Appendices for

A strategy to link the change of quality traits of Japanese sea bass (*Lateolabrax japonicus*) muscle and proteins in its exudate during cold storage by mass spectrometry

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A. The protein content in muscle exudate at different storage periods

To determine the content of trypsin needed for enzymatic hydrolysis of each exudate protein sample of LJ, the absorbance of the protein sample was measured to determine the concentration of the protein sample. The protein concentration in muscle exudate of LJ was determined by using bovine serum albumin (BSA) as reference. The calibration equation of BSA is $y = 0.4758x - 0.005718$ under the utilized conditions and UV/vis spectroscopic setup, where y and x represent the absorbance of the protein sample and the concentration of the protein sample, respectively. The measured absorbance and calculated corresponding protein concentration of the protein exudate during studied cold storage period are detailed in Table A.1.

Table A.1. The absorbance of LJ exudate, the corresponding protein concentration after 100-fold of dilution, and the protein content in LJ exudate samples at different cold storage periods.

storage periods at 4 °C (d)	absorbance after 100-fold of dilution	protein concentration after 100-fold of dilution (mg/mL)	protein content in exudate (mg/mL)
0	0.549±0.002	1.165±0.004	116.5±0.2
1	0.573±0.002	1.217±0.003	57.3±0.2
4	1.143±0.013	2.415±0.027	114.3±1.3
7	1.180±0.008	2.492±0.016	118±0.8
12	1.106±0.007	2.336±0.015	110.6±0.7

B. Mass peaks with S/N≥3 in the MALDI-TOF mass spectra of muscle exudates at different storage periods

Table B.1. Mass peaks for the enzymatic hydrolysate of muscle tissue exudates of Japanese sea bass (*Lateolabrax japonicus*, LJ) during different storage periods (0 d, 1 d, 4 d, 7 d, 12 d) at 4 °C, with signal-to-noise ratio (SNR)≥3 using sinapinic acid (SA) as matrix. The ions highlighted in green color correspond to the peptides of LJ muscle exudate identified by LC-MS/MS.

storage periods at 4 °C (d)	mass peaks with S/N≥3 in the mass spectra with
0	436.680, 459.446, 1055.326, 1071.680, 1084.950, 1095.311, 1116.347, 1129.191, 1142.061, 1195.789, 1218.420, 1240.708, 1263.353, 1442.509, 1465.703, 1489.330, 1601.789, 1647.000, 1667.028, 1739.007, 1819.569, 2071.323, 2140.004, 2153.674, 2163.115, 2181.439, 2203.062, 2221.171, 2264.132, 2280.438, 2303.289, 2339.858, 2362.574, 2388.691, 2406.849, 2423.850, 2457.574, 2480.690, 2488.577, 2526.371, 2543.229, 2564.319, 2574.291, 2589.202, 2596.145, 2614.555, 2636.374, 2646.781, 2670.404, 2685.716, 2695.537, 2734.876, 2793.209, 2804.509, 2820.300, 2840.196, 2852.562, 2877.650, 2960.508, 3019.088, 3065.453, 3084.113, 3129.197, 3147.108, 3205.407, 3307.465, 3411.221, 3488.352, 3502.115, 3573.137, 3613.059, 3692.519, 3708.963, 3742.924, 3756.923, 3788.367, 3826.448, 3899.237, 3913.615, 4056.287, 4269.998, 4458.313, 4569.332, 4585.997, 4607.044, 4619.871, 4683.424, 4725.863, 5109.628, 5125.578, 5569.005, 5729.718, 11460.305, 11534.407, 11564.914, 11607.664, 11622.311, 11664.986, 11754.055
1	423.521, 441.427, 463.645, 479.755, 1048.175, 1059.867, 1075.561, 1090.442, 1135.219, 1224.325, 1405.542, 1609.984, 1654.965, 1673.932, 2080.373, 2149.206, 2162.827, 2190.173, 2212.551, 2273.293, 2289.203, 2433.182, 2482.769, 2532.237, 2546.495, 2571.711, 2584.379, 2624.325, 2661.593, 2713.510, 2777.307, 2818.287, 2850.647, 2860.121, 2907.052, 2920.396, 2978.041, 3094.651, 3162.627, 3357.015, 3406.288, 3571.614, 3755.183, 3769.268, 3885.399, 3909.444, 3926.569, 4386.138, 4618.412, 4632.412, 4901.613, 5136.040, 5169.737, 5289.195, 5509.337, 5743.861, 5794.212, 5847.377, 5888.671, 5900.667, 11485.701, 11594.950, 11697.868, 11801.311, 11873.490, 11889.749, 11905.017, 12109.936, 12232.800, 12388.913
4	439.575, 462.346, 1057.656, 1072.990, 1084.353, 1100.631, 1126.939, 1132.298, 1139.179, 1157.088, 1181.563, 1199.754, 1224.194,, 1237.295, 1244.505, 1267.174, 1274.136, 1297.198, 1330.856, 1379.541, 1422.897, 1446.242, 1468.707, 1481.250, 1486.769, 1492.075, 1496.507, 1504.806, 1521.768, 1537.089, 1545.524, 1555.928, 1594.795, 1610.489, 1645.040, 1652.358, 1658.995, 1669.248, 1692.104, 1708.906, 1714.033, 1738.188, 1758.179, 1771.962, 1784.052, 1790.931, 1840.004, 1852.780, 1869.620, 1886.820, 1893.511, 1901.597, 1935.049, 1988.980, 2007.669, 2075.412,

	2102.008, 2117.961, 2136.820, 2149.833, 2158.089, 2167.020, 2190.180, 2207.412, 2249.442, 2280.290, 2320.080, 2402.898, 2468.426, 2496.417, 5336.677, 5436.820, 5466.938, 5537.602, 5566.408, 5597.478, 5636.506, 5665.949, 5699.214, 5735.290, 5765.321, 5783.335, 5800.732, 5839.122, 11472.296, 11518.264, 11575.382, 11680.829
7	423.904, 441.685, 463.866, 480.117, 649.800, 1060.338, 1075.084, 1094.608, 1117.823, 1135.371, 1152.534, 1240.774, 1484.645, 1525.239, 1560.114, 1673.108, 1791.677, 1856.831, 1905.874, 1996.026, 2027.541, 2054.056, 2078.876, 2122.438, 2153.042, 2162.302, 2211.814, 2236.965, 2254.210, 2276.202, 2334.446, 2448.338, 2502.887, 2637.859, 2651.966, 2670.240, 2745.527, 2818.867, 2954.129, 3096.111, 3164.778, 3258.562, 3272.895, 3755.298, 5089.345, 5218.911, 5319.876, 5347.407, 5450.622, 5475.235, 5532.463, 5553.316, 5580.683, 5609.528, 5681.934, 5710.300, 5746.035, 5802.554, 5855.516, 8779.087, 8790.750, 8828.148, 11486.532, 11711.279, 11918.313, 12093.935, 12135.964, 12152.687, 12233.282, 12289.569, 12315.256, 12354.698, 12416.695, 12441.465
12	1223.681, 423.535, 441.399, 463.630, 1074.046, 1087.574, 1094.925, 1117.809, 1135.757, 1159.225, 1223.681, 1241.605, 1448.973, 1472.580, 1486.327, 1490.574, 1525.760, 1560.785, 1628.650, 1663.283, 1674.094, 1686.097, 1696.233, 1706.142, 1714.433, 1793.801, 1906.777, 1941.109, 1994.074, 2028.630, 2054.253, 2077.660, 2123.254, 2143.758, 2155.909, 2211.432, 2237.784, 2272.457, 2335.930, 2410.501, 2449.530, 2502.607, 2651.265, 2671.149, 2746.409, 2771.064, 2819.225, 3164.616, 5221.267, 5285.924, 5318.929, 5349.404, 5421.668, 5449.582, 5469.531, 5521.754, 5550.057, 5576.500, 5612.825, 5650.721, 5678.173, 5751.471, 5779.375, 5835.939, 5851.555, 5880.935, 5908.408, 5951.680, 5980.828, 6016.039, 8920.131, 8931.817, 8944.763, 11490.059, 11592.022, 11703.097, 11783.817, 11884.501, 11907.048, 12180.994, 12295.602, 12362.559

C. Score plots of PCA and PLS-DA respectively for the mass spectra of the enzymatic hydrolysates of exudates of Japanese sea bass (*Lateolabrax japonicus*, LJ) muscle tissue at different storage periods

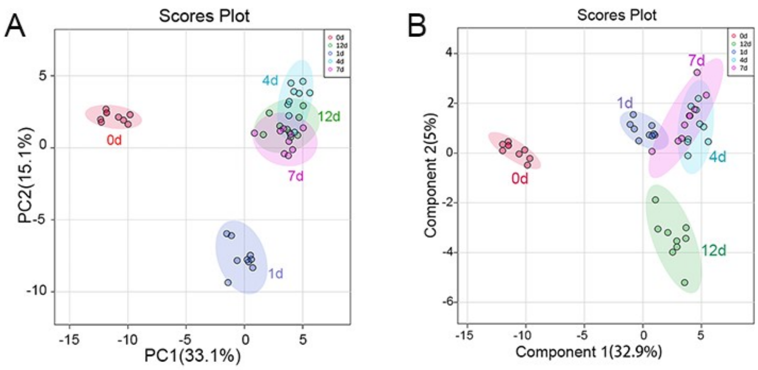

Fig. C.1. A) Principal component analysis (PCA) scores plot for the mass spectra of the enzymatic hydrolysates of muscle tissue exudates of Japanese sea bass (*Lateolabrax japonicus*, LJ) at different storage periods (0 d, 1 d, 4 d, 7 d, and 12 d) at 4 °C obtained in positive MS mode; B) Plot of partial least squares regression-discriminant analysis (PLS-DA) for mass spectra datasets of Japanese sea bass (*Lateolabrax japonicus*, LJ) with different storage periods (0 d, 1 d, 4 d, 7 d, and 12 d) at 4 °C obtained in positive MS mode.

Table C.1. List of ions with a VIP score greater than 1 shown in Fig. 2. The ions highlighted in green color correspond to the peptides of LJ muscle exudate identified by HPLC-MS/MS.

peptide mass	**VIP score**
3744.617	2.3316
441.0149	1.9785
3084.926	1.9784
1525.479	1.9532
423.29	1.9364
1315.334	1.8993
1271.698	1.8507
479.5391	1.8365
2200.97	1.8212
5214.492	1.7662
3789.639	1.7655
689.0226	1.7201
649.0126	1.6783
1299.274	1.6701
2630.13	1.6627

463.5315	1.6545
2121.651	1.6448
2095.572	1.6339
3465.159	1.624
705.9853	1.6238
1104.006	1.6229
1955.274	1.621
2262.641	1.6132
5372.313	1.5794
4730.231	1.5716
408.5831	1.5709
1791.831	1.5642
2839.818	1.542
1184.15	1.5394
1647.235	1.4807
2573.428	1.4613
3403.11	1.448
2695.873	1.4447
2423.316	1.4388
1256.627	1.4233
5469.33	1.4132
11698.72	1.4013
1134.603	1.3815
3828.376	1.3692
2154.183	1.3621
1224.986	1.3354

D. The link of the quality traits of Japanese sea bass (*Lateolabrax japonicus*, LJ) fillet and the relative intensity of identified proteins during cold storage

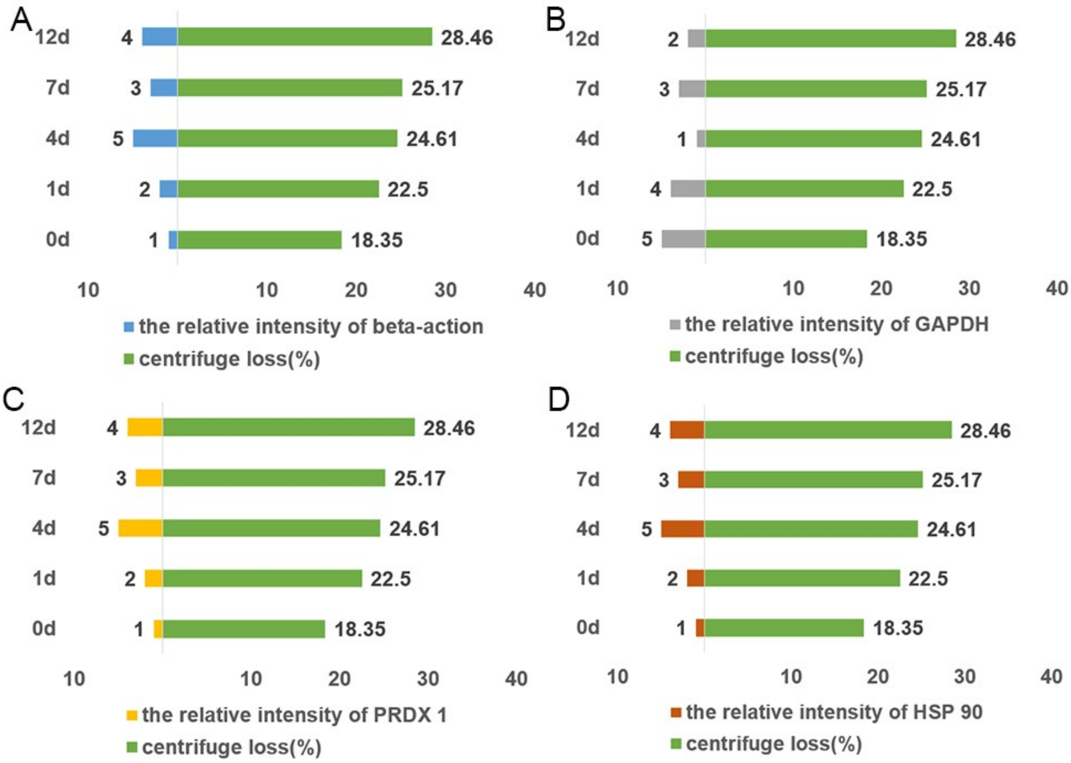

Fig. D.1. A) Pyramid chart of the relationship between the centrifugal loss of Japanese sea bass (*Lateolabrax japonicus*, LJ) fillet and the relative intensity of beta-action from the exudate of Japanese sea bass (*Lateolabrax japonicus*, LJ); B) Plot of the relationship between the centrifugal loss of Japanese sea bass (*Lateolabrax japonicus*, LJ) fillet and the relative intensity of glyceraldehyde-3-phosphate dehydrogenase (GAPDH) from the exudate of Japanese sea bass (*Lateolabrax japonicus*, LJ); C) Pyramid chart of the relationship between the centrifugal loss of Japanese sea bass (*Lateolabrax japonicus*, LJ) fillet and the relative intensity of peroxiredoxin 1 (PRDX1) from the exudate of Japanese sea bass (*Lateolabrax japonicus*, LJ); D) Pyramid chart of the relationship between the centrifugal loss of Japanese sea bass (*Lateolabrax japonicus*, LJ) fillet and the relative intensity of heat shock protein 90 (HSP90) from the exudate of Japanese sea bass (*Lateolabrax japonicus*, LJ). It can be seen that glyceraldehyde-3-phosphate dehydrogenase (GAPDH) may correlated to the centrifugal loss of Japanese sea bass (*Lateolabrax japonicus*, LJ) fillet during the storage.

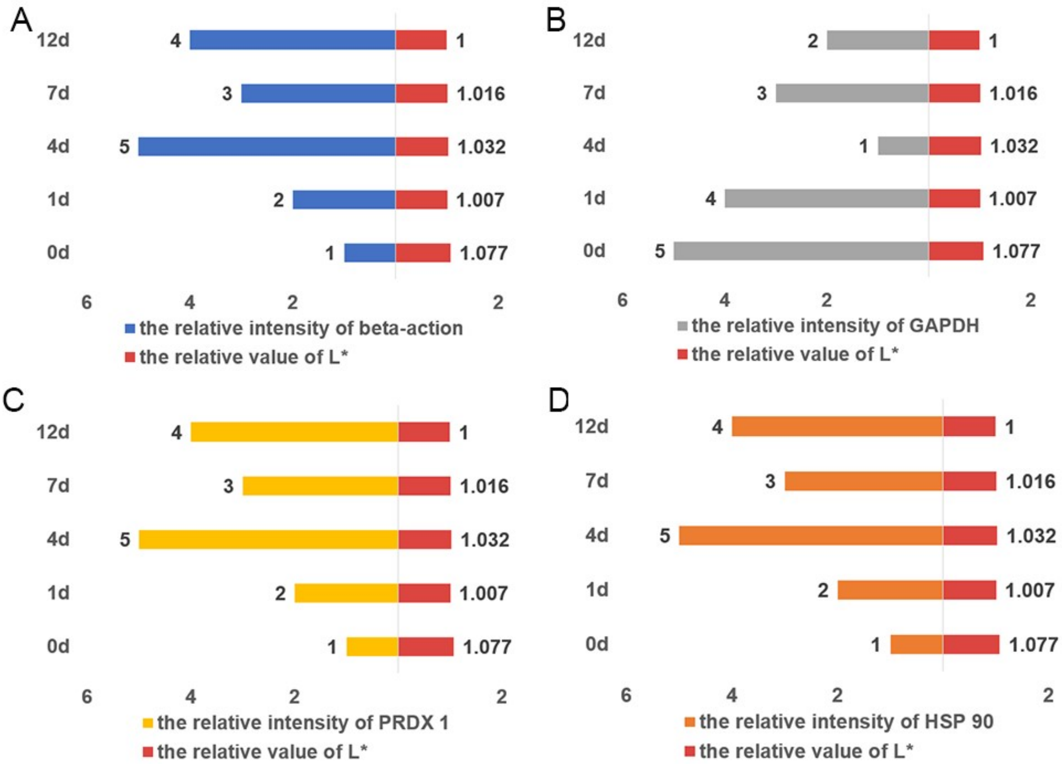

Fig. D.2. A) Pyramid chart of the relationship between the lightness value (*L**) of Japanese sea bass (*Lateolabrax japonicus*, LJ) fillet and the relative intensity of beta-action from the exudate of Japanese sea bass (*Lateolabrax japonicus*, LJ); B) Plot of the relationship between the lightness value (*L**) of Japanese sea bass (*Lateolabrax japonicus*, LJ) fillet and the relative intensity of glyceraldehyde-3-phosphate dehydrogenase (GAPDH) from the exudate of Japanese sea bass (*Lateolabrax japonicus*, LJ); C) Pyramid chart of the relationship between the lightness value (*L**) of Japanese sea bass (*Lateolabrax japonicus*, LJ) fillet and the relative intensity of peroxiredoxin 1 (PRDX1) from the exudate of Japanese sea bass (*Lateolabrax japonicus*, LJ); D) Pyramid chart of the relationship between the lightness value (*L**) of Japanese sea bass (*Lateolabrax japonicus*, LJ) fillet and the relative intensity of heat shock protein 90 (HSP90) from the exudate of Japanese sea bass (*Lateolabrax japonicus*, LJ).

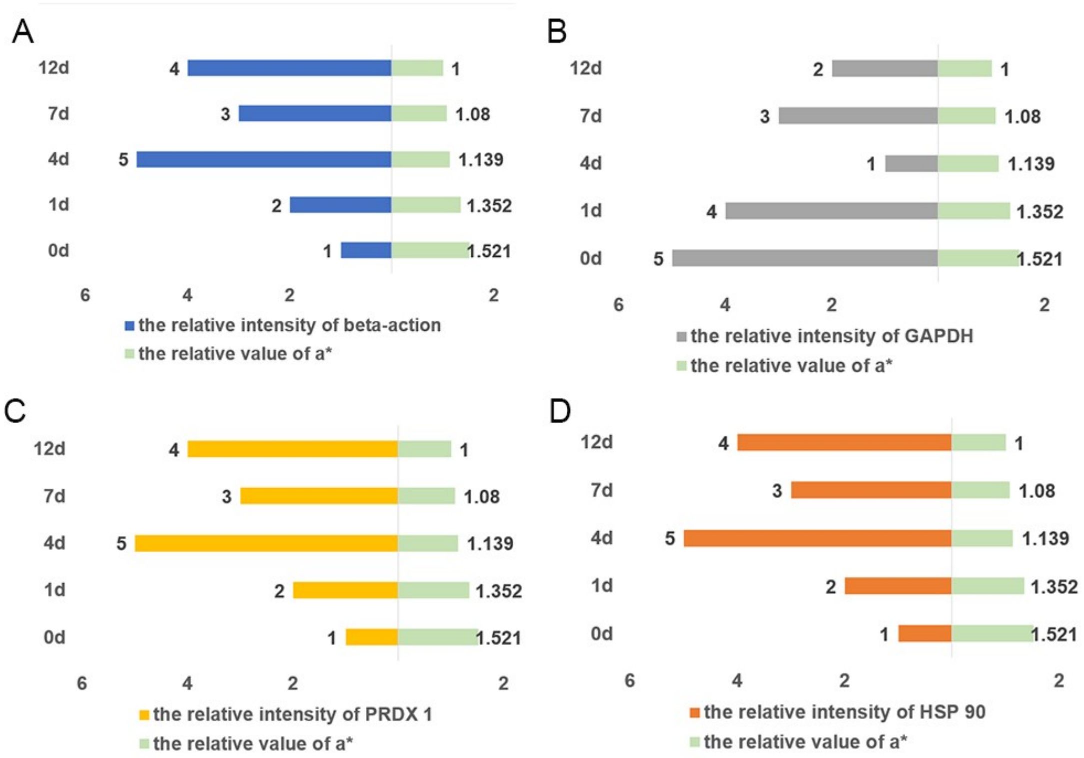

Fig. D.3. A) Pyramid chart of the relationship between the redness value (*a**) of Japanese sea bass (*Lateolabrax japonicus*, LJ) fillet and the relative intensity of beta-action from the exudate of Japanese sea bass (*Lateolabrax japonicus*, LJ); B) Plot of the relationship between the redness value (*a**) of Japanese sea bass (*Lateolabrax japonicus*, LJ) fillet and the relative intensity of glyceraldehyde-3-phosphate dehydrogenase (GAPHD) from the exudate of Japanese sea bass (*Lateolabrax japonicus*, LJ); C) Pyramid chart of the relationship between the redness value (*a**) of Japanese sea bass (*Lateolabrax japonicus*, LJ) fillet and the relative intensity of peroxiredoxin 1 (PRDX1) from the exudate of Japanese sea bass (*Lateolabrax japonicus*, LJ); D) Pyramid chart of the relationship between the redness value (*a**) of Japanese sea bass (*Lateolabrax japonicus*, LJ) fillet and the relative intensity of heat shock protein 90 (HSP90) from the exudate of Japanese sea bass (*Lateolabrax japonicus*, LJ). It can be seen that beta-action, peroxiredoxin 1 (PRDX1), and heat shock protein 90 (HSP90) may correlate with the red color change of Japanese sea bass (*Lateolabrax japonicus*, LJ) fillet during storage.

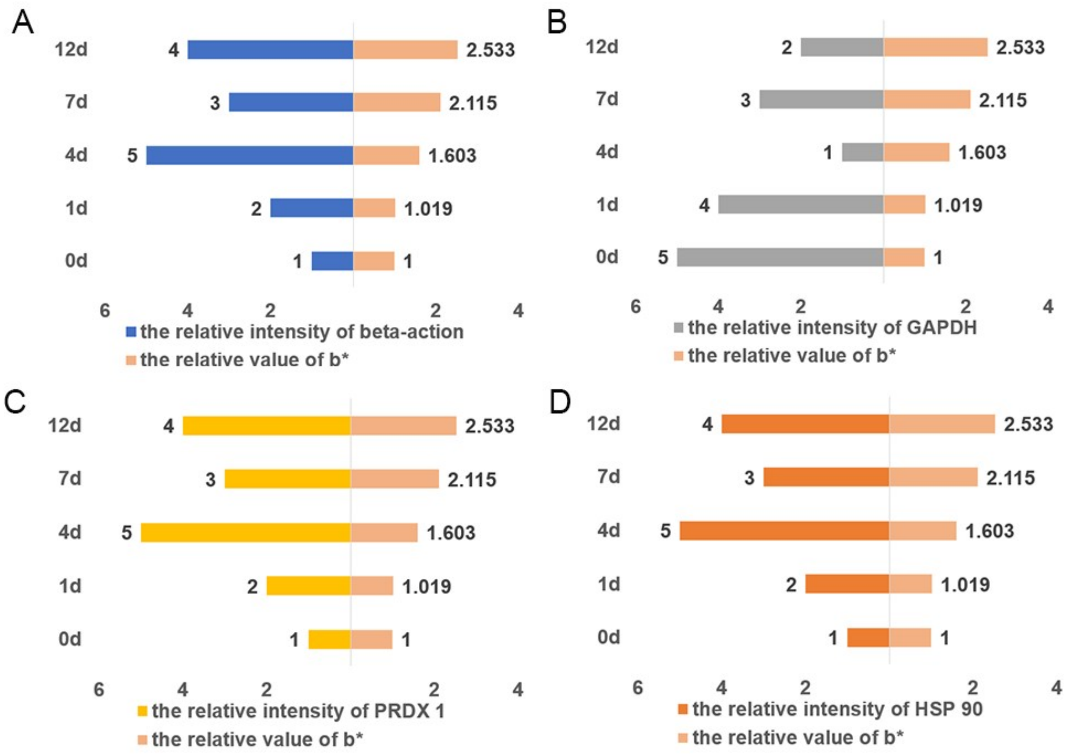

Fig. D.4. A) Pyramid chart of the relationship between the yellow value (*b**) of Japanese sea bass (*Lateolabrax japonicus*, LJ) fillet and the relative intensity of beta-action from the exudate of Japanese sea bass (*Lateolabrax japonicus*, LJ); B) Plot of the relationship between the yellowness value (*b**) of Japanese sea bass (*Lateolabrax japonicus*, LJ) fillet and the relative intensity of glyceraldehyde-3-phosphate dehydrogenase (GAPDH) from the exudate of Japanese sea bass (*Lateolabrax japonicus*, LJ); C) Pyramid chart of the relationship between the yellow value (*b**) of Japanese sea bass (*Lateolabrax japonicus*, LJ) fillet and the relative intensity of peroxiredoxin 1 from the exudate of Japanese sea bass (*Lateolabrax japonicus*, LJ); D) Pyramid chart of the relationship between the yellow value (*b**) of Japanese sea bass (*Lateolabrax japonicus*, LJ) fillet and the relative intensity of heat shock protein 90 (HSP90) from the exudate of Japanese sea bass (*Lateolabrax japonicus*, LJ). It can be seen that glyceraldehyde-3-phosphate dehydrogenase (GAPDH) may be correlated with the yellow color change of Japanese sea bass (*Lateolabrax japonicus*, LJ) during storage.

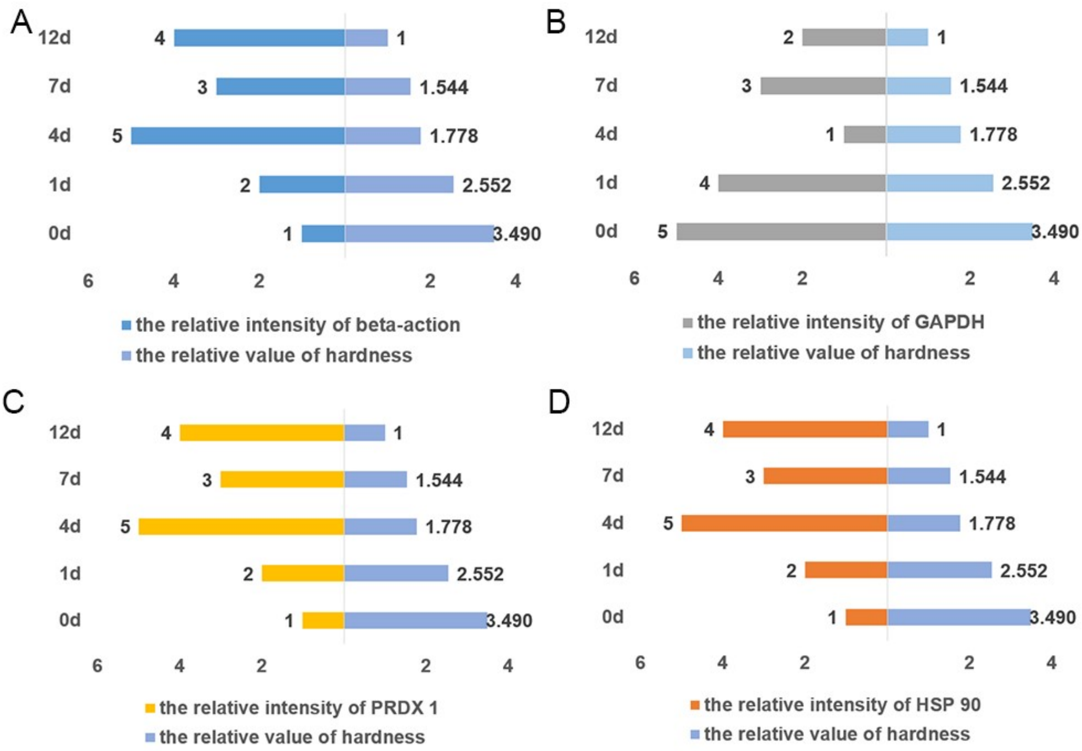

Fig. D.5. A) Pyramid chart of the relationship between the hardness of Japanese sea bass (*Lateolabrax japonicus*, LJ) fillet and the relative intensity of beta-action from the exudate of Japanese sea bass (*Lateolabrax japonicus*, LJ); B) Plot of the relationship between the hardness of Japanese sea bass (*Lateolabrax japonicus*, LJ) fillet and the relative intensity of glyceraldehyde-3-phosphate dehydrogenase (GAPDH) from the exudate of Japanese sea bass (*Lateolabrax japonicus*, LJ); C) Pyramid chart of the relationship between the hardness of Japanese sea bass (*Lateolabrax japonicus*, LJ) fillet and the relative intensity of peroxiredoxin 1 (PRDX1) from the exudate of Japanese sea bass (*Lateolabrax japonicus*, LJ); D) Pyramid chart of the relationship between the hardness of Japanese sea bass (*Lateolabrax japonicus*, LJ) fillet and the relative intensity of heat shock protein 90 (HSP90) from the exudate of Japanese sea bass (*Lateolabrax japonicus*, LJ). It can be found that peroxiredoxin 1 (PRDX1) and heat shock protein 90 (HSP90) may be correlated with the hardness change of Japanese sea bass (*Lateolabrax japonicus*, LJ) fillet during storage.

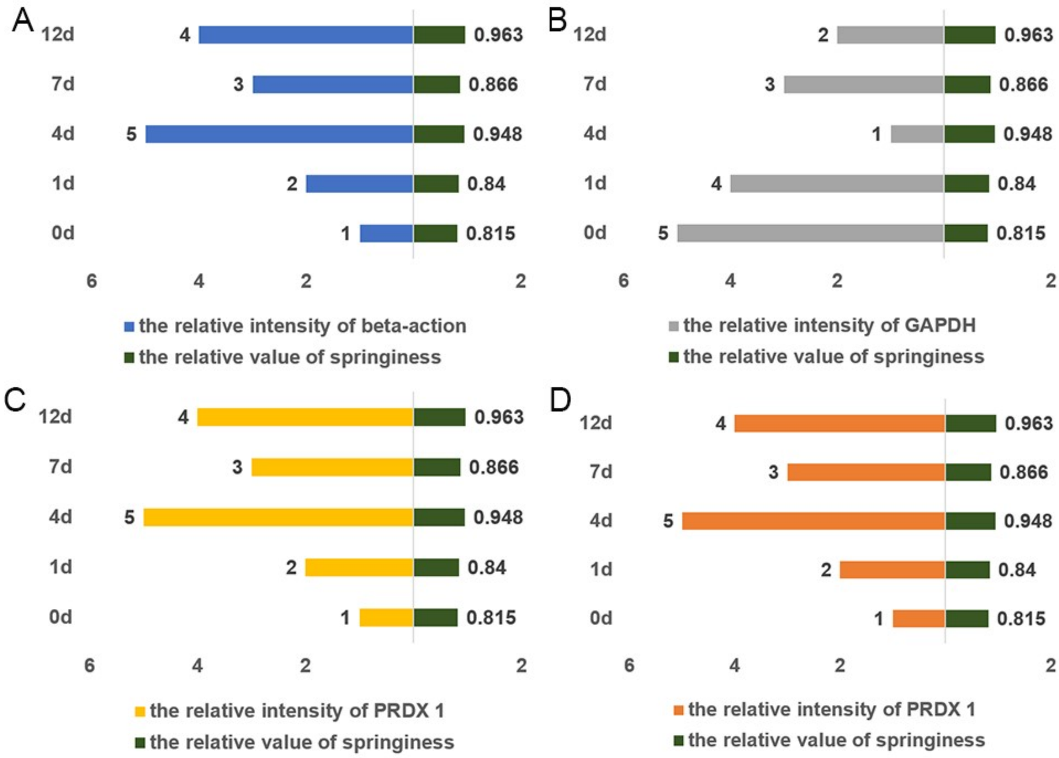

Fig. D.6. A) Pyramid chart of the relationship between the springiness of Japanese sea bass (*Lateolabrax japonicus*, LJ) fillet and the relative intensity of beta-action from the exudate of Japanese sea bass (*Lateolabrax japonicus*, LJ); B) Plot of the relationship between the springiness of Japanese sea bass (*Lateolabrax japonicus*, LJ) fillet and the relative intensity of glyceraldehyde-3-phosphate dehydrogenase (GAPDH) from the exudate of Japanese sea bass (*Lateolabrax japonicus*, LJ); C) Pyramid chart of the relationship between the springiness of Japanese sea bass (*Lateolabrax japonicus*, LJ) fillet and the relative intensity of peroxiredoxin 1 (PRDX1) from the exudate of Japanese sea bass (*Lateolabrax japonicus*, LJ); D) Pyramid chart of the relationship between the springiness of Japanese sea bass (*Lateolabrax japonicus*, LJ) fillet and the relative intensity of heat shock protein 90 (HSP90) from the exudate of Japanese sea bass (*Lateolabrax japonicus*, LJ). It can be found that glyceraldehyde-3-phosphate dehydrogenase (GAPDH) may be correlated with the springiness of Japanese sea bass (*Lateolabrax japonicus*, LJ) fillet during storage.

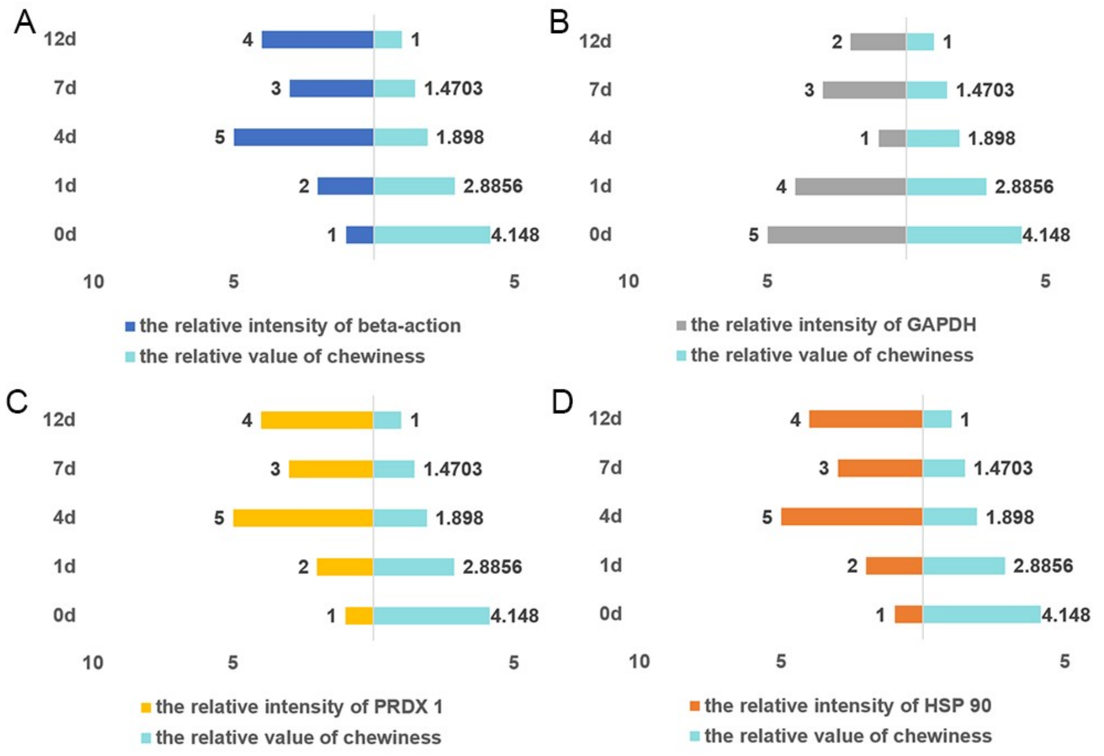

Fig. D.7. A) Pyramid chart of the relationship between the chewiness of Japanese sea bass (*Lateolabrax japonicus*, LJ) fillet and the relative intensity of beta-action from the exudate of Japanese sea bass (*Lateolabrax japonicus*, LJ); B) Plot of the relationship between the chewiness of Japanese sea bass (*Lateolabrax japonicus*, LJ) fillet and the relative intensity of glyceraldehyde-3-phosphate dehydrogenase (GAPDH) from the exudate of Japanese sea bass (*Lateolabrax japonicus*, LJ); C) Pyramid chart of the relationship between the chewiness of Japanese sea bass (*Lateolabrax japonicus*, LJ) fillet and the relative intensity of peroxiredoxin 1 (PRDX1) from the exudate of Japanese sea bass (*Lateolabrax japonicus*, LJ); D) Pyramid chart of the relationship between the chewiness of Japanese sea bass (*Lateolabrax japonicus*, LJ) fillet and the relative intensity of heat shock protein 90 (HSP90) from the exudate of Japanese sea bass (*Lateolabrax japonicus*, LJ). It can be found that peroxiredoxin 1 (PRDX1) is correlated with the chewiness change of Japanese sea bass (*Lateolabrax japonicus*, LJ) fillet during storage. It can be found that heat shock protein 90 (HSP90) may be correlated with the chewiness of Japanese sea bass (*Lateolabrax japonicus*, LJ) fillet during storage.