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Microbial behavior and changes in food constituents during fermentation of Japanese sourdoughs with different rye and wheat starting materials

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Sourdough is a food item made by kneading grain flour and water together and allowing fermentation through the action of lactic acid bacteria (Lactobacillales) and yeast. Typically, Japanese bakeries make sourdough with rye flour, wheat flour, malt extract, and water and allow spontaneous fermentation for 6 days. We compared the microbial behavior and food components, such as organic acids, sugars, and free amino acids, of sourdoughs made using two different rye and wheat flours during the 6-day fermentation period. Comparisons were made for two types of rye and wheat flours, using different production sites and different milling, distribution, and storage conditions. The microbial count was evaluated using different culture media. All sourdough types showed a significant increase in lactic acid levels on fermentation day 2 and a decrease in free amino acid levels on day 4. Low overall lactic acid production and little fluctuation in sugar levels occurred in sourdough made from French ingredients. For sourdough made from Japanese ingredients, sugar levels (chiefly glucose, sucrose, and maltose) declined on fermentation day 1, increased on day 2, and declined by day 5. With the French ingredients, no yeast cells were detected until day 3, and many acid precursors of sourdough flavor components were detected. Yet with the Japanese ingredients, 10^6/g yeast cells were detected on days 3–5, as well as sourdough-flavor esters and alcohols. Differences in raw material quality affected the microbial behavior and changes in food constituents during the fermentation process and, consequently, the sourdough flavor.

[**Key words:** Lactobacillales; Fermentation; *Saccharomyces cerevisiae*; Lactic acid; Sourdough]

During the late Edo period in Japan, bread became familiar in areas where foreigners resided, including Nagasaki, Yokohama, and Kobe (1). Traditionally, bread was made using hops broth to raise the bread dough during this era. In the Meiji Era, Japanese people established bakeries. Since domestic hops were unobtainable; yeast mass (called sakadane) from the Japanese saké (rice wine) production process was used to create *anpan—azuki* (red bean) sweet jam bread, which is popular with Japanese people (1). Sakadane is made by fermenting *koji* (rice malt) and yeast, and used to raise bread and add a fruity *ginjo* flavor to the bread (*ginjo* is top-quality saké made by low-temperature fermentation). This fruity *ginjo*-tasting bread was first developed in Japan. In recent years, bakeries throughout Japan have also been using *pain au levain* and San Francisco sourdough bread types introduced from Europe and North America. In particular, these sourdoughs are different from sakadane in that raw materials and manufacturing methods (2). However, these sourdoughs resemble sakadane at the point having favorable aromas by the function of yeast (2).

Sourdough is a fermented food item made by kneading a mixture of milled grain and water into dough, which is then fermented by lactic acid bacteria and yeast (3–7). Sourdough has been used to improve bread qualities such as flavor (8), taste, texture (9), and shelf life (10–12). A traditional method for making sourdough involves incorporating rye flour, wheat flour, malt extract, water, and other ingredients (5,7), and allows 6 days for natural fermentation. This method is popular in many Japanese bakeries (13). Involvement of lactic acid bacteria and yeast in traditional sourdough processes has been reported (5,7,14–18). Several studies explored the microbial behavior during the fermentation process of French and Italian sourdoughs (19–21). Popular sourdough fermentation processes applied in Japanese bakeries are different from those used in France and Italy in terms of temperature control and duration of fermentation. However, details of the microbial behavior and changes in ingredients during the process used in Japanese bakeries are scant.

This study compared the microbial behaviors and food constituents, such as organic acids, sugars, and free amino acids, of sourdoughs made from rye flour and wheat flour milled in Japan and

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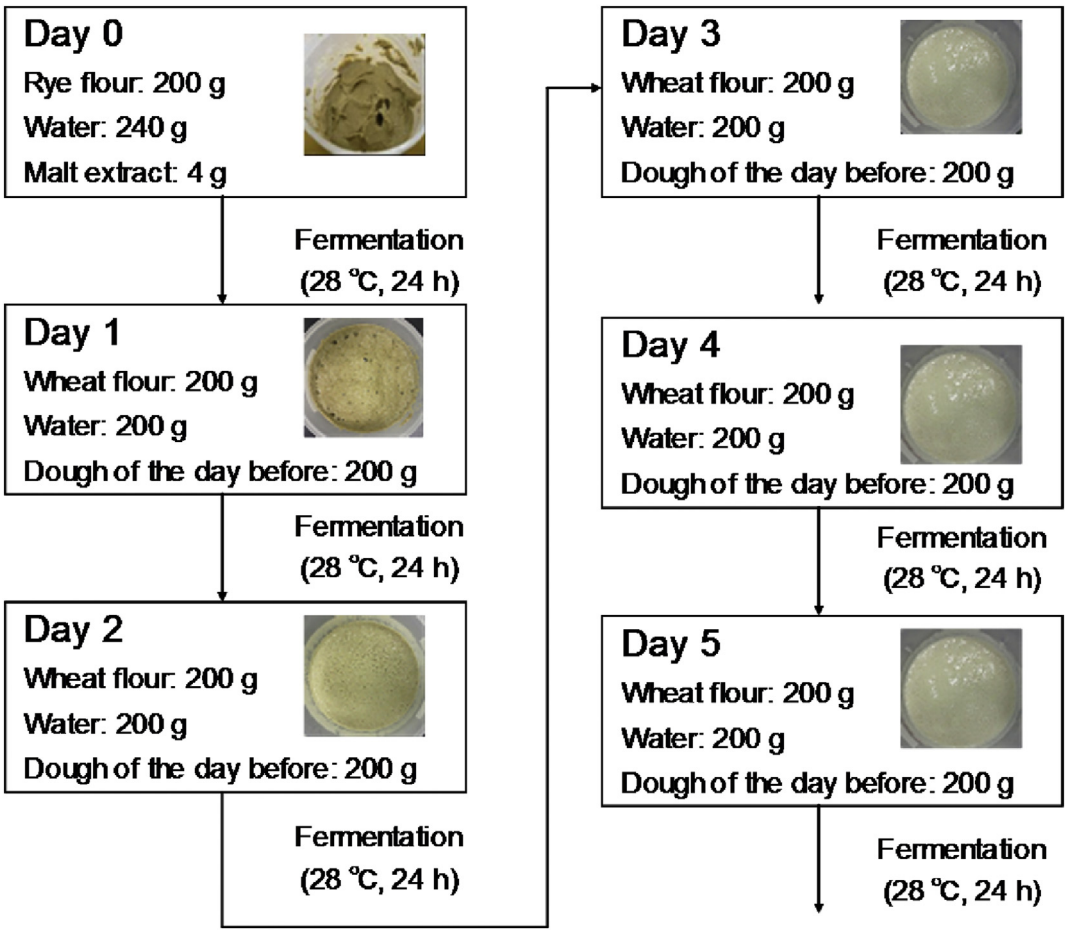

FIG. 1. Typical sourdough production method used in Japan.

France. The volatile compounds of the two sourdoughs were analyzed by gas chromatography/mass spectrometry (GC/MS) to evaluate flavor and fragrance of the sourdoughs from the two countries.

MATERIALS AND METHODS

Flour, malt extract, and water The starting materials for making sourdough were as follows. Grain milled in France and imported to Japan included rye flour F (product name: Seigle type 130, Minoteries Viron; protein: 8.5%, ash: 1.5%, the cultivation area: France) and wheat flour F (product name: La Traditionnelle Française, Minoteries Viron; protein: 10.5%, ash: 0.55%, the cultivation area: France). Grain milled and distributed in Japan included rye flour J (product name: Aare Fine, Nisshin Seifun Group; protein: 11%, ash: 1.3%, the cultivation area: Germany) and wheat flour J (product name: Lys d'Or, Nisshin Seifun Group; protein: 10.7%, ash: 0.45%, the cultivation area: Canada or USA). Malt extract was EuroMalt (made in Italy, Nichifutsu Shoji). Deionized water was sterilized by autoclaving at 121°C for 15 min. Commercially available rye and wheat flours were purchased and used promptly.

Viscographic analysis of enzyme activity in rye flour and wheat flour Enzyme activity of flours was measured by monitoring gelatinization behavior (22). Sixty-five grams of wheat flour or rye flour mixed with 450 mL water was placed into a Viscograph-E device (Brabender, Duisburg, Germany). Viscosity at 95°C was used as the index of enzyme activity.

Sourdough preparation procedure The sourdough was made as illustrated in Fig. 1. On the first day (day 0), 200 g rye flour, 240 g sterile water, and 4 g malt extract were mixed thoroughly and warmed until the dough temperature reached 30°C. The dough was allowed to ferment for 24 h at 28°C. On day 1, 200 g of fermented dough was mixed thoroughly with 200 g wheat flour and 200 g sterile water until the dough temperature reached 28°C, followed by another 24-h fermentation at 28°C. On day 2 and every day thereafter to day 5, 200 g dough fermented from the previous step was combined with an additional 200 g of wheat flour and 200 g sterile water, mixed thoroughly until the dough temperature reached 28°C, and incubated for 24 h at 28°C. Sampling on day 0 was performed immediately after mixing the ingredients. From day 1 and thereafter, sampling and evaluation were performed immediately after fermentation.

The rye flour F and wheat flour F milled in France were used to prepare the F-sourdough. Rye flour J and wheat flour J milled in Japan were used to prepare the J-sourdough. Sourdoughs were made independently three times and evaluated using the average score from the three trials.

pH measurement Sourdough samples were diluted five-fold with deionized water. The pH of each suspension was measured using a Seven Easy pH meter (Mettler-Toledo, AG, Switzerland).

Free amino acid analysis Fifty milliliters of 2% sulfosalicylic acid solution was added to 5 g of sourdough sample and homogenized to remove proteins by centrifugation (8947 ×*g*, 5 min, 20°C). The supernatant was transferred to a new tube and left to settle overnight. The upper phase of the supernatant was filtered through a 0.45-μm DISMIC-13CP membrane filter (Advantec, Tokyo, Japan). Free amino acids were quantified using a JCL-500/V fully automated amino acid analyzer (JEOL Ltd., Tokyo, Japan).

Organic acid analysis Sourdough samples (5 g) were diluted five-fold in deionized to form suspensions. Each was centrifuged (8947 ×*g*, 5 min, 20°C) and 1 mL of supernatant was transferred to a new tube. Twenty microliters of 20% sulfosalicylic acid was mixed with each 1-mL supernatant sample, which was then filtered through a 0.45-μm membrane filter. Organic acid analysis was performed by high-performance liquid chromatography (HPLC) using an LC10A Series device (Shimadzu, Kyoto, Japan), using an organic acid column (7.8 mm × 300 mm; Waters, Milford, MA, USA), column temperature of 40°C, solvents that comprised an A buffer phase (9.51 g *p*-toluenesulfonic acid dissolved in 100 mL distilled water) and B buffer phase (9.51 g *p*-toluenesulfonic acid, 41.85 g Bis–Tris, 0.29 g EDTA-2Na dissolved in 100 mL distilled water), a flow rate of 0.8 mL/min for both A and B phases, and a refractive index (RI) detector.

Sugar analysis Five grams of sourdough sample was homogenized with 25 mL of 50% aqueous acetonitrile. The homogenized solution was placed in a 50-mL test tube and centrifuged (8947 ×*g*, 5 min, 20°C). Twenty microliters of 20% sulfosalicylic acid was mixed with each 1-mL supernatant, which was filtered through a 0.45-μm membrane filter. Sugar analysis was performed by HPLC using

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