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2-DE and MALDI-TOF MS-based proteomic analysis of dry- and wet-aged beef (#88)

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Introduction

As meat and meat products are originated from animal muscle, it is important to understand its biological changes in relation to meat quality traits [1]. As one of scientific approaches, muscle proteins in meat and meat products have been analyzed for different purposes using 2-dimensional electrophoresis (2-DE) and/or matrix assisted laser desorption ionization-time of flight mass spectrometer (MALDI-TOP MS) based proteomic analyses.

Meanwhile, aging, which indicates meat storage for a certain time, can improve meat palatability on the basis of biological changes by muscle proteolytic enzymes [2]. It is generally classified as dry and wet aging. Dry aging exposes raw meat without vacuum packaging under the controlled condition (temperature, relative humidity, and air flow velocity), whereas wet aging stores meat vacuum-packaged. Due to the different conditions in both aging methods, there could be different biological changes between dry and wet aging. In addition, Lee et al. (2019) [2] suggested microorganisms on the crust (dried surface) of dry-aged beef could affect the changes in protein with their proteolytic enzymes; however, the scientific reports for biological difference within dry and wet aging are still scarce. Therefore, the objective of this study was to investigate the muscle proteins of dry- and wet-aged beef using 2-DE and MALTI-TOF MS-based proteomic analyses and compare their differences.

Methods

Raw material and aging process

A total of six strip loins (*longissimus lumborum*) were obtained from the both sides of three different carcasses (Holstein steer, quality grade 3) [3]. The samples were randomly assigned to two different aging methods (dry and wet aging, n=3 for each aging group). Prior to aging process, wet-aged group was vacuum-packaged and aged at 4°C, whereas dry-aged group was placed at 4°C, 75% relative humidity, and 2.5 m/s air flow velocity for 28 days of aging period. The crust of dry-aged beef was trimmed off and its internal meat was used for the analyses.

Protein identification

The samples from each aging group were pooled and ground for further 2-DE and MALDI-TOF MS proteomic analyses. All procedures from protein extraction to identification were conducted based on the method of Lee et al. [4]. The extracts of muscle proteins from dry- and wet-aged beef were loaded onto immobilized pH gradient strips ranged pH 3-10 in two different gels(n=2 for each aging group). Four different proteins were used as the land marks in this study: myosin light chain 1, glycogen phosphorylase, phosphatidylinositol transfer protein isoform, and 3-hydroxyisobutyrate dehydrogenase. The detected spots in 2-DE gels were identified based on the databases from SWISS-PROT and NCBI.

Results

In 2-DE gels, different muscle proteins from the pH 3-10 range were detected in each aging group (data not shown). Among them, we selected a total of 16 consistent spots in the same locations of two different gels and identified 15 muscle proteins using MALDI-TOP MS analysis (Table 1). As a result, two antioxidative metabolism-related proteins (P00442 and GSTP), one cellular metabolism-related protein (UPK1B), five glycolysis proteins (PGK1, ALDOB, G3P, TPIS, and ENO), one immune system-related protein (C1QB), three nucleotide metabolism-related proteins (HINT1, GDIR1, and P00570), and three protein metabolism-related proteins (A6QP89, FETUA, and Q0P571) were found in this study with two different aging methods.

Then, we compared the differences in muscle protein of dry- and wet-aged beef using the image analysis of detected spots (Fig. 1). In this study, 10 (DS3-370, WD1-300, WD2-510, DU3-157, DU3-174, WD1-361, WD1-514, WD1-350, WD1-244, and DU3-353) out of 16 spots in dry-aged beef showed higher intensities than those in wet-aged counterpart, whereas the other six spots (WU1-578, WU1-528, WU1-536, WU1-368, WU2-518, and WU4-469) were stronger in wet-aged one (Fig. 1). Dry aging occurred more proteins in relation to cellular metabolism, glycolysis, immune system, while wet aging had proteins associated with nucleotide metabolism and protein metabolism (Fig. 2). The differences between dry- and wet-aged beef may be attributed to moisture evaporation, microbial enzymes, and, possibly, oxidation occurred during the dry aging process.

Conclusion

Dry- and wet-aged beef had different muscle proteins with different functions in biological metabolism, possibly due to their different processing conditions. The results in this study suggest different aging methods could be contributable to different meat quality traits as well as their functionality.

Notes

Spot ID	Est'd Z*	Accession Uniprot	Protein Information	Coverage %	pI†	kDa†
DS3-370	1.90	UPK1B	Uroplakin-1b	19	5.5	30.5
WD1-300	1.81	C1QB	Complement C1q sub-component subunit-B	42	9.2	47.9
WD2-510	2.43	C1QB	Complement C1q sub-component subunit-B	24	9.5	26.6
DU3-157	1.09	PGK1	Phosphoglycerate kinase 1	13	7.8	44.9
DU3-174	1.35	ALDOB	Fructose-bisphosphate aldolase B	13	8.6	39.5
WD1-361	2.19	G3P	Glyceraldehyde 3-phosphate dehydrogenase	15	8.2	36.1
WU1-578	1.38	HINT1	Histidine triad nucleotide-binding protein 1	34	5.4	13.9
WU1-528	1.45	GSTP	Glutathione S-transferase P	20	6.8	23.8
WU1-536	1.44	GDIR1	Rho GDP-dissociation inhibitor 1	60	5.1	23.4
WD1-514	2.06	TPIS	Triosephosphate isomerase (fragment)	24	6.0	21.8
WU1-368	2.43	FETUA	Alpha-2-HS-glycoprotein (fragment)	42	5.5	38.4
WD1-350	1.24	ENO	Enolase	22	8.1	47.1
WD1-244	1.56	A6QP89	MYBPC1 Myosin-binding protein C (fragment)	23	5.0	59.8
WU2-518	1.22	P00570	Adenylate kinase isoenzyme 1	32	7.4	21.8
DU3-353	1.69	P00442	Superoxide dismutase, mitochondrial precursor		7.8	24.8
WU4-469	2.1	Q0P571	Myosin regulatory light chain2, skeletal muscle isoform	14	4.8	19.1

Table 1.
MALDI-TOF MS-identified muscle proteins extracted from dry- and wet-aged beef

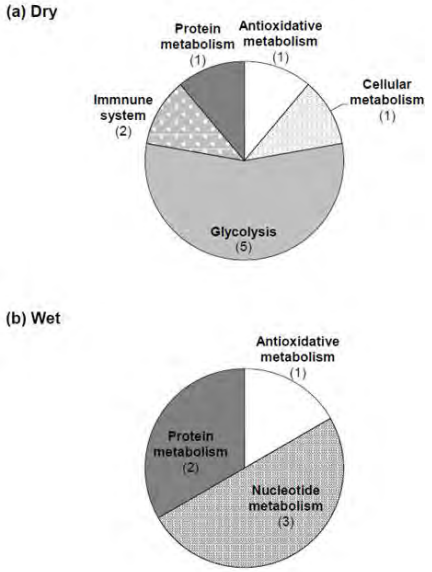

Fig 2.
Functional categorization of MALDI-TOF MS-identified muscle proteins higher in (a) dry- or (b

Notes

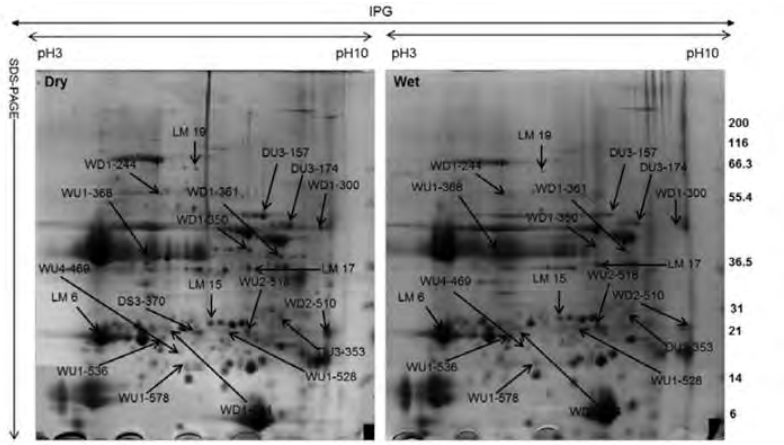

Fig 1.
2DE-page gel pattern of muscle proteins extracted from dry- and wet-aged beef.

Notes