber 80 g/100 g, protein 2 g/100 g, ash 7 g/100 g (Srichamroen & Chavasit, 2011a). Malva nut gum (MNG) extracted with alkaline solution had carboxylic bonds with the

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reduction of galacturonic acid contents. This is correlated with the increased storage moduli (G') of alkaline-extracted gum with the value being higher than that of water-extracted MNG. The previous study also showed the α-amylase inhibitory effect of alkaline-extracted MNG in starch solution (Srichamroen & Chavasit, 2011b). The alkaline-extracted MNG is new to scientific report. It is interesting to investigate the ability of MNG to inhibit α-amylase activity in solid matrix and to determine the effect of extracted MNG on physical properties of bread.

2. Materials and methods

2.1. Materials

Malva nut seeds harvested during March and April in 2010 were obtained from local markets in the East of Thailand. Malva nut gum was extracted in the laboratory at Naresuan University (Srichamroen & Chavasit, 2011a). Briefly, the seeds were ground (0.5 mm mesh size) and dispersed in distilled water at a ratio of 1:100 (w/v) and placed in a boiling water bath for 1.5 h. The slurry was then cooled to room temperature, and added with NaOH to a final concentration of 0.05, 0.1, or 0.2 mol/L, then treated with absolute ethanol at a ratio of 1:1 (v/v). Precipitate was removed to recover MNG. The gum was adjusted to pH 7.4 and dried in hot air oven at 60 °C. All of extracted MNGs were ground to pass through a 100 μm sieve before use.

All chemicals, pepsin and α-amylase used in this study were of analytical grade and purchased from Sigma Chemical Co. (St. Louis MO, USA). Dialysis membrane (molecular weight cutoff of 12,000) was obtained from Membrane Filtration Products Inc. (Seguin Texas, USA).

2.2. Bread preparation

Wheat bread, called control bread, consisted of 60 g wheat flour, 1.5 g dried yeast, 3.5 g soybean oil, 0.5 g salt, 0.5 g sugar, and 34 g water. Because of high water binding capacity of extracted MNG (Srichamroen & Chavasit, 2011a), water level of MNG-containing bread formulation had to be adjusted in order to avoid underdeveloped gluten network due to increased dietary fiber content. Previous research showed that MNG-containing bread should have an additional 6 g of water when MNG was used 1.7 g/100 g flour. Extracted MNG was boiled to fully hydrate and cooled to room temperature prior to be added into the mixture of bread formulation. Dough was fermented at 40 °C and 90% relative humidity for 2 h in the chamber of incubator (ShelLab model TC2323, Cornelius OR, USA), after which they were kneaded and divided into 210 g portions and put in a bakery stainless steel tin (19 cm length, 8 cm width, 8 cm height). Breads were baked in an air oven (Kluay Nam Thai Trading Group Co., Ltd., Bangkok, Thailand) at 170 °C for 20 min. After baking, breads were cooled at room temperature for 1 h before analyses. For the study of effect of storage on physical properties of bread, bread loaf was kept in a polypropylene bag at 4 °C to prevent mold. After certain time of storage (on days 1, 2 and 3) bread loaf in a food-grade polypropylene bag (Goldenpack Co., Chonburi, Thailand) was transferred to room temperature before further analyses. The reason to choose three days of storage was to imitate the use of bread in a local hospital store for diabetic patients.

2.3. Loaf volume and specific volume

Loaf volume was determined by rapeseed displacement according to AACC approved Method 10-05 (AACC, 2000). Loaf height was determined using calibrated calipers which measured from the center of the loaf. Specific volume was calculated by dividing loaf volume by weight.

2.4. Moisture content and water activity

Moisture content of bread crumb was determined by heating samples in an oven at 105 °C for 12 h. Three bread slices were cut from the central loaf and a crumb was taken from the centre of each slice and torn into small pieces for moisture determination. Water activity of bread crumb was measured at 25 °C using a Novasina AWC200 water activity meter (Axair AG, Pfäffikon, Switzerland).

2.5. Crumb firmness

The crumb firmness measurement was performed with a QTS Brookfield Texture Analyser (Middleboro, MA, USA). Three bread slices were cut from the central loaf. The central part of each slice was cut into cubes (2 × 2 × 2 cm) and subjected to one compression test. The sample was compressed to 40% original height at a compression load of 25 kg with a cross-head speed for 60 mm/min.

2.6. Scanning electron microscopy (SEM)

Bread crumb was cut into fine pieces with a blade in order to create a clean fracture surface to observe in the scanning electron microscope (LEO model 1455 VP, LEO Electron Microscopy Ltd., Cambridge, England). Sample was mounted on an aluminum sample holder (12 mm diameter) with double sided conductive carbon tape and a line of carbon paint was painted around the base of the sample to improve conductivity from the top of the seed to the taped surface. The sample was then sputter coated with gold (Sputter Coater Model SC 7620, Quorum Technologies Ltd., UK) and placed in the SEM chamber for examination and photographed using a 10 kV of electron beam-accelerating voltage.

2.7. In vitro digestibility of starch of the breads

To simulate the hydrolysis reaction in human stomach, pepsin (5.75 units/g starch, Sigma Chemical Co., St. Louis MO, USA) was added into 1 mol/L sodium phosphate buffer solution (pH 6.9) which contained fine pieces of bread equivalent to 40 g of wheat flour (Symons & Brennan, 2004). The mixture was adjusted to pH 1.5 (with HCl) at 37 °C for 30 min. The pH of mixture was readjusted to pH 6.9 (with NaOH) prior to addition of porcine pancreatic α-amylase (110 units/g starch, Sigma Chemical Co., St. Louis MO, USA). The mixture was transferred to a dialysis bag against 250 mL of deionized water in erlenmeyer flask, and stirred at 37 °C in a water bath shaker. Dialysate (2 mL of deionized water in erlenmeyer flask) was collected after 15, 30, 60, 120, and 180 min in order to determine glucose and maltose contents.

2.8. Sugar determination

Glucose and maltose contents in the dialysate were measured by using HPLC system (Shimadzu, Kyoto, Japan). The dialysate (2 mL) was filtered through a 0.45 μm filter and a portion (10 μL) of filtrate was injected into the HPLC column (Inertsil® NH_2 5 μm, dimension 4.6 × 250 mm). The mobile phase was CH_3CN/H_2O:75/25 at flow rate 1.0 mL/min, and 40 °C. The HPLC was performed using a refractive index detector with detection limit of 0.01 mmol/L.

2.9. Statistical analysis

All experiments were performed in three replications. Results were presented as the mean ± SE. Statistical analysis was