Development of Gluten-Free Bread Baked with Banana (Musa spp.) Flour

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The purpose of this study was to create gluten-free bread for patients with celiac disease. In this experiment we looked for a viscous material as an alternative to gluten protein, and banana (Musa spp.) flour was selected. Gluten-free bread was baked with banana flour, starch, sugar (sucrose), compressed yeast, and water. Green (un-ripe, 0 day) and yellow (ripe, 5 days) banana flours did not exhibit good breadmaking properties (bread height (mm) and specific volume (cm³/g)). However, black (over-ripe, 44 days) banana flour showed good breadmaking properties. The suspension of black banana flour/water was dialyzed against a large amount of water, and was separated to nondialyzable (high-molecular-weight (HMW)) and dialyzable (low-molecular-weight (LMW)) fractions. The HMW or LMW fraction alone did not show good breadmaking properties, but a mixture of them did. When black banana flour or the HMW/water was heated at 127°C for 100 min in an autoclave, the enhancement effect of black banana flour or HMW/water with LMW fraction on breadmaking was lost, suggesting that the enzymes in black banana flour act as key materials. High amylase and protease activities in black banana flour could be ascertained by RVA (rapid visco analyzer) and mixograph tests, respectively.

Keywords: gluten-free bread, banana, Celiac disease (CD), enzymes, HMW fraction, LMW fraction

Introduction

Celiac disease (CD) is an intestinal intolerance to gluten or gluten-like proteins in wheat, rye, barley, and oats that causes mucosal lesions and nutrient malabsorption in genetically predisposed individuals (Wieser et al., 2012; Catassi and Yachha, 2009). In countries populated by individuals of European origin, CD affects approximately 1% of the general population. In many developing countries, the frequency of CD is likely to increase in the near future, given the widespread tendency to adopt a Western, gluten-rich diet. Toxicity of gluten traces (10 – 50 mg daily) in the celiac diet was reported (Catassi and Fasano, 2008). With the availability of improved and more accessible diagnostic tools, CD is now more frequently recognized in many countries, in both children and adults.

To produce a new gluten-free bread, banana flour was selected in this study. Bananas are easily obtained and popular throughout the world. Black (over-ripe) banana flour was selected for use in this gluten-free bread. While the green (un-ripe) banana contains 20% starch and the ratio of starch/sugar is 20/1, the ratio becomes 1/20 with ripeness (Noro et al., 2011), i.e., the starch granules almost completely disappear and sweetness increases with ripeness. When the banana is over-ripe the polysaccharides in cell walls degrade and the texture of the banana becomes softer. It was reported that pectin in the banana cell walls dissolves at the same time as starch degradation to sugar, and the cell walls contain lower levels of polysaccharide than other fruits (Kojima et al., 1994). Mbeguie-A-Mbeguie et al. (2009) monitored the expression of cell-wall modifying genes in peel tissues during post-harvest ripening of Cavendish banana fruit, and three pectin methylesterase (PME) and seven xyloglucan endotransglycosylase/hydrolase (XTH) genes were isolated. The accumulation of their RNAs and those of polygalacturonase, expansin, and pectate lyase genes...
already isolated from bananas was examined. During post-harvest ripening, transcripts of all genes were detected.

In this work we used bananas that were black in color and had a heavy viscous texture. The resulting black banana flour had a high viscosity, similar to gluten proteins, and was used in gluten-free breadmaking. The breadmaking properties such as bread height (mm) and specific volume (cm³/g) were comparable with those of wheat bread.

Materials and Methods

Storage of banana fruits and preparation of banana flour

Green (un-ripe) banana fruits (6.4 kg) were purchased from commercial sources and were stored at room temperature. Yellow (ripe) and black (over-ripe) banana fruits were obtained after 5 and 44 days, respectively. After peeling, bananas were crushed in a Waring blender (Hamilton Beach/Proctor-Silex, Inc., Southern Pines, NC, USA) and freeze-dried at −15°C for 70 hrs in a Kyowa freeze-dryer RLE-120 (Kyowa Vacuum Engineering Co., Ltd., Tokyo, Japan). The moisture, ash, and protein contents were measured (Table 1). Each dried sample was crushed into flour in a mortar and pestle, and stored in a freezer until use. Nagata Sangyo Co., Ltd. (Hyogo, Japan) donated a wheat starch sample. General analysis (protein, ash, and moisture contents) of the wheat starch were 0.15, 0.13, and 10.7%, respectively. We used a wheat starch sample for comparison because wheat starch granules are known to contain minor components such as non-starch polysaccharides, proteins such as friabilin, and lipids (Galliard et al., 1989). The lipids and proteins at the surface of starch granules have been implicated in the behavior of starch with respect to pasting properties, wetting and dispersion, and the stability of starch suspensions (Galliard et al., 1989). Moisture content was determined by the method of Tsutsui and Nagahara (1961). Protein conversion was carried out using the formula N x 5.7 (Approved Methods 46 – 10, AACC International 2000). Ash was determined by the AACC International Method (08 – 01, 2000) at 14.0% moisture content.

Microscopic observation of banana starch granules

Fresh banana fruit contains 20% starch granules (diameter: 3 – 88 μm) (Noro et al., 2011). Banana starch granules were observed under an Olympus BX50 microscope (Tokyo, Japan).

Bread baking

Higher bread height (mm) and larger specific volume (cm³/g) are important characteristics for wheat bread (Eliasson and Larsson, 1993), and our goal was to obtain gluten-free bread having these characteristics without wheat flour. In this experiment, bread was baked with flour from bananas stored for three different periods: green (un-ripe), yellow (ripe) and black (over-ripe) banana flour. For baking bread with banana flour, a method was developed to achieve bread similar to that made with wheat flour. To bake one loaf: banana flour (30 g), sugar (8.86 g), and compressed yeast (10 g); 10 mL of water were mixed in a 3.6-L bowl using a Kitchen Aid mixer (Kenmix Chef Aicoh Mixers and Aicoh Systems Co., Ltd., Japan) for 9 min at 116 rpm. Wheat starch (30 g) and water (40 mL) were added, and the mixture was further homogenized for 9 min at the same speed. The entire homogenized dough, composed of banana flour 30 g (green banana flour, 29.01 g; yellow banana flour, 27.54 g; and black banana flour, 26.04 g in dry matter at 0% moisture content, respectively), wheat starch 30 g, sugar 8.86 g, compressed yeast 10 g, and water 50 mL (actual total water when green, yellow, and black banana flours were used, 51.0 mL, 52.5 mL, and 54.0 mL, respectively), was placed into a pan (5.5 x 9.5 x 6.6 cm³), proofed in an oven at 40°C for 20 min, and baked at 210°C for 10 min in an oven (Sanyo Drying Oven MOV-212, Sanyo Co., Ltd., Japan). After baking, the bread was removed from the pan and cooled for 1 hr at room temperature (26°C) and a relative humidity of 43%. Bread height (mm), weight (g), and volume (cm³) were measured, and crumb grains were evaluated visually. The color (L, a, and b values) of the bread crumb was evaluated using an L, a, and b color specification system chromaticity diagram and a Hunter Lab Color Meter NE 2000 (Nippon Denshoku Co., Ltd., Tokyo, Japan). Positive values for L, a, and b indicate white, red, and yellow, respectively.

Dialysis of black banana flour/water suspension

Black banana flour (30 g) was dialyzed against a large amount of water overnight and separated into nondialyzable (HMW) (8.22 g) and dialyzable (LMW) fractions (21.2 g). A dialysis tube (regenerated cellulose, 21.4 mm diameter; MWCO (molecular weight cut off), 12,000 – 14,000 Daltons; and pore size, 24 Å) (Nihon Medical Science Co., Ltd., Takasaki, Japan) was used to dialyze 30 g of black banana flour suspended in 200 mL of water against 10 L of water overnight in a cold room (4°C). The non-dialyzable fraction

<p>| Table 1. General analysis (moisture, ash, and protein contents (%)) of green (0 days), yellow (5 days), and black (44 days) banana flours. |</p>
<table>
<thead>
<tr>
<th>Banana flour, days</th>
<th>Moisture content (%)</th>
<th>Ash content (%)</th>
<th>Protein content (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Green banana flour, 0 days</td>
<td>3.3a (0.50)</td>
<td>3.5a (0.21)</td>
<td>5.0a (0.34)</td>
</tr>
<tr>
<td>Yellow banana flour, 5 days</td>
<td>8.2b (0.00)</td>
<td>3.6a (0.21)</td>
<td>4.9a (0.46)</td>
</tr>
<tr>
<td>Black banana flour, 44 days</td>
<td>13.2c (1.20)</td>
<td>4.4b (0.00)</td>
<td>7.0b (0.90)</td>
</tr>
</tbody>
</table>

Values represent means of two measurements with SD in parentheses. Means followed by different letters in columns are significantly different (P < 0.05) according to Duncan’s multiple range test.
(in the dialysis tube) composed of banana starch, polysaccharides and proteins was freeze-dried at −15°C for 70 hrs and crushed; this was termed the HMW fraction. The dialyzable fraction, composed mainly of peptides and sugars, was concentrated to a syrup at 65°C using a rotary evaporator (RE-51, Yamato Scientific Co., Ltd., Tokyo, Japan), and was termed the LMW fraction.

**Autoclave treatment of black banana flour or HMW fraction/water suspension** Black banana flour (30 g) or HMW fraction /40 mL water was heated at 127°C for 100 min in an autoclave (Shimadzu, Kyoto, Japan) and cooled until use. Weights (g) of samples were adjusted before and after autoclave treatment by the addition of water.

**RVA (Rapid visco analyzer) tests of black banana flour** Wheat starch (2.0 g) and banana flour (2.0 g) were mixed in 25 mL of water, and RVA testing of the mixture was performed. An RVA-4 (Newport Scientific Pty. Ltd., Foss Japan, Tokyo, Japan) was employed to measure the pasting properties of starch samples. Their weights (g) as dry matter (0%) and total water (mL) were as follows: green banana flour, 1.93 g and 25.1 mL; yellow banana flour, 1.84 g and 25.2 mL; black banana flour, 1.74 g and 25.3 mL. Experiments were performed using the STD 1 profile (Approved Method 76 – 21, AACC International 2000), in which the sample (4.0 g per 25.0 mL of water) was equilibrated at 50°C for 1 min, heated at 12.2°C/min to 95°C, held at 95°C for 3.5 min, cooled at 11.8°C/min to 50°C, and held at 50°C for 2 min. The speed was 960 rpm for the first 10 sec, and 160 rpm for the remainder of the experiment. Peak viscosity, final viscosity, and pasting temperature of these starch samples were compared with the pasting curve. The reported values were the means of three measurements.

**Mixograph tests of black banana flour** The mixograph test of black banana flour was performed in a 10-g mixograph (National MFG Company, NE, U.S.A.) according to the method of Nakamura et al. (2008). Black banana flour was examined for protease activity. Black banana flour (1.0 g) was mixed with wheat flour (9.0 g) in 5.5 mL of water, and preliminarily incubated at 40°C for 20 min. If protease was present in black banana flour and digested the gluten proteins in wheat flour, it would be possible to see a change in viscosity of the mixograph profiles due to gluten protein would be lower than that before incubation. As a control, 10 g of wheat flour was mixed with water and incubated (not shown). The mixing speed of the mixograph was set to 86 rpm.

**Results and Discussion**

**Storage of bananas** The moisture, ash, and protein contents of banana flours are shown in Table 1. The moisture content in banana flour increased from 3.3 to 8.2 and 13.2% during ripening, causing a texture change from potato-like with green banana to jam-like with black banana. The protein content increased from 4.9 to 7.0% during ripening, which suggested increased enzyme formation.

**Breadmaking with banana flour** The baking results were as follows: for green banana flour, 0 day (un-ripe) bread height and specific volume, 44.0 mm and 1.54 cm³/g, respectively; yellow banana flour, 5 days (ripe), 35.0 mm and 1.60 cm³/g; and black banana flour, 44 days (over-ripe), 67.1 mm and 2.72 cm³/g (Table 2 and Fig. 2). The difference in water content was negligible since the bread height and specific volume increased when black banana flour was used even though the weight of dry matter was the lowest. The L values of breads baked with banana flours decreased from 55.0 to 49.2, which indicated darkening of the bread with banana storage time. Black banana flour, at 44 days, was the most suitable for breadmaking, yielding almost the same breadmaking properties as Haruyutaka wheat flour (bread height, 69.4 mm and specific volume, 3.45 cm³/g, Table 2). Next, the leavening mechanism by black banana flour was assessed with respect to breadmaking. To determine the effect of the black banana flour fraction on breadmaking properties, black banana flour/water was separated into HMW (high molecular weight) and LMW (low molecular weight) fractions by dialysis, and the breadmaking properties of these fractions were compared with those of whole black banana flour.

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**Fig. 1a.** Image of banana starch granules in green banana flour

**Fig. 1b.** Image of gelatinized starch in black banana flour
Fractionation of black banana flour and breadmaking tests
with these banana flour fractions  Black banana flour (30 g) was
dialyzed against a large amount of water overnight and separated
into nondialyzable (HMW) (8.22 g) and dialyzable (LMW)
fractions (21.2 g). Each of these fractions was used in breadmaking
(Table 3 and Fig. 4). The breadmaking properties, bread height
(mm) and specific volume (cm$^3$/g), of the mixture of LMW and
HMW fractions were 69.6 mm and 2.52 cm$^3$/g, respectively, which
was similar to bread made with black banana flour (67.1 mm and
2.72 cm$^3$/g, respectively). The individual LMW or HMW fractions
did not exhibit good breadmaking properties (31.8 mm and
1.13 cm$^3$/g; 48.6 mm and 2.22 cm$^3$/g, respectively). When black
banana flour/water was heated at 127°C for 100 min, the good
breadmaking properties were lost (38.6 mm and 1.62 cm$^3$/g) (Table
3 and Fig. 4). The $L$ value of black banana flour decreased from
49.2 to 28.5, indicating a decrease of whiteness due to the Maillard
reaction by heat. When the HMW fraction/water was heated under
the same conditions and mixed with the LMW fraction, poorer
breadmaking results were obtained (37.2 mm and 1.52 cm$^3$/g,
respectively) (Table 3, and Figs. 3 and 4). These results suggested
that black banana flour contains the enzymes necessary for
breadmaking, but those enzymes are denatured by autoclave
treatment.

RVA (Rapid visco analyzer) tests of banana flour  When green
banana flour was used (Fig. 5-1, 660 RVU), almost no change of
RVA was observed compared to 4.0 g wheat starch in 25 mL water
(Fig. 5-4, 630 RVU), which suggested that the amylase activity
was low in green banana flour. When yellow and black banana
flours were mixed with wheat starch (2.0 g), the
maximum viscosity of green banana flour (660 RVU) decreased to 204 RVU
(yellow banana flour) (Fig. 5-2) and 144 RVU (black banana flour)
(Fig. 5-3), respectively. However, the black banana flour mixed
with 2.0 g of wheat starch was higher (144 RVU) than 2.0 g of
wheat starch alone (60 RVU) (Fig. 5-5). This indicated that in
black banana flour the starch granules were not completely
digested. In this experiment the weights of dry matter and water
were not equal for all the samples. A smaller dry matter content
does not mean that the amount of enzymes decreased, it merely
indicates an increase in water content. The decrease in RVU cannot
be described by the increase in water content alone, and suggests
an increase in starch degradation enzymes.

Mixograph profiles of black banana flour  Black banana flour
(1.0 g), wheat flour (9.0 g) and 5.5 mL of water were mixed and
incubated at 40°C for 20 min and subjected to mixograph testing.
Table 3. Effects of banana flour fractions (autoclaved black banana flour, LMW (low molecular weight) fraction, HMW (high molecular weight) fraction, HMW + LMW fractions, and LMW + autoclaved HMW fractions, respectively) on breadmaking properties.

<table>
<thead>
<tr>
<th>Fraction</th>
<th>Bread height (mm)</th>
<th>Specific volume (cm$^3$/g)</th>
<th>Bread crumb L, a, b</th>
</tr>
</thead>
<tbody>
<tr>
<td>Black banana flour</td>
<td>67.1a(1.2)</td>
<td>2.72a(0.04)</td>
<td>49.2, 12.6, 19.4</td>
</tr>
<tr>
<td>Autoclaved black banana flour (127°C, 100 min)</td>
<td>38.6b(0.8)</td>
<td>1.62b(0.05)</td>
<td>28.5, 8.04, 8.20</td>
</tr>
<tr>
<td>LMW fr.</td>
<td>31.8c(1.1)</td>
<td>1.13c(0.64)</td>
<td>39.5, 1.08, 11.2</td>
</tr>
<tr>
<td>HMW fr.</td>
<td>48.6d(4.9)</td>
<td>2.22d(0.08)</td>
<td>46.3, 5.40, 15.2</td>
</tr>
<tr>
<td>LMW fr. + HMW fr.</td>
<td>69.6a(2.9)</td>
<td>2.52e(0.20)</td>
<td>35.5, 11.6, 12.9</td>
</tr>
<tr>
<td>LMW fr. + autoclaved HMW fr.</td>
<td>37.2b(1.4)</td>
<td>1.52b(0.04)</td>
<td>33.49, 8.36, 8.13</td>
</tr>
</tbody>
</table>

Values represent means of two measurements with SD in parentheses. Means followed by different letters in columns are significantly different (P < 0.05) according to Duncan’s multiple range test.

Fig. 3. Image of sectioned banana bread baked with black banana flour, and autoclaved black banana flour. Left: black banana flour, right: autoclaved (127°C, 100 min) black banana flour

Fig. 4. Image of sectioned breads baked with various banana fractions. Left to right: black banana flour, HMW fraction, LMW fraction, HMW + LMW fractions, and autoclaved HMW fraction + LMW fraction.
The amplitudes of the mixograph profiles were smaller with incubation (Fig. 6-b) than without incubation (Fig. 6-a), which suggested that the enzyme actions in black banana flour would decrease the viscosity of wheat flour proteins. The level of the amplitudes of control was almost the same as in Fig. 6-a.

Conclusions

Good gluten-free bread could be baked with black banana flour. Trace levels of gluten (several mg) may be derived from wheat starch granules present in a single loaf, but will be below the toxic level of gluten (10 – 50 mg daily). However, in the future, it will be necessary to search for other starch sources. Black (over-ripe) banana flour was fractionated into HMW and LMW fractions using dialysis, and bread was baked with the HMW or LMW fractions. Although the individual HMW or LMW fractions showed poor breadmaking properties, the combined HMW and LMW fractions provided favorable bread height (mm) and specific volume (cm$^3$/g). The enzymes in the HMW fraction act as key materials in this gluten-free breadmaking.

References


