LIPOLYTIC AND PROTEOLYTIC ACTIVITIES OF MOLD AND YEAST ISOLATED FROM DRY-AGED BEEF AND THEIR APPLICATION FOR DRY AGING PROCESS

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I. INTRODUCTION

Dry-aged beef has a more intense beefy and roasted flavor, which is absent in fresh and wet-aged beef [1]. Therefore, there has been an effort to investigate the mechanism of the flavor development for dry-aged beef. In previous study, we found that different microbial composition, especially mold and yeast, may change flavor development of dry-aged beef (unpublished data). Then, we isolated mold (*Pilaira anomala*) and yeast (*Debaryomyces hansenii*) from the dry-aged beef to identify the characteristic of each microorganism. In consequence, the lipolytic and proteolytic activities of *P. anomala* and *D. hansenii* and their effect on free fatty acid (FFA) and free amino acid (FAA) of dry-aged beef were investigated in this study.

II. MATERIALS AND METHODS

The lipolytic and proteolytic activities of *P. anomala* and *D. hansenii* were analyzed by an agar model system based on the method from Atanassova *et al*. [2]. To analyze their effect on dry-aged beef, a total of 24 sirloins (Holstein steer, quality grade 3) were obtained and each 12 sirloins were inoculated with different microorganism at the final concentration of 10^6 CFU/mL. After the inoculation, all samples were dry aged for 0, 14, 21, and 28 days (temperature, 4°C; relative humidity, approximately 75%; n=3 for each aging day), then trimmed off their crust for further analyses of FFA and FAA following the methods from Wang *et al*. [3] and Graham *et al*. [4], respectively. For statistical analysis, the general linear model was performed using SAS 9.4 (SAS Institute Inc., Cary, NC, USA) on the basis of Tukey's multiple comparison test at a level of $P<0.05$.

III. RESULTS AND DISCUSSION

Based on this result by the agar model system, *P. anomala* had a higher lipolytic activity than that in *D. hansenii*, whereas similar activity of proteolysis was shown by both microorganisms (Fig. 1).

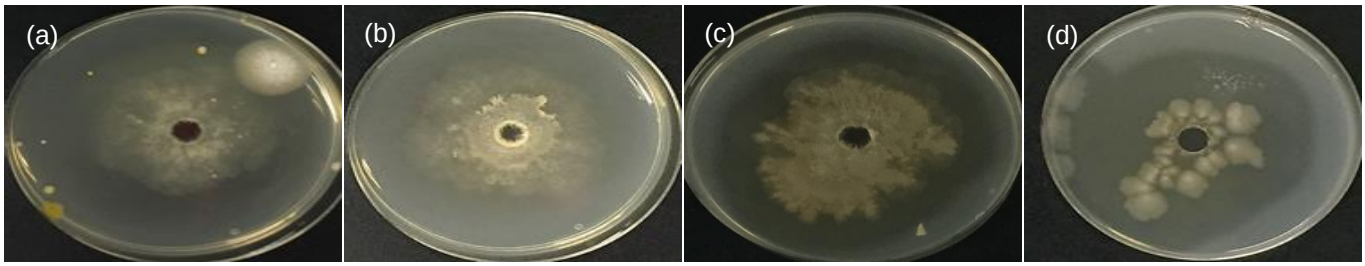

Figure 1. Lipolytic (a, b) and proteolytic (c, d) activities of *Pilaira anomala* and *Debaryomyces hansenii* isolated from dry-aged beef, respectively.

P. anomala and *D. hansenii* were inoculated on dry-aged beef and analyzed the changes in total FFA and FAA contents during 28 days of aging (Fig. 2). In total FFA content, the pattern of FFA change was similar in both microorganisms as it reached the highest value and decreased thereafter, probably by the degradation to short-chain FFA or the oxidation [5], however, 2-4 folds higher with *P. anomala* ($P<0.05$, Fig. 2a). This could suggest a higher lipolytic activity of *P. anomala*, compared to that of *D. hansenii*, as shown in Fig. 1. Meanwhile, although similar activity of proteolysis by *P. anomala* and *D. hansenii* was found on

the agar model system, the pattern of change in total FAA content was varied with different microorganism during dry aging process (Fig. 2b). *P. anomala* gradually increased FAA content of dry-aged beef only until day 21, whereas *D. hansenii* resulted in an insignificant change from days 14 to 21 and, again, increased thereafter (P<0.05). This phenomenon indicates that *P. anomala* and *D. hansenii* has different effect on FAA of dry-aged beef by different aging period.

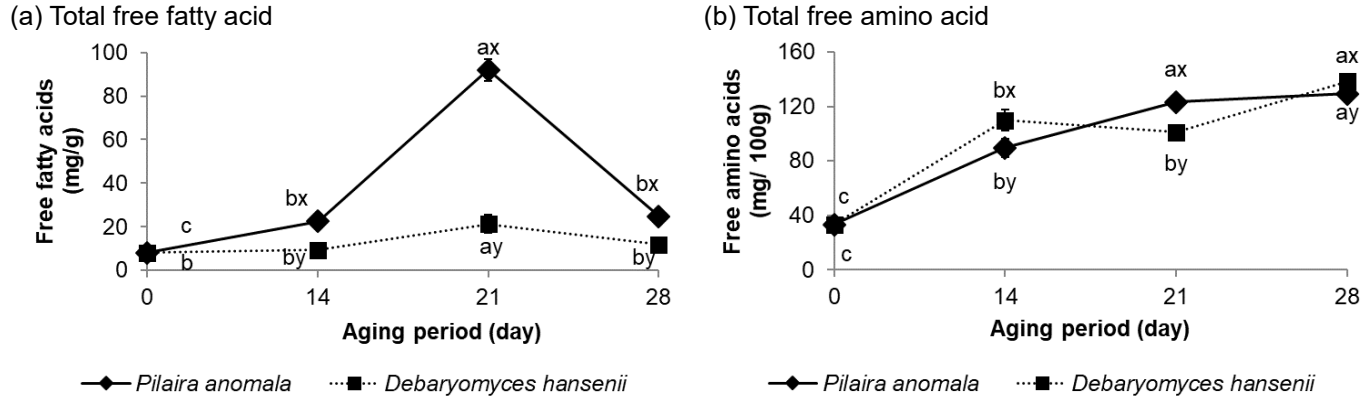

Figure 2. Total contents of free fatty acid (a) and free amino acid (b) of dry-aged beef inoculated with *Pilaira anomala* and *Debaryomyces hansenii* during 28 days of aging period. $^{a-c}$Different letters in the same microorganism indicate significantly different (P<0.05). ^{x,y}Different letters in the same aging period indicate significantly different (P<0.05).

IV. CONCLUSION

The lipolytic and proteolytic activities of *P. anomala* and *D. hansenii* and their role in the increase of FFA and FAA were proven by this study. However, the application of these microorganisms for dry aging process should be carefully investigated as they had significant but different roles by different aging period.

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