

Effect of wet ageing on the composition of non-esterified fatty acids in Japanese Wagyu beef

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Abstract: The non-esterified fatty acids (NEFA) derived from bovine fat are responsible for flavour formation in aged beef after slaughter. In this study, we investigated the degree of lipid oxidation, composition of NEFA and phospholipids, and lipase activities in loins (*M. Longissimus thoracis*, LT) and topsides (*M. semimembranosus*, SM) of the Japanese Wagyu beef aged at 2°C for up to 50 days. Although no significant differences were observed in the total NEFA and thiobarbituric acid reactive substance (TBARS) levels of both the muscles during ageing, total phospholipid content showed a significant reduction in LT. The NEFA composition exhibited increased ratios of polyunsaturated fatty acids (PUFA) and saturated fatty acids (SFA) in LT and SM, respectively, with ageing. Phospholipase activity was higher than neutral lipase activity in LT. Contrarily, in SM, neutral lipase activity was higher than phospholipase activity. This study provides an insight into the differences in the meat flavours, derived from NEFA, of LT and SM after ageing.

Introduction: It is widely known that Japanese Wagyu beef has a high level of marbling owing to the accumulation of intramuscular fat (IMF). The degree of marbling is associated with the formation of a beefy flavour derived from the fatty acids present in IMF. Polyunsaturated fatty acids (PUFA) constituting non-esterified fatty acids (NEFA), which are derived from beef fat, serve as the precursors of volatile aldehydes, such as hexanal, as free unsaturated fatty acids are more susceptible to oxidation and degradation upon heating. Post-mortem ageing is one of the most important steps in the formation of a beefy flavour from NEFA. However, the effect of ageing on NEFA generation from the IMF of Japanese Wagyu beef and the difference in the composition of NEFA between loins and topsides are not fully understood. Therefore, we investigated the generation of NEFA by endogenous lipases and the oxidation of NEFA in two beef muscles [*M. Longissimus thoracis* (LT) and *M. semimembranosus* (SM)] after ageing.

Materials and Methods: Meat samples (LT and SM), obtained from Japanese Wagyu cattle raised in Ibaraki Prefecture (Japan), were wet aged under vacuum packaging conditions at 2°C for 10-50 days after slaughter. The samples were then subjected to various quality tests. Lipid oxidation in the samples was evaluated using the 2-thiobarbituric acid test. Total NEFA content in the sample fat isolated via thin-layer chromatography (TLC) was determined using a NEFA assay kit (FUJIFILM Wako Pure Chemical, Osaka, Japan). NEFA composition was determined via gas chromatography (GC) after esterification. Total phospholipids in the fat were measured with a UV-Vis spectrophotometer as the phosphomolybdic acid complex. The compositions of phosphatidylcholine and phosphatidylethanolamine were determined via GC after the isolation of phospholipids from fat using TLC. The activities of acid and neutral lipases and phospholipase were determined using 4-methylumbelliferyl oleate.

Results: The total NEFA levels in the LT and SM groups did not increase during ageing. The ageing process did not influence thiobarbituric acid reactive substance (TBARS) levels in both the muscles. The total phospholipid content in LT decreased significantly during ageing. The NEFA composition showed an increased ratio of PUFA, such as C18:2 and C20:2n6, in LT and an increased ratio of SFA in the SM during ageing. The LT muscle had high enzymatic activity, in the order of acid lipase, phospholipase, and neutral lipase. Lipase activity in the LT did not change with ageing. SM exhibited the highest enzymatic activity, in the order of acid lipase, neutral lipase, and phospholipase. Furthermore, the acid lipase and phospholipase activities of SM decreased with an increase in ageing for up to 50 days. Phospholipase activity was higher than neutral lipase activity in LT. In SM, neutral lipase activity was higher than phospholipase activity.

Discussion: In this study, the effects of ageing on lipid oxidation, the composition of NEFA and phospholipids, and various lipase activities were investigated in the LT and SM of Japanese Wagyu beef. The TBARS and total NEFA levels in beef were not affected by wet ageing, which suggested that cold storage with vacuum packaging inhibited lipid oxidation and NEFA production. However, the ratio of PUFA to NEFA increased as phospholipid content decreased in LT. Phospholipase activity was higher than the activities of acid and neutral lipases in LT. On the other hand, the ratio of SFA to NEFA increased upon the hydrolysis of triglycerides in SM. Furthermore, in SM, acid and neutral lipase activities were higher than phospholipase activity. These results indicate that the NEFA composition can be altered by degree of activities of various endogenous lipases in each muscle type. This study provides a new insight into the mechanism of NEFA formation in beef during long-term ageing.

Key words: Japanese Wagyu beef, Lipase activity, Non-esterified fatty acids, Wet-ageing