EFFECTS OF SMOKING AND LIQUID SMOKE ON THE SUPPRESSION OF LACTIC ACID BACTERIA IN MEAT PRODUCTS

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I. INTRODUCTION

Lactic acid bacteria (LAB), although widely beneficial for human health, are known to promote discoloration and slime production in meat products. Thus, it is important to control the LAB count during meat processing. Smoking is a popular method of processing meat. Liquid smoke, produced by condensation of wood smoke created by pyrolysis of wood chips is also used for meat processing. It contributes towards flavor enhancement and storage stability of the meat products. However, to the best of our knowledge, only few studies have investigated the anti-microbial effects of smoking and liquid smoke on the LAB in meat products. In this study, we investigated the anti-microbial effects of smoking with some wood tips on the microbe in meat products. In addition, the anti-microbial effects of several liquid smoke products on the spoilage LAB found in meat products were also assayed.

II. MATERIALS AND METHODS

The tested sausages in this study were prepared with pork meat, 2% NaCl, 1% sucrose, 0.1% L-sodium ascorbate, and 0.1% spice mixture (each component was added (w/v)). Because the anti-microbial effects of smoking were to be assessed, sodium nitrite ($NaNO_2$) was not added to the sausages in this study. The sausages were smoked with the wood chips from *Prunus* (sakura), *Fagus* (beech), and *Juglans* (walnut) trees (Watanabe Rinsan Inc., Tokyo, Japan), separately, at 50°C for 1 hour. After smoking, each sausage was heated such that the temperature at its center reached 75°C. The sausages were kept at 10°C for 20 days, following which the microbial count was enumerated. The microbial assay was carried out using the culture method. Briefly, the aerobic bacterium, LAB, psychrophilic bacteria, staphylococci, coliform bacteria, and yeast and molds were enumerated with standard plate count agar, BCP agar, mannitol salt agar, CVT agar, desoxycholate agar, and the modified PDA agar, respectively (EIKEN Chemical co. ltd, Japan). In addition, sausages were kept at 30°C for 7 days and the spoilage LAB were isolated on a modified GYP agar (Takeda et al. 2011). The isolated LAB were identified by 16s rDNA sequencing. Then, they were subjected to the anti-microbial assay with liquid smoke products. The liquid smoke products (Smoke EZA, C35, Enviro, Cherry smoke, and Light smoke) were kindly provided from the Logos Corp. (Kyoto, Japan). The anti-microbial properties of liquid smoke against the meat microbial isolates were measured by a paper disk diffusion assay.

III. RESULTS AND DISCUSSION

As shown in Table 1, the standard plate counts of the unsmoked and smoked sausages with respective wood chips were between 5.2 to 6.7 $\log_{10}$ CFU/g at day 10 after preparation. The LAB were not detected in any smoked sausage samples (< 2.0 $\log_{10}$ CFU/g), although the LAB count in unsmoked samples was 5.0 $\log_{10}$ CFU/g. This could be attributed to the effects of smoking because smoking is a known preservation technique (Goto et al. 2014). The levels of other microbes detected in the smoked and unsmoked sausages were the almost same, perhaps because of the absence of $NaNO_2$, often used as a preservative in the meat products, rather than the effect of smoking.

We isolated 20 LAB isolates from the sausages kept at 30ºC for 7 days. The isolates belonged to *Enterococcus malodoratus* (12 strains), *Streptococcus thermophiles* (7 strains), and *Carnobacterium maltaromaticum* (1 strain). These isolates were considered to be the spoilage LAB in tested sausages and

were subjected to paper disk diffusion assay with the liquid smoke products. The results of paper disk diffusion assay for *S. thermophiles* strain no. 1, *E. malodoratus* strain no. 2 and *C. maltaromaticum* strain no. 15 are shown in Figure 1 (The data for other strains are not shown). The inhibition areas produced by liquid smokes of 'Smoke EZA' and 'Enviro' tend to be larger than those by the other liquid smokes in strain No.1, 2, and 15. These results may be attributed to the different levels of phenols and/or carbonyls in the liquid smoke products (data not shown), as suggested in a previous report (Lingbeck et al. 2014). Variation in diameters of inhibition zones was observed between different strains of the same LAB species (data not shown). The anti-microbial effects of liquid smoke on the spoilage LAB would vary depending on the liquid smoke product used and the LAB species and strains. Next, to confirm the antimicrobial effects of liquid smoke products, we intend to determine the viable percentage of LAB in nutrient broth supplemented with liquid smoke products.

Table 1 Number of microbe from the sausages in smoking with different tips

Microbe	Number of microbe (log_{10} CFU/g)			
	Unsmoked	Sakura tip	Beech tip	Walnut tip
Aerobic bacterium	6.2±0.5	5.4±1.3	5.2±1.4	6.7±0.1
Lactic acid bacteria	5.0±0.7	ND	ND	ND
Psychrophilic bacteria	4.8±0.1	4.9±0.0	3.4±0.1*	4.5±0.2
Staphylococci	ND	ND	3.6±0.2	ND
Coliform bacteria	3.8±0.1	3.9±0.2	4.0±0.1	4.4±0.3
Yeast and Mold	ND	5.2±0.6	5.0±0.9	5.1±0.2

The data were yielded from the respective sausages incubated at 10ºC for 15 days. ND: < 2.0 log_{10} CFU/g
*: $P < 0.05$ vs unsmoked sample by one-way ANOVA, followed by Tukey's test

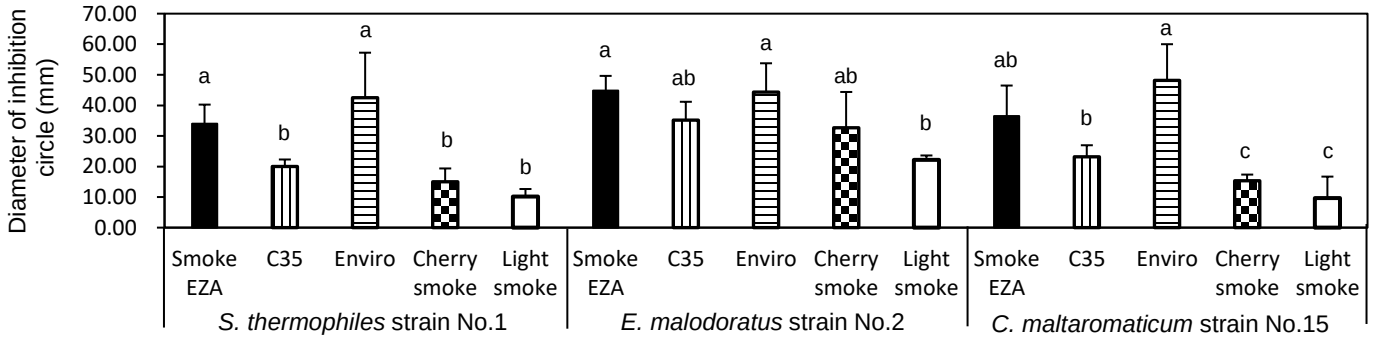

Figure 1. Antimicrobial effects of liquid smokes on the spoilage LABs
The different letters in each strain are significantly different by one-way ANOVA, followed by Tukey's test ($P < 0.05$).

IV. CONCLUSION

In conclusion, smoking contributes to prevention of an increase in the LAB number in the sausages. Moreover, the spoilage LAB isolates were suppressed with liquid smoke. Hence, smoking and addition of liquid smoke to meat products is believed to be useful for meat product preservation from spoilage LAB.

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