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Low-Calorie Bread Baked with Charred Cellulose Granules and Wheat Flour to Eliminate Toxic Xanthene Food Dye in the Alimentary Canal

Aya TABARA, Chihiro YAMANE, and Masaharu SEGUCHI[†]

Faculty of Home Economics, Laboratory of Food Technology, Kobe Women's University,
2-1 Higashi Suma Aoyama, Suma-ku, Kobe 654-8585, Japan

We baked low-calorie bread by mixing charred cellulose granules with wheat flour, using the charred cellulose granules to eliminate toxic xanthene food dyes contained in processed foods from the alimentary canal. The size of the charred cellulose granules played an important role in determining good breadmaking properties in respect of the bread height (mm) and specific volume (SV, cm^3/g). Charred cellulose granules with a diameter above 270 μm were blended with wheat flour at 10% to obtain bread with a lower caloric content (1020 kcal/gram of bread) than the control bread (1126 kcal) made solely from wheat flour. The charred cellulose granules taken out from the bread adsorbed toxic xanthene food dyes at around pH 6.5, such that toxic food dyes taken into the alimentary canal were excreted in the feces with the non-digestible cellulose granules.

Key words: charred cellulose granule; low calorie; xanthene food dye; breadmaking

The insoluble fibers of cellulose are pure polymers of glucose molecules linked through non-digestible β-1,4-bonds and are non-caloric. Cellulose has beneficial effects on bowel transit, thereby reducing the risk of colonic cancer.[1] Ang and Crosby[2] have studied the application of powdered cellulose to minimize the caloric intake, and cellulose blended with wheat flour has been used to make low-calorie bread.[3] However, such breadmaking properties as bread height (mm) and specific volume (SV, cm^3/g) deteriorate with increasing percentage of cellulose in bread. Seguchi *et al.*[3] have reported the diameter of cellulose granules in bread to be an important factor in determining breadmaking properties, and that good properties could be maintained by using cellulose granules with a diameter above 154 μm. They applied a mixiograph test of cellulose granule-blended wheat flour dough to elucidate the effect on breadmaking properties of larger cellulose granules.[3] Cellulose granules with a diameter above 154 μm blended with wheat flour resulted in almost the same profile for the mixogram as that of wheat flour; *i.e.*, the profile had a short mixing requirement and showed a viscous gluten matrix,[3] these being necessary for good breadmaking properties. However, cellulose granules with a diameter below 81 μm blended with wheat flour produced a different curve showing non-continuous and non-viscous dough due to breakdown of the gluten

protein, as confirmed by microscopy of the cellulose granule/wheat flour dough stained with protein dye (coomassie brilliant blue).[3]

Such xanthene food dyes as erythrosine, phloxine, and rose bengal are used in processed foods because of their low cost, good spread of color, and aesthetic function. Erythrosine is listed in the US as FD & C Red no. 3 (erythrosine), in the EU as E127, and in many other countries. However, phloxine and rose bengal are not used as food additive dyes in many countries. They are permitted for use in Japan as a food additive dye (Japanese Specifications and Standards for Food Additives, 1999).[4] The acceptable daily intake (ADI) level of erythrosine evaluated by the Joint FAO/WHO Expert Committee on Food Additives (JECFA) is 0–0.1 mg/kg of body weight.

These dyes have been clarified for their toxicity by many studies. Levitan[5] has reported that erythrosine (0.4 mM) increased the resting membrane potential and conductance of neurons, and was highly correlated with their lipid solubility, toxicity and brain uptake in vertebrates. Augustine and Levitan[6–8] have hypothesized erythrosine as a neurotoxin, because a 10 mM or greater level of erythrosine stimulated transmitter release without depending on the presence of calcium ions in the frog test. Dees *et al.*[9] have reported that erythrosine had estrogen-like growth-stimulatory properties and may be genotoxic, representing a significant risk factor in human breast carcinogenesis. Erythrosine can induce DNA damage in the gastrointestinal organs, even at a low dose (10 or 100 mg/kg).[10] Uysal *et al.*[11] have suggested that erythrosine may cause atopic diseases from the results of animal tests, in which rats were treated with 2 mg/mL of erythrosine for 7 to 11 d. Mpountoukas *et al.*[12] have reported that erythrosine at 1, 2, 4 and 8 mM in human peripheral blood cells showed high cytotoxicity and cytostaticity, and indicated potential toxicity to human lymphocytes *in vitro* and seemed to bind directly to DNA. Ganesan *et al.*[13] have found that erythrosine showed inhibitory activity with a large variety of protein-protein interactions (PPIs) within the tumor necrosis factor superfamily, TNF-R-TNFα and CD40–CD154, in the 2–20 μM range (approximately 2–20 mg/L). Bamforth *et al.*[14] have reported that sulphation of the xenobiotic steroid, 17α-ethinyloestadiol (EE$_2$), in human liver was inhibited by 6.7 μM of erythrosine. Uesugi *et al.*[15] have reported that halogenated xanthene food dyes strongly inhibited human

uridine 5-diphosphate (UDP)-glucuronosyltransferase 1A6 (UGT1A6) activity with IC50 values of 0.04–0.015 mM, while Furumiya and Mizutani[16] and Mizutani[17] have reported that human cytochrome P450 3A4 (CYP3A4) and P-glycoprotein were inhibited by basic xanthene food dyes with IC50 values of 5.6–21.2 μM. It has been reported[15–17] that approximately 2 mg of total pigment/day was ingested and the concentration in the body was estimated to be 2 nM. This is lower than the poisonous level toward the human body presented in many reports. However, there are many warnings to exclude these dyes from processed foods, while it is also necessary to exclude as far as possible taking dyes into the body.

Tabara *et al.* have observed that cellulose granules charred at 250 °C for 20–120 min could adsorb toxic xanthene food dyes,[18] the mechanism for the adsorption of xanthene food dyes to charred cellulose granules by ionic and hydrophobic interactions having been elucidated.[19]

We report in this study a novel low-calorie bread baked with charred cellulose granules and wheat flour having the function of adsorbing toxin xanthene food dyes taken into alimentary canal from processed foods and excreting these dyes *via* the charred cellulose granules. It was necessary to learn whether (i) charred cellulose granules could be used in low-calorie bread instead of uncharred cellulose granules, (ii) charred cellulose granules in the bread could adsorb the xanthene food dye, and (iii) charred cellulose granules could adsorb xanthene food dye in the alimentary canal. These aspects were therefore examined, and low-calorie and xanthene food dye-eliminating bread was baked.

Materials and Methods

Chemical reagents. Erythrosine ($C_{20}H_6I_4Na_2O_5 = 879.86$) and phloxine ($C_{20}H_2Br_4Cl_4Na_2O_5 = 829.63$) were purchased from Merck, and rose bengal ($C_{20}H_2Cl_4I_4Na_2O_5 = 1{,}017.64$) was purchased from Aldrich.

Wheat flour and cellulose granule samples. Kameria bread wheat flour (Nisshin Flour Milling Co., Tokyo, Japan) was used in this experiment. D50 dry particle size of the flour was 65–68 μm, this being determined by a laser diffraction analysis with an MT-3000 II instrument (Nikkiso Co., Tokyo, Japan). The protein content was obtained with conversion factors of N × 5.7 for Kameria wheat flour and 6.25 for the cellulose granules. The ash content was determined by the AACC international method[20] on a 14.0% moisture basis. The respective protein, ash, and moisture contents of Kameria wheat flour were 14.9, 0.37, and 12.6%, while the respective protein and ash contents of the cellulose granules were 0.12 and 0.03%. The respective water absorption capacity (mL/g) and bulk density (g/mL) of the cellulose granules were 1.00 and 0.93. The water absorption capacity (mL/g) of the cellulose granules was independently determined. The cellulose granules (10 g) were taken into water (30 mL) in a glass beaker and left for 1 h at room temperature. The granules were then removed from the water by passing though filter paper, and any water remaining on the cellulose granules was wiped off with dry filter paper. The wiped cellulose granules were weighed (W), and the water absorption capacity was calculated as W ÷ (the initial 10 g weight of the cellulose granules). The bulk density (g/mL) of the cellulose granules was then determined. The cellulose granules (30 g) were taken into a graduated 100-mL-cylinder, and the cylinder was tapped about 30 times. The volume (V) was read and calculated as 30 ÷ V (g/mL).[21] Five cellulose granule samples were then prepared: no. 1 (6 μm average granule diameter), no. 2 (154 μm), no. 3 (270 μm), no. 4 (464 μm), and no. 5 (650 μm). Microcrystalline cellulose (1.5 kg) produced by hydrolyzing leaf bleached kraft pulp (LBKP from Rayonier) with 2.5 N HCl at 90 °C[22] was kneaded with 1.5 L of water for 5 min in a granulator with a high-speed disc rotor (Fukae Powtec Co., Kobe, Japan), and the resulting wet granules (1.0 kg) were rotary-shaken (500 rpm) for 10 min in Marumerizer-Q-230 apparatus (Fuji Paudal Co., Osaka, Japan) to form balls, while constantly adding water (200 mL) to the balls at a rate of 20 mL/min. The sample was left at 40 °C overnight to dry and separated by iron-sieving to various diameters according to Kamata.[21] The granule size of cellulose was determined by BX 50–34-DIC microscopy (Olympus, Tokyo, Japan). The number of μm per division in the eyepiece was determined by using an objective micrometer.

Charring of the cellulose granules. The cellulose granules were charred to increase their adsorption of the xanthene food dye. The cellulose granules (15 g) were placed in a crucible (15 cm diameter and 6.5 cm depth), charred in an FA-21 electric furnace (Yamato Scientific Co., Tokyo, Japan) at 250 °C for 20 min, and then taken out and cooled at room temperature. The color of the charred cellulose granules determined the color of the bread, so the change in color (from yellow to brown) of the cellulose granules was measured by using the chromaticity diagram for the *L, a, b* color specification system with a Hunter *Lab* colorimeter of the NE 2000 color meter (Nippon Denshoku Co., Tokyo, Japan). The *L, a,* and *b* values respectively indicate white-black, red-green, and yellow-blue. The degree of polymerization and crystallinity of the charred cellulose granules were 199 and 55.2%, respectively, as determined by the method of Yamane *et al.*[23] It was known that more than 50% crystallinity of the charred cellulose granules was necessary to maintain the granular form from a physiological action such as peristaltic motion of a body.[21]

Brabender faringograph and breadmaking tests. It was necessary to know the water absorption (%) of flour to add the correct amount of water for breadmaking. Each charred cellulose granule sample was blended with wheat flour at 10% and 20%. Adding the charred cellulose granules to wheat flour changed the water absorption of the blended flour, so the water absorption of the blended flour was determined by a faringograph with 300 g of flour.[20] The water absorption of flour is defined as the amount of water necessary to bring the dough to a specified consistency (normally 500 Brabender units, BU) at the point of optimum development and best results in baking.[24] Bread was made by the method of Seguchi *et al.*[25] Kameria wheat flour (290 g), compressed yeast (8.7 g; Kaneka Co., Osaka, Japan), sugar (14.5 g), salt (NaCl, 2.9 g), and water (estimated from the faringograph at 500 BU) were mixed in a National SD-BT6 computer-controlled automatic bread mixer (Matsushita Electric Ind. Co., Osaka, Japan) with the 1st proof performed for 2 h 20 min at 30 °C. The timing cycle was 15 min for the 1st mixing, 50 min of resting, 5 min for the 2nd mixing, and 70 min of fermentation. The precise mixing action (time and temperature) was computer-controlled by the bread maker. The bread dough was then removed from the bread maker and divided into 120-g pieces, rounded, molded, and placed in baking pans.[20] The bread dough was further proofed for 22 min at 38 °C (2nd fermentation), and the heights of the bread dough before and after proofing were measured. Baking was performed at 210 °C for 30 min in a DN-63 deck-style oven (Yamato Scientific Co., Tokyo, Japan), and the bread was removed from the pan and cooled for 1 h at a room temperature of 26 °C and relative humidity of 43%. The bread height (mm), weight (g), and volume (cm^3) were measured, and crumb grains were visually evaluated. The bread volume was measured by the rapeseed displacement method.[26] The color (*L, a,* and *b* values) of the bread crust was measured as already described. Bread springiness was measured by avoiding variation of the bread cells, the whole loaf (10.4 × 6.3 × 6.5 cm^3) being pressed for 3 min by a plunger of 11.72 kg after measuring the bread volume (V1), which was equivalent to applying a pressure of 0.18 kg/cm^2 on the bread surface. After releasing the pressure, the bread volume was measured again (V2), and the bread springiness was calculated as V2/V1 × 100%. The loaf was left for one day in a plastic bag at room temperature and the bread springiness was again measured.

Mixograph test and microscopic observation of the charred cellulose granules and wheat flour dough. A mixiograph test and microscopic observation were preformed according to Seguchi *et al.*[3]

to evaluate the dough made from charred cellulose granules and wheat flour. The granules and flour (10 g) were mixed with an amount of water determined by the farinograph, and the resulting dough was subjected to a mixograph test (National Mfg. Co., Lincoln, NE, USA) for 30 min. The dough was also observed under a microscope after the 30-min-mixograph test. Protein was stained with a 0.25% Coomassie brilliant blue solution.[27]

Sensory test of bread baked from charred cellulose granules and wheat flour. The sensory test was performed by the method of Hayashi and Seguchi.[28] Bread baked from charred cellulose granules and wheat flour was cut into pieces of $2.5 \times 2.5 \times 1.0 \, cm^3$ and then subjected to a sensory test by panelists (n = 10). The panelists were asked to rate the color, odor, hardness, wetness, mouth-feel roughness and overall evaluation of the baked bread using the following points system: better (+2, +1), normal (0), and worse (−1, −2).

Treatment of the charred cellulose granules in the bread crumb and crust. It was necessary to collect the charred cellulose granules from the bread to examine the adsorption of xanthene food dyes to the granules taken from the bread. Dialysis tubes were used in this experiment to easily separate the charred cellulose granules from the bread. One gram of charred cellulose granules was placed in a dialysis tube (6.4 mm diameter and 25–45 mm length) with a pore size of 24 Å (Nihon Medical Science Co., Gunma, Japan). The tubes containing charred cellulose granules were buried at the center of the dough and under the surface of the dough. The tubes were removed from the bread crumb and crust after baking, and the charred cellulose granules were taken out from each tube.

Adsorption of xanthene food dyes (i.e., erythrosine, phloxine, and rose bengal) to the charred cellulose granules taken out from the bread crumb and crust. An adsorption test was performed by column chromatography to ascertain the adsorption of xanthene food dyes to the charred cellulose granules taken out from the bread crumb and crust. Column chromatography was performed according to the method of Tabara et al.[19] The charred cellulose granules (1 g) were packed into a glass column (11 cm height and 0.4 cm diameter). Erythrosine (0.40 mg/mL), phloxine (0.40 mg/mL), and rose bengal (0.40 mg/mL) were respectively charged into the column at a flow rate of 86–100 μL/min. Each dye was eluted with water (7 mL) and 0.1 N NaOH (4 mL). Each fraction (1 mL) obtained was diluted with 2 mL of water, and the color density (OD 510 nm) was measured with a Shimadzu UV-1200 instrument (Kyoto, Japan).

pH dependence and effect of a pH 6.0 sucrose (10%) solution containing 10 mM Tris–HCl on the adsorption of erythrosine to the charred cellulose granules. It was necessary to learn whether adsorption of the xanthene food dye occurred under the pH conditions of the alimentary canal. We therefore examined the pH dependence for the adsorption of erythrosine to the charred cellulose granules. The adsorption of erythrosine for 10 mM Tris–HCl at pH 6.00, 6.60, 6.70, 6.80, 6.90, and 7.00 was studied, a fecal pH value of 6.5 being general in healthy people.[29–37] The adsorption test was performed by column chromatography according to the method of Tabara et al.[18]

To examine the effect of concentrated food materials on the adsorption of erythrosine to charred cellulose, a sucrose (10%) solution containing 10 mM Tris–HCl at pH 6.0 was tested for the adsorption of erythrosine to charred cellulose granules as one model for food components.

Scanning electron microscopy-electron probe micro-analysis (SEM-EPMA) of charred cellulose granules. Charred cellulose granules (1 g) with adsorbed erythrosine were subjected to SEM-EPMA under an acceleration voltage of 15 kV and pressure of 1–10 Pa, respectively. The X-ray analysis of EPMA was under an acceleration voltage of 15 kV and measurement time of 100 s.

Calorie calculation. The colorie content of bread containing charred cellulose granules was determined. Low-calorie bread was obtained by blending 10% and 20% charred cellulose granules with wheat flour. The total calorie content of the control bread was calculated by multiplying the energy values of wheat flour (3.66 kcal/g), sugar (3.84 kcal/g), and compressed yeast (1.03 kcal/g) by the respective weights of wheat flour (290 g), sugar (14.5 g), and compressed yeast (8.7 g), and summing the products. The respective weights of wheat flour were 261 and 232 g in the bread samples with 10% and 20% charred cellulose granules.

Statistical analysis. A statistical software package (SPSS, Chicago, III, USA) was used for the statistical analysis. Four bread samples were baked for each treatment, the bread height (mm) and SV (cm^3/g) being measured four times for each sample and the measurements obtained being averaged. All the experiments were run at least in duplicate. The analysis produced significant F values by an analysis of variance that was followed by Duncan's multiple-range test for comparing means.

Results and Discussion

Preparation of differing charred cellulose granule samples

The color of the bread became darker by blending the charred cellulose granules with wheat flour, which would not be favorable to many people, and less darkening by the charred cellulose granules was necessary for this breadmaking exercise. The lowest charring conditions (250 °C for 20 min) for the color of the cellulose granules were therefore selected. Cellulose granule samples of various sizes, i.e., nos. 1 (6 μm average diameter), 2 (154 μm), 3 (270 μm), 4 (464 μm), and 5 (650 μm), were charred at 250 °C for 20 min. The color of the granules changed from white to yellow and brown (Fig. 1), the granule color being measured according to the L, a, b specification system. The smallest cellulose granules, i.e., no. 1, became yellowish (*Lab*: 84.6, 1.3, and 13.1), and the largest cellulose granules, no. 5, became dark brown (*Lab*: 40.3, 8.9, and 16.2) (Fig. 1). The proportion of brownish granules therefore increased with increasing diameter of the cellulose granules.

Effect of charred cellulose granules on the bread-making properties

Charred cellulose granule samples of various sizes (nos. 1–5) were blended with wheat flour at 10% and 20%, and bread was baked. Table 1 indicates the baking absorption level (%) of the charred cellulose granules and wheat flour, and the results [bread height (mm) and SV (cm^3/g)] of the bread-making test. The respective baking absorption of 71.9% and 75.6%, with 10% and 20% of 6-μm charred cellulose granules in wheat flour, was higher than that from 55.8% to 62.2% with other sizes of charred cellulose granules in wheat flour. This may have been due to the higher surface area of charred cellulose granules which was necessary to obtain the 500 BU value. Table 1 shows the relationship between the bread-making properties and the diameter of the charred cellulose granule samples; similar bread-making properties to those for the control bread were apparent in the loaves baked with 10% charred cellulose granules of 270 μm (no. 3), 464 μm (no. 4), and 650 μm (no. 5) diameter. Figure 2 shows the cross-sectional appearance of the loaves. It is apparent that the bread height increased from no. 1 (70.3 mm) to nos. 2 (78.0 mm) and 3 (82.0 mm), and that nos. 4 (82.5 mm) and 5 (80.2 mm) had a similar bread height to that of the control (81.9 mm). This suggests that the diameter of the charred cellulose granule samples was an important factor for obtaining similar bread-making properties to
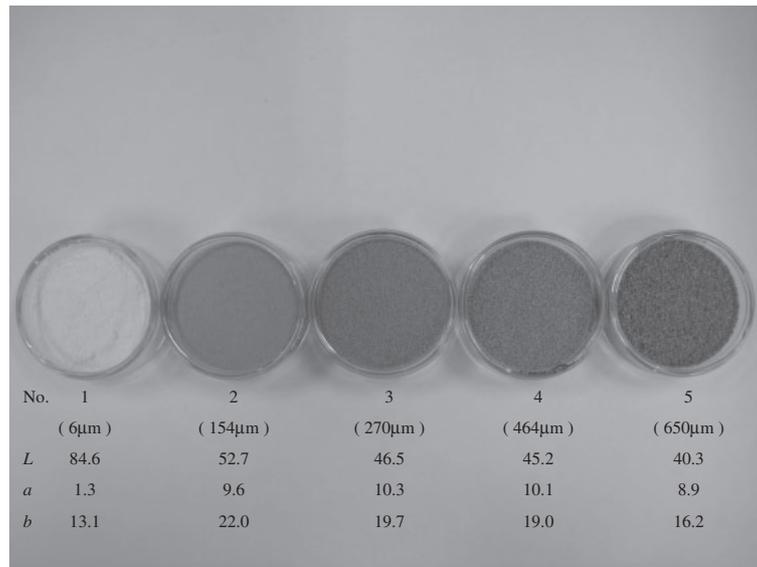
Fig. 1. Photographs of Various Cellulose Granule Samples Charred at 250 °C for 20 min: No. 1 (6 μm average diameter), No. 2 (154 μm), No. 3 (270 μm), No. 4 (464 μm), and No. 5 (650 μm).

The *L*, *a*, and *b* values for the various samples respectively indicate white, red, and yellow.

Table 1. Effects of Charred Cellulose Granules on Bread-Making Properties

Sample no.	Diameter of charred cellulose granules (μm)	Blend (%)	Baking absorption (%)	Bread height (mm)	Specific volume (cm³/g)
1	6	0	65.5a (0.76)	81.9a (2.64)	3.54a (0.05)
		10	71.9b (0.33)	70.3b (2.02)	2.78b (0.04)
		20	75.6c (2.09)	61.8c (2.27)	1.93c (0.03)
2	154	0	65.5a (0.76)	81.9a (2.64)	3.54a (0.05)
		10	62.2b (0.46)	78.0b (2.96)	3.33b (0.08)
		20	56.8c (0.61)	72.0c (1.29)	2.95c (0.03)
3	270	0	65.5a (0.76)	81.9a (2.64)	3.54a (0.05)
		10	62.0b (0.08)	82.0a (0.92)	3.58a (0.09)
		20	56.4c (0.38)	78.9a (1.39)	3.22b (0.02)
4	464	0	65.5a (0.76)	81.9a (2.64)	3.54a (0.05)
		10	61.7b (0.35)	82.5a (0.51)	3.66b (0.09)
		20	55.9c (0.12)	75.4b (2.11)	3.08c (0.02)
5	650	0	65.5a (0.76)	81.9a (2.64)	3.54a (0.05)
		10	61.4b (0.51)	80.2a (0.60)	3.39b (0.07)
		20	55.8c (0.48)	74.2b (1.20)	3.01c (0.02)

Values represent the mean of 4 replicates with the standard deviation in parentheses.
Means followed by the different letters in columns are not significantly different at $p = 0.05$ according to Duncan's multiple-range test.

those of the control. These results are in accordance with those of the bread-making tests for uncharred cellulose granules and wheat flour.[3] It was thus concluded that good bread-making properties could be obtained when charred cellulose granules with a diameter above around 270 μm were used. Seguchi *et al.*[3] have reported that the reason why bread baked with smaller uncharred cellulose granules (6–154 μm) and wheat flour gave inferior bread-making properties to those baked with larger uncharred cellulose granules (154–650 μm) and wheat flour was that the smaller uncharred cellulose granules interfered with the formation of a gluten matrix which was apparent from a mixograph test and microscopic observation; they also observed from a fermograph test that the amount of gas released from uncharred cellulose granules contained in the bread dough increased with decreasing size of the uncharred cellulose granules

below 154 μm. The mixograph test and microscopic abservation in this experiment were performed with charred cellulose granules of 6 or 650 μm and wheat flour and the same results were obtained (data are not shown here).

A fermentation test was performed measuring the height of the bread dough after the 2nd fermentation of the dough made with charred cellulose granules (6 or 650 μm) and wheat flour (Table 2). The height of the bread dough containing 6-μm charred cellulose granules was 11.31 mm, which is less than that of the dough containing 650-μm charred cellulose granules (13.23 mm) and the control bread dough (14.23 mm), suggesting that the smaller charred cellulose granules (6 μm) interfered with the formation of the gluten matrix and leaked gas produced by the yeast; however, larger cellulose granules did not interfere as much with the
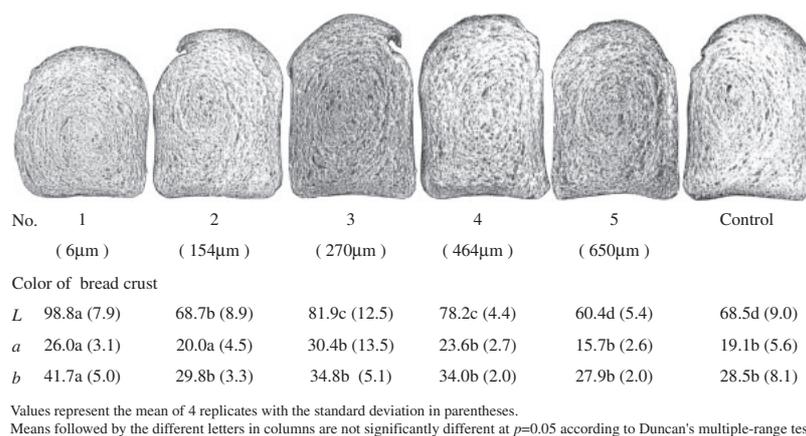
No.	1	2	3	4	5	Control
	(6 μm)	(154 μm)	(270 μm)	(464 μm)	(650 μm)	
Color of bread crust						
L	98.8a (7.9)	68.7b (8.9)	81.9c (12.5)	78.2c (4.4)	60.4d (5.4)	68.5d (9.0)
a	26.0a (3.1)	20.0a (4.5)	30.4b (13.5)	23.6b (2.7)	15.7b (2.6)	19.1b (5.6)
b	41.7a (5.0)	29.8b (3.3)	34.8b (5.1)	34.0b (2.0)	27.9b (2.0)	28.5b (8.1)

Values represent the mean of 4 replicates with the standard deviation in parentheses.
Means followed by the different letters in columns are not significantly different at $p=0.05$ according to Duncan's multiple-range test.

Fig. 2. Appearance of Bread Sections Baked with Various Samples of Charred Cellulose Granules (10%) and Wheat Flour: No. 1 (6 μm average diameter), No. 2 (154 μm), No. 3 (270 μm), No. 4 (464 μm), and No. 5 (650 μm).
Control indicates bread baked with only wheat flour. The L, a, and b values for the crust respectively indicate white, red, and yellow.

Table 2. Effect of Particle Size (diameter) of Charred Cellulose Granules on the Height of Charred Cellulose Granules and Wheat Flour Bread Dough after the 2nd Fermentation

Particle size (μm)	Height of dough (mm)
control	14.23a (1.32)
650	13.23a (0.63)
6	11.31b (0.26)

Values represent the mean of 4 replicates with the standard deviation in parentheses.
Means followed by different letters in columns are not significantly different at $p = 0.05$ according to Duncan's multiple-range test.

formation of the gluten matrix and the production of gas, resulting in a larger loaf. This was almost the same result as that obtained by a fermograph test with dough of uncharred cellulose granules and wheat flour.[3] However, the effect on the bread-making properties of the size of the cellulose granules was different between uncharred and charred cellulose granules. Seguchi *et al.*[3] have reported that SV of bread made with uncharred cellulose granules and wheat flour increased with increasing diameter, indicating a plateau of more than 154 μm in diameter; however, in the case of charred cellulose granules, SV of the bread increased with increasing diameter, to a maximum of 464 μm in diameter. Tabara *et al.*[18] have reported that charring of the cellulose granules increased the positively charged amino group on the surface of the granules, and that this would interact with the gluten matrix resulting in a different increase in SV from the bread samples baked with uncharred cellulose granules and wheat flour. The calculated calorific values of the bread samples baked with 0, 10, and 20% charred cellulose granules and wheat flour were 1126, 1020, and 914 kcal, respectively, indicating respective decreases calories of 9.4% and 18.8% due to the use of 10% and 20% charred cellulose granules.

Sensory and storage tests of bread baked with charred cellulose granules and wheat flour

Uncharred or charred cellulose granules (10%) and wheat flour were baked, and the bread subjected to a sensory test (Table 3). The odor, hardness, and wetness of the sample loaves were almost the same, although the difference in color, mouth-feel roughness and overall evaluation was more marked. The bread recipe did not contain milk and fat, and the proportions of charred cellulose granules and wheat flour could substantially affect the resulting taste when these components were added.

The effect of storing for 1 d at room temperature on the hardness of the bread baked from charred cellulose granules and wheat flour was studied. The hardness of the bread was evaluated by the springiness of a whole loaf of bread baked with uncharred or charred cellulose granules and wheat flour according to the pancake springiness test.[38] The springiness of bread baked with charred cellulose granules (10%) and wheat flour was 67.4% just after baking and 99.7% after 1 d of storage (Table 3) which may have been due to the movement of moisture from the bread crumb to crust. These results are almost the same as those for uncharred cellulose granules and wheat flour. However, the springiness after 1 d of storage was not different, suggesting that the effects of cellulose granules on the starch fraction of wheat flour in stored bread, and of charring of the cellulose granules on the stored bread were small. The RVA profiles of both uncharred and charred cellulose granules and wheat flour suggest almost no effect of the cellulose granules on the flour profile (data not shown here). Both uncharred and charred cellulose granules maintained the granular form and may not have interacted with the starch fraction in wheat flour.

Adsorption of xanthene food dyes (i.e., erythrosine, phloxine, and rose bengal) to charred cellulose granules taken out from the bread crumb or crust

Tabara *et al.*[19] have reported the adsorption of xanthene food dye to charred cellulose granules by a column method. However, it is unclear whether xanthene food dye was also adsorbed to the charred cellulose granules contained in bread. The charred cellulose granules were therefore removed from the bread baked with charred cellulose granules and wheat flour, and adsorption tests were performed. Table 4 shows the results for the adsorption of all the xanthene food dyes to the charred cellulose granules from the bread crumb and crust. In the case of the bread crumb, each xanthene food dye (erythrosine, phloxine, and rose bengal) was eluted with 7 mL of water (48.9, 59.4, and

Table 3. Sensory Test and Storage Test of Bread Baked with Charred Cellulose Granules and Wheat Flour

	Color	Odor	Hardness	Wetness	Mouth feel roughness	Overall evaluation	Springiness	
							0 day	1 day
Control	0.00a	0.00a	0.00a	0.00a	0.00a	0.00a	71.9a	99.5a
							(3.11)	(0.87)
Uncharred	−0.20a	−0.30a	−0.30a	−0.40a	−0.70b	−0.60b	63.4b	99.7a
	(0.63)	(0.67)	(1.16)	(0.84)	(0.82)	(0.70)	(1.47)	(0.58)
Charred	−1.20b	−0.01a	−0.30a	−0.60a	−1.70c	−1.50c	67.4c	99.7a
	(0.63)	(0.47)	(0.95)	(0.84)	(0.67)	(0.71)	(1.12)	(0.58)

Values represent the mean of 10 replicates with the standard deviation in parentheses.
Means followed by the different letters in columns are not significantly different at $p = 0.05$ according to Duncan's multiple-range test.
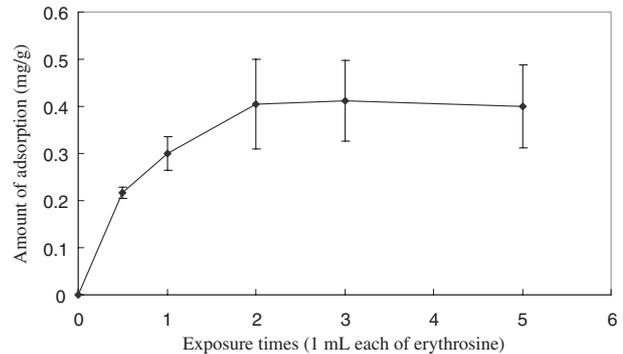
Table 4. Adsorption of Xanthene Dyes to Charred Cellulose Granules Removed from the Bread Crust or Crumb and Tested by Column Chromatography

Xanthene dye	Bread	Eluted with H_2O		Eluted with 0.1 N NaOH solution	
		Total OD value	%	Total OD value	%
Erythrosine					
	crust	1.79 (0.07)	28.0	4.59 (0.62)	72.0
	crumb	2.72 (0.59)	48.9	2.84 (0.52)	51.1
Phloxine					
	crust	1.88 (0.19)	37.5	3.13 (0.21)	62.5
	crumb	3.08 (0.20)	59.4	2.10 (0.53)	40.6
Rose bengal					
	crust	0.57 (0.08)	18.9	2.47 (0.09)	81.1
	crumb	0.96 (0.04)	24.3	2.97 (0.20)	75.7

Erythrosine, phloxine, or rose bengal were charged into a column of charred cellulose granules which had been removed from bread crust or crumb.
Values represent the mean of 3 replicates with the standard deviation in parentheses.

Fig. 3. Effect of the Contact Frequency of Erythrosine on the Adsorption to Charred Cellulose Granules Soaked in Distilled Water.

24.3%, respectively), and for each remaining solution was eluted with 0.1 N NaOH (51.1, 40.6, and 75.7%, respectively). For the bread crust, the dyes were also eluted with 7 mL of water (28.0, 37.5, and 18.9%, respectively), and for each remaining solution were all eluted with 0.1 N NaOH (72.0, 62.5, and 81.1%, respectively). However, for all the dyes, the water-eluted dye from the charred cellulose granules taken out from the bread crust was slightly less than that from the bread crumb. It was thus confirmed that xanthene food dyes were more substantially adsorbed to charred cellulose granules from the bread crust than from the crumb. This can be explained by considering that the temperature near the center of the bread dough increased from 30 °C to approximately 90 °C in the oven (230 °C), but did not exceed 90 °C. The moisture content of the resulting bread crumb was approximately 40–50%.[24] It therefore seems that the charred cellulose granules in the tubes would have remained wet throughout the bread-making process, suppressing dye adsorption. Tabara *et al.*[19] have reported that the water in charred cellulose granules suppressed the adsorption of the erythrosine dye. We found that the temperature of the surface of the bread dough increased to 90 °C in a few minutes, and then gradually increased to approximately 170 °C after being placed in the oven for 20 min. The charred cellulose granules in the tubes buried below the surface of the bread dough would therefore initially have been wet but would soon have dried. This would have resulted in the charred cellulose granules from the bread crust maintaining their dry state, enabling them to substantially adsorb all the xanthene dyes.

It was then necessary to determine whether the charred cellulose granules in the bread crumb could also adsorb xanthene dyes when they were in continuous contact with such a dye in the alimentary canal. Column chromatography of the charred cellulose granules was performed to determine the possible adsorption of xanthene food dyes to charred cellulose granules taken from the bread crumb. The experiment was conducted by repeatedly charging erythrosine in the column (Fig. 3). Charred cellulose granules taken from the bread crumb could adsorb the dye to the same level as those of bread crust by repeated charging. These results suggest that charred cellulose granules in the bread crumb adsorbed dyes when passing through the alimentary canal through peristaltic action.

SEM-EPMA observation of erythrosine and charred cellulose granules from the bread crust

Tabara *et al.*[18] have observed the adsorption of erythrosine to charred cellulose granules by a scanning electron microscopy-electron probe micro-analysis (SEM-EPMA), indicating carbon, oxygen, and iodine elements originating from erythrosine. The adsorbed erythrosine in the charred cellulose granules taken from the bread crust was also observed by SEM-EPMA (Fig. 4b) and showed the presence of iodine in erythrosine. Figure 4b shows that erythrosine was not homogenously adsorbed to the surface of the charred cellulose granules, but was clearly localized instead. White areas due to erythrosine were apparent on the charred cellulose granules taken from the bread crust, suggesting that the dye on the charred cellulose granules was erythrosine. The reason for this is likely that the
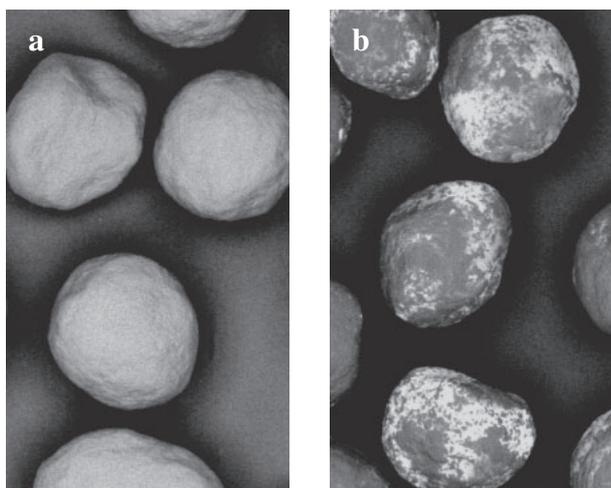
Fig. 4. SEM-EPMA Photographs of Charred Cellulose Granules from the Bread Crust (a) and Erythrosine Adsorbed to Charred Cellulose Granules from the Bread Crust (b).
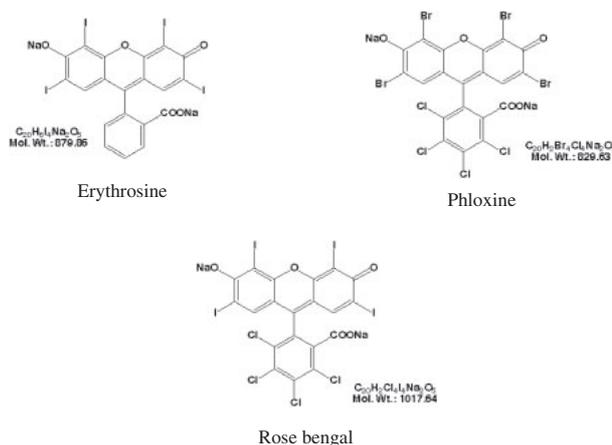

Fig. 5. Structures of Synthetic Xanthene Food Dyes: Erythrosine, Phloxine, and Rose Bengal.

Table 5. Effect of pH on the Adsorption of Erythrosine to Charred Cellulose Granules

pH value	Adsorption of erythrosine (mg/g)
6.00	0.92 (0.01)
6.60	0.88 (0.01)
6.70	0.58 (0.31)
6.80	0.14 (0.05)
6.90	0.06 (0.02)
7.00	0.08 (0.02)

Values represent the mean of 2 replicates, with the standard deviation in parentheses.

charred cellulose granules were packed into a glass column, as mentioned in Materials and Methods, and that erythrosine was charged *in situ*.

The adsorption of erythrosine to charred cellulose granules was studied in a laboratory column test, rather than in the alimentary canal. Such circumambient conditions as the pH value, ionic strength, and food materials in the alimentary canal are more complex. It is possible not to adsorb xanthene food dyes to the charred cellulose granules under such conditions in the alimentary canal, so we examined whether the adsorption of the xanthene food dye (erythrosine) to the charred cellulose granules would occur at around pH 6.5, which is the fecal pH value in healthy people,[29–37] and under other conditions.

pH dependence of the adsorption of erythrosine to charred cellulose granules

It was necessary to know whether the adsorption of erythrosine to charred cellulose granules would occur or not at the same pH values as that in the alimentary canal, particularly at around pH 6.5 which is the fecal pH value for healthy people. Xanthene food dyes such as erythrosine, phloxine, and rose bengal have two negative charges in the molecules (Fig. 5) that yield to precipitation in the stomach (pH 1.0–1.8), but that would be dissolved in the neutral canal by pancreatic juice and bile. The dye is soluble in small intestinal segments such as the duodenum, intestinal jejunum, and intestinal ileum, and in the large intestine. It is known that foods are fermented by lactic acid bacteria and the coli-aerogenes group in the large intestine, and that organic acids are produced.[30,33] The fecal pH value in healthy people is therefore generally known to be around 6.5.[29–37] The pH dependence of the adsorption of erythrosine to charred cellulose granules was examined in a 10 mM Tris–HCl buffer at pH 6.0, 6.6, 6.7, 6.8, 6.9, and 7.0, the respevtive amounts of erythrosine adsorbed to the charred cellulose granules being 0.92, 0.88, 0.58, 0.14, 0.06, and 0.08 mg/g (Table 5). Although the adsorption of erythrosine was sharply decreased when the pH value approached 7.0, erythrosine was adsorbed to the charred cellulose granules at around pH 6.5.

Erythrosine could therefore be adsorbed to charred cellulose granules in the large intestine and extruded with the charred cellulose granules from the anal canal.

A 10% sucrose solution containing 10 mM Tris–HCl at pH 6.0 was examined for the adsorption of erythrosine to charred cellulose granules and showed that the amount of erythrosine adsorbed to the charred cellulose granules was almost the same (0.85 mg/g), suggesting that a concentrated food material (sucrose) would be effective at a low level for the adsorption of erythrosine to charred cellulose granules. Since ionic and amphipathic molecules are known[19] to inhibit the adsorption of erythrosine to charred cellulose granules, the presence of such food materials would negatively act on the adsorption.

Xanthene food dyes could be trapped in the alimentary canal where they may cause disease. When we eat bread baked with charred cellulose granules and wheat flour, the non-digestible charred cellulose granules will pass through the alimentary canal from the mouth to stomach and small and large intestine, and will be excreted through the anus. The charred cellulose granules could be in contact with xanthene food dyes, adsorb them through peristaltic motion, and finally be excreted with feces throughout this passage. Using charred cellulose granules to eliminate xanthene food dyes from the body may therefore be achieved. We applied one model for excluding toxic xanthene dyes in this experiment by adsorbing to charred cellulose granules. It will be necessary to further examine this in the human body.

Conclusions

Almost the same bread-making properties as those of control bread baked with wheat flour were obtained when charred cellulose granules with a diameter above 270 μm were used in a bread-making test. The bread showed a respective 9.4% and 18.8% decrease in calorie content when 10% and 20% wheat flour was replaced by charred cellulose granules. The charred cellulose granules removed from the bread indicated adsorbed xanthene food dyes (erythrosine, phloxine, and rose bengal). It was apparent that the charred cellulose granules could adsorb xanthene food dye (erythrosine) at pH 6.6 which is close to the fecal pH value in healthy people. Charred cellulose granules having more than 50% crystallinity were not disrupted by such physiological force as peristaltic motion,[21] so that a xanthene food dye would be adsorbed to the granules in the alimentary canal. Bread baked with charred cellulose granules and wheat flour had a lower-calorie content than conventional bread, and the granules in the bread could adsorb toxic xanthene food dyes contained in processed foods and excrete them from the body.

Acknowledgments

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