A simple method for measurement of carnosine, anserine, balenine, histidine, N^{π}-methylhistidine and N^{τ}-methylhistidine in meats by using HPLC

Ayumi Katahuchi [1], Saki Shimamoto [2], Shogo Matsunaga [3,4], Akira Ohtsuka [1,4], Daichi Ijiri [1,4]

[1] Graduate School of Agriculture, Forestry and Fisheries, Kagoshima University, [2] Graduate School of Science and Technology, Niigata University, [3] Nagasaki Agricultural and Forestry Technical Development Center, Nagasaki, [4] The United Graduate School of Agricultural Science, Kagoshima University, Japan

Objective: The imidazole dipeptides (IDPs) such as carnosine (β-alanyl-L-histidine), anserine (β-alanyl-N^{π}- methyl-L-histidine), and balenine (β-alanyl-N^{τ}-methyl-L-histidine) are abundant in skeletal muscle of the vertebrates. These IDPs are well known to have bioregulatory functions such as metal chelate, antioxidant, antiglycation, and antifatigue. To date, either high-performance liquid chromatography (HPLC) or liquid chromatography mass spectrometry (LC-MS) has been used to determine IDPs. However, si- multaneous analytical methods for IDPs and their components are limited. The aim of this study was to establish a simple method for determination of carnosine (Car), anserine (Ans), balenine (Bal), and their components following histidine (His), N^{π}- methylhistidine (N^{π}-MeHis), and N^{τ}-methylhistidine (N^{τ}-MeHis) by using HPLC methods in meats. Materials and Method We vali- dated a HPLC method for measurement of Car, Ans, Bal, His, N^{π}-MeHis and N^{τ}-MeHis by confirming the peak specificity, the lin- earity of calibration curve, the intra- and inter-day repeatability, and the recovery percentage. HPLC analysis was performed by the HPLC system (LC-2000 Plus HPLC System; JASCO Co. Ltd., Tokyo, Japan) with a YMC-Triart C18 column (4.6 × 150 mm, 5 μm; YMC CO., LTD., Kyoto, Japan). The mobile phase was 15 mmol/L sodium 1-octanesulfonate in 20 mmol/L KH2PO4. The column was attached to an oven at 50 °C. A fluorometric detector set at an excitation wavelength of 365 nm and an emission wave- length of 460 nm was used to monitor the fluorescence derived from the reaction with ortho-phthalaldehyde. In this study, we at- tempted to determine concentrations of Car, Ans, Bal, His, N^{π}-MeHis and N^{τ}-MeHis in meat, i.e., several edible parts (i.e., breast, tenderloin, thigh, drumette, and wingette) of commercial broiler chickens, breast of Kuro-Satsuma (a cross between a Japanese memorial native chicken, Satsuma dori, and Barred Plymouth Rock), and commercial pork fillet. These meats were purchased from supermarkets. One gram of meat sample was weighed and homogenized with 10 mL of 100 mmol/L HCl. Then, 10 mL of hexane was added and mixed by vortexing, and centrifuged at 5,000 × g for 10 min, and the lower water layer was collected. Fifty microli- ters of the lower layer were mixed with 450 μL of water and 500 μL of acetonitrile by vortexing, and then centrifuged at 20,000 × g for 5 min. After centrifugation, the supernatant was filtered using a 0.2 μm pore size filter. Then, IDPs and Histidine derivatives de- termined by the validated HPLC method.

Results and Discussion: We confirmed the detection ranges of Bal, His, N^{π}-MeHis and N^{τ}-MeHis were 1.56-25.00 μmol/L and those of Car and Ans were 1.56~1600.00 μmol/L, respectively. The accuracies were acceptable since the calculated intra- and inter-day repeatability of them in pectoral muscle of chickens fitted within the range of our criteria (RSD≦7.3). Furthermore, the recovery percentage ranges of the four different concentrations of Car, Ans, Bal, His, N^{π}-MeHis and N^{τ}-MeHis were within the range of our criteria (80-110%), respectively, and thus the results of recovery tests in the HPLC method were acceptable. The concentrations of Car and Ans in Kuro-Satsuma were higher than those in broiler chickens and pork fillet, respectively. On the other hand, the concentrations of Bal in the pork fillet were higher than breast meat of chickens. Furthermore, we analyzed concentrations of IDPs in the edible part of broiler chickens. The breast, tenderloin, thigh, drumette, and wingette contained higher amount of Ans compared with the other IDPs. The amount of Car and Bal were higher in the breast than the other edible parts. Furthermore, histidine derivatives were able to be detected and determined in all meat samples examined in this study.

Conclusions: This HPLC methods are reliable to measure the concentrations of Car, Ans, Bal, His, N^{π}-MeHis and N^{τ}-MeHis in chicken and pork meat. These results contribute to not only determination of IDPs and their components in meat samples but also utilization for research into imidazole dipeptide metabolism (synthesis and/or degradation) in skeletal muscle of the vertebrates.

Key words: Imidazole dipeptide, Nτ -methylhistidine, HPLC, Method validation