Application of Potassium Myristate as an Antifungal and a Dough Improving in Bread-Making

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Surface-acting agents are used as bread improvers in bread making to increase the specific loaf volume. We focused on the use of fatty acid salts as surface acting agents and new food additives. Fatty acid salts are the main components of soaps and display antibacterial activity. In this study, we investigated the mold-proofing activity and baking property with fatty acid salts. We examined the influence of fatty acid salts with different lengths of carbon chains on the dough expansion ability and found that the dough expansion ability increased significantly in the presence of more than 5% potassium myristate (C14K). No significant difference was observed in the dough fermentation abilities between the control dough and C14K-treated dough, indicating of the absence of any effect of C14K on the fermentation ability. The antifungal test showed that C14K effectively inhibited fungal growth. Thus, C14K may serve as a promising bread improver.

Key words : Bread making / Fatty acid salt / Bread improver / Dough expansion ability.

In recent years, bread has been produced on a large-scale with heavy machinery and often using bread improvers. Bread improvers make the production process simple and safer, enabling bakers to prepare quality and standardized end products. As a dough improver, dough conditioner plays a very important role (Tanaka and Nakae, 1982). Examples of dough conditioners include emulsifiers, potassium bromate, oxidants, and enzymatic agents. Glycerin fatty acid ester, sorbitan fatty acid ester, propylene glycol fatty acid ester, sucrose fatty acid ester, and soybean phospholipid are the five approved emulsifiers in Japan (Tanaka and Nakae, 1982). Emulsifiers are extensively used in the baking industry for improving the dough machinability and to extend the shelf life of the products (Gomez et al., 2004).

Fatty acid salt is one of the surface-acting agents used as a new food additive to replace emulsifiers and comprises fatty acid and alkali. On the other hand, fatty acid salts are the main components of soaps and have been reported to show some antibacterial and antifungal activities (Kollanoor et al., 2007; Galbraith et al., 1971; Ababouch et al., 1992). Fungi generated in bread include Aspergillus spp., Penicillium spp., and Rhizopus spp. (Tanaka and Matsumoto, 1991) and fatty acid salts are known to be effective against fungi (Era et al., 2015). Therefore, fatty acid salts can be used as antifungal agents. Here, we propose the application of fatty acid salts for increasing the specific loaf volume and as a mildew-proofing agent.

The use of fatty acid salts as food additives is not approved in Japan but is permitted to be used in the certain foods in the other countries. According to JECFA (FAO/WHO Joint Expert Committee on Food Additives), salts of myristic (C14), palmitic (C16), and stearic (C18) acids may be used as food additives because they have been evaluated ADI was “not specified” by JECFA (JECFA, 1997; JECFA, 1986). In particular, calcium stearate is used in the medical industry in Japan and there are no reports related to safety issues (Food Safety Commission of Japan, 2004). Therefore, fatty acid salts of potassium myristate (C14K), potassium palmitate (C16K), and potassium stearate (C18K)
may be used as dough improvers for bread making.

The antifungal activity of fatty acid salts may be partly attributed to the length of their carbon chains. The antimicrobial effects of fatty acids, which are the raw materials for the production of fatty acid salts, reduce with an increase in the chain length; medium chain fatty acids exhibit stronger activity than longer chain fatty acids (Wang and Johnson, 1992).

Isaacs et al. (1995) reported stronger antiviral and antibacterial activities for fatty acids and monoglycerides containing 8-12 carbons as compared with those containing long carbon chains. It has been suggested that fatty acid salts with short carbon chains may display strong antibacterial effects. Therefore, we selected potassium myristate, which has a short carbon chain, as a bread improver, and investigated its baking property and fungus-proofing activity.

Potassium salt of myristic acid was prepared by mixing myristic acid, potassium hydroxide (KOH), and distilled water. Myristic acid (C14:0) was obtained from Tokyo Chemical Industry Co., Ltd and KOH pellets, from Wako Pure Chemical Industries, Ltd., Osaka, Japan. Samples were prepared at a concentration of 350 mM and stirred for 2 h at 75°C. Potassium myristate (C14K) was adjusted using a pH-adjusted KOH solution (pH 10.5). Potassium caprylate (C8K), potassium caprate (C10K), potassium laurate (C12K), potassium palmitate (C16K), and potassium stearate (C18K) were used for comparison with potassium myristate. Caprylic acid (C8:0), capric acid (C10:0), and lauric acid (C12:0) were obtained from Tokyo Chemical Industry Co., Ltd. Palmitic acid (C16:0) and stearic acid (C18:0) were supplied by Wako Pure Chemical Industries, Ltd., Osaka, Japan. These fatty acid salts were prepared along with potassium myristate. Also, fatty acid salts is not in a state of dehydrating.

The blending ratio of bread dough with fatty acid salts are shown as follows. Bread dough comprised 100 g flour (Camellia, Nisshin Flour Milling Inc., Japan), 5 g sugar, 1.7 g salt, 1.7 g dry yeast (Super Camellia, Nisshin Flour Milling Inc., Japan), 68 ml water, and fatty acid salt. The dry yeast contains emulsifier and vitamin C. The ingredients other than fatty acid salt were mixed 300 times by hand. Additionally, fatty acid salt was added and mixed 500 times in total. The concentration of the fatty acid salt relative to the wheat flour weight was 1%, 3%, 5%, 8%, and 10%.

Dough was fermented at 30°C and 85% humidity for 120 min-on an incubator (FLI-2000H, Tokyo Rikakikai Co, LTD, Japan). Furthermore, leavened bread dough of 100 g was degassed, quickly rounded, and baked at 180°C for 15 min in an oven (RMC-S12E, Rinnai Inc., Japan). After baking, the bread was allowed to cool to room temperature.

The dough expansion ability test was performed to measure the physical property of bread dough according to the methods of Baker’s Yeast by Japan Yeast Industry Association (Tanaka and Matsumoto, 1991). In this test, a cylinder was filled with 150 g of dough from its bottom at 30°C and 85% humidity for 120 min on an incubator (FLI-2000H, Tokyo Rikakikai Co, LTD, Japan). The height of the dough expansion was measured to determine its expansion ability.

In order to investigate the effect of fatty acid salts on baker’s yeast, we examined the dough fermentation ability and viable count. Using the immediate prepared dough, these tests were performed by the fermentation at an initial stage.

Dough fermentation ability test was performed to measure the physical property of bread dough according to the methods of Baker’s Yeast by Japan Yeast Industry Association (The Japan Yeast Industry Association, 1996). The gas substitution apparatus was filled with saturated saline solution dyed with methylene blue. Additionally, gas inlet in the gas substitution apparatus was connected to 300 mL Erlenmeyer flask including the dough 50 g by rubber tubes. Similarly, liquid exit in the gas substitution apparatus was connected to 300 mL measuring cylinder by rubber tubes.

In this test, 50 g of bread dough was fermented in conical flasks at 30°C (water temperature) and constant shaking (100 r/min; 4.5 cm amplitude) using a shaking apparatus. The gas yield during fermentation was measured every 20 min, while the leaving power was determined as the total gas yield for 2 h.

The viable count was evaluated as follows: a sample of 0.5 g-dough was suspended in 10 ml of 0.85% saline solution. The suspension was used as a stock solution, diluted, and 0.1 ml of the diluted sample was plated on the yeast malt peptone agar plates. Following incubation at 30°C for 24 h, the number of yeast colonies was counted. The composition of yeast malt peptone medium was as follows: 10.0 g/L glucose, 5.0 g/L peptone, 3.0 g/L yeast extract, 3.0 g/L malt extract, 15.0 g/L agar, and 1 L distilled water.

We investigated the antifungal effects of fatty acid salts added to the bread. We baked bread in the presence 0%, 5%, and 10% C14K at 180°C for 15 min in the oven. After baking, the bread was allowed to cool to room temperature. Bread samples of 3 × 3 × 1 cm dimension were placed on the potato dextrose agar (PDA: Nissui Pharmaceutical Co., Ltd., Tokyo, Japan) plates and incubated at 30°C for 7 d. Samples were visually observed every 24 h.

All results were expressed as mean ± standard deviation. The data were obtained from at least three independent experiments. A P value less than 0.05 was considered statistically significant.
APPLICATION OF POTASSIUM MYRISTATE FOR BREAD-MAKING

We investigated the bread-making quality of the bread by adding C14K as an anionic surfactant. The result of the dough expansion ability test is shown in Fig.1. The addition of C14K at 10% resulted in a sufficient increase in the dough expansion ability. The dough expansion ability was 540, 568, and 577 ml in the presence of 5%, 7%, and 10% C14K, respectively, for 120 min. The dough expansion ability increased by 14.9% to 22.8% with increase in C14K (5% to 10%) as compared with that of the control sample (470 ml). A significant difference (P<0.05) was observed between the control groups and groups with more than 5% C14K, as analyzed using t-test. Thus, maximum dough expansion at the time of 120 min was observed with the addition of 10% C14K.

As the various fatty acid salts were used in their liquid forms, the increase in the water content of the dough may cause the increase in the expansion ability of the bread. Hence, we measured the dough expansion ability by adding the same amount of water without fatty acid salts. In comparison with C14K, water remarkably decreased the dough expansion ability (data not shown). Thus, the increase in the dough expansion ability in the presence of C14K was associated with the components of C14K. Therefore, C14K was revealed to be most effective for improving the dough quality.

We investigated the yield of CO₂ gas released during the fermentation of the dough in the presence of C14K. Fig.2 shows the dough fermentation ability with the addition of C14K. No significant difference was observed in the yield of CO₂ generated every 20 min with or without C14K. Furthermore, the dough fermentation ability with C14K for 120 min was same as that without C14K. Thus, C14K had no effect on the fermentation ability. These results suggest that the volume of the dough increased in presence of C14K, although the amount of CO₂ produced during fermentation was constant. Thus, C14K had no effect on CO₂ gas but affected on the dough structure.

Anionic surfactants such as sodium stearoyl lactylate (SSL) mediate the binding between gliadin and glutenin, which account for about 47% of wheat proteins (Bushuk and Wrigley 1974). Surfactants neutralize the positive charge of proteins and promote protein aggregates. Therefore, it is presumed that the bread-making property of the bread dough would be improved depending on the change in the structure of the dough as well as the cooperative interactions between proteins (Tanaka and Matsumoto 1991).

The bread-making property was thought to be related with C14K and gas film of the dough. Gas film is a thin film of gluten formed by the mixing of gliadin and glutenin that confines carbon dioxide produced during fermentation (Tanaka and Matsumoto 1991). There are countless gas films within the expanded dough that result in the expansion of the whole bread (Tanaka and Matsumoto 1991). Therefore, gas film may break and leak, leading to insufficient expansibility (Tanaka and Matsumoto 1991). As explained above, it is considered that C14K mediate the binding of gliadin and glutenin, which may increase the dough expansion ability.

Table 1 shows the results of the viable count of the
dough with C14K, as dough fermentation ability test. These results suggested that the reduction of viable count and addition of C14K had no effect on the fermentation ability of CO₂.

Fig.3 shows the result of the antifungal test using bread treated with fatty acid salts. No change was observed in the all samples incubated for 0 and 1 d. However, fungal growth was observed in control samples incubated for 2 d. On the other hand, no fungal growth was detected for samples treated with 5% and 10% C14K for 2 d. After incubation for 3 d, fungi were observed all over the plate in the control samples, but no fungal growth was observed in the samples treated with 5% and 10% C14K. A few colonies were observed from 4 d onward, but the fungal growth was suppressed as compared with that of the control sample. These results are indicative of the effect of C14K as an antifungal agent.

Additionally, regarding the influence of mold spores, fatty acid salts are considered to inhibit fungal growth by penetrating the cell membrane (Era et al., 2015). Walters et al. (2003) and Rihakove et al. (2001) studied the antifungal activity of fatty acids and their derivatives against highly pathogenic plant fungi, such as Aspergillus niger. Accordingly, the antifungal activity of C14K is assumed to relate to influence of mold spores by fatty acid salts on antifungal activity.

Pyler et al. (1988) reported that the extension of the keeping quality by 1-2 d has profound significance in the distribution and consumption of bread. Therefore, C14K may not only display dough expansion ability but also act as an antifungal agent. In the future, it is neces-

**TABLE 1.** Effect of baker’s yeast in the dough by addition of C14K

| Sample                | Before fermentation | After fermentation |
|-----------------------|---------------------|--------------------|
|                       | viable count        | standard deviation | viable count  |
|                       | (cfu/g-dough)       | (−)                | (cfu/g-dough) |
|                       | standard deviation  | (−)                | standard deviation |
| Control               | 7.2 × 10⁸           | 2.1 × 10⁷          | 8.2 × 10⁸  |
| C14K addition of 5%   | 9.5 × 10⁸           | 1.7 × 10⁸          | 7.2 × 10⁸  |
| C14K addition of 10%  | 1.3 × 10⁹*          | 3.0 × 10⁷          | 9.0 × 10⁸  |

*P < 0.05
sary to examine about of factor increased the dough expansion, further studies are needed in order to baking condition and relationship with gluten network in the dough.

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