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Proteome changes on water-holding capacity of yak *longissimus lumborum* during postmortem aging

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ABSTRACT

To study differentially expressed proteins on water-holding capacity (WHC) during postmortem aging of *longissimus lumborum* muscle, samples were classified according to drip loss into high and low drip loss groups. Fifty-five proteins were differentially abundant at days 0, 1 and 7 during postmortem aging and identified by MALDI TOF/TOF. The identified proteins can be divided into four main categories: metabolic enzymes, cell structural proteins, stress related proteins and transport proteins. Myosin light chain, heat shock protein 27 and triosephosphate isomerase showed a major difference between the two groups and may have the potential to be biological markers for WHC prediction. Furthermore, bioinformatics analysis revealed that the identified proteins were related to carbon metabolism, glycolysis and biosynthesis of amino acids and pyruvate metabolism. The functions of the identified proteins contribute to a more detailed molecular view of the processes behind WHC and are a valuable resource for future investigations.

1. Introduction

Yak (*Bos grunniens*) are predominantly located in Qinghai, Gansu, Tibet, Sichuan, Xinjiang, and Yunnan provinces of China, accounting for 95% of the entire worldwide yak population (Zhang et al., 2015). The annual yak meat yield of these regions is approximately 300,000 tons. Yak meat is rich in protein and low in fat, and does not contain anabolic steroids. Yak meat products are attractive because of its high quality. It is well known meat qualities change significantly during postmortem aging, and water-holding capacity (WHC) is among these qualities. It is the ability of meat to retain moisture when force is applied (Schafer, Rosenvold, Purslow, Andersen, & Henckel, 2002). Poor WHC is a common problem during postmortem aging and the processing of yak meat. Poor yak meat quality correlates with significant financial losses for the industry by influence on the appearance, palatability and processability (Di Luca, Elia, Hamill, & Mullen, 2013).

Water-holding capacity is often assessed by determining drip loss (Pearce, Rosenvold, Andersen, & Hopkins, 2011). Previous work reported that two types of extracellular space might act as a trigger for drip loss, and it includes the spaces between fibre bundles and the spaces between fibres (Offer & Cousins, 1992). Some researches demonstrated that the time-dependent changes in WHC were related to the cytoskeletal proteins (Kristensen & Purslow, 2001). The degradation of integrin could contribute to the formation of drip channels, and intact desmin helps to transfer the shrinkage of myofibrils to whole cell level and force the water out of the myofibrils (Lawson, 2004; Zhang, Lonergan, Gardner, & Huff-Lonergan, 2006). However, Moeseke and Smet (1999) concluded that reduced drip loss, when meat was sampled at a later time, was likely related to the "leaking out" effect, and aging itself did not improve WHC. Bertram, Schafer, Rosenvold, and Andersen (2004) suggested that the initial event be a cellular swelling caused by the increased intracellular osmolarity postmortem, and then water be expelled from the myofibrillar matrix. Farouk, Mustafa, Wu, and Krsinic (2012) hypothesized that with ageing, the muscle structure breaks and debris begins to fall within the microtubes creating hindrances ("sponge effect") with aging. Changes in meat during postmortem aging are a highly coordinated, genetically programmed and an irreversible phenomenon involving a series of physiological, biochemical, and proteomic changes (Isfort, 2002). Due to the numerous variables influencing WHC, the molecular mechanisms of these variations are yet to be completely understood.

The study of the muscle proteome is of great value for understanding the molecular mechanisms associated with WHC. Bioinformatics analysis constructs complex networks to achieve functions through protein-protein interactions, modifications, and regulation of expression relationships (Zheng et al., 2012). The more functional information acquired from differentially abundant proteins study, the more thoroughly proteomics and bioinformatics analysis can demonstrate. Recently, proteomic technique has been extensively used to study meat quality, such as tenderness (Laville et al., 2009; D'Alessandro et

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al., 2012, color (Suman, Rentfrow, Nair, & Joseph, 2014; Wu et al., 2015) and pre-slaughter stress (Franco et al., 2015). While proteomics has already earned a forefront place in the field of meat science research, the integration of biochemical knowledge about WHC, proteomics and bioinformatics is scant. The main objective of this work is to combine proteomics and bioinformatics to determine the most important proteins and pathways on WHC.

In this study, proteome profile via 2DE gels of yak muscle at specific postmortem time points was created. In addition, gene ontology (GO), Kyoto encyclopedia of genes and genomes (KEGG) pathway, and protein-protein interaction analyses were performed to support several key node proteins at the gene level. Since WHC is an important quality characteristic for yak, our research objectives are screening the differential expression proteins and investigating the molecular mechanisms related to WHC in yak muscles.

2. Materials and methods

2.1. Animals and sample preparation

The *M. longissimus lumborum* (LL, the anterior 12th rib to the last lumbar vertebrae) were randomly extracted from a commercial abattoir (Qingheyuan Hala Food Ltd., Linxia City, Gansu Province, China). Thirty Tianzhu yak bulls (weight 241–280 kg) were of the same age (36–38 months old) and fed on the same diet in the same batch. The animals were kept off feed before slaughtering, but given free access to water. Animals were stunned by captive bolt pistol and their blood was drained. The carcasses were hung by the Achilles tendon and chilled at 4 °C. Samples of LL tissue were taken during postmortem period at three different time points (d 0, 1, & 7). Samples were washed with PBS to remove any blood and contaminants on the surface, and kept frozen in liquid nitrogen, and then stored at −80 °C until the extraction of muscle proteins.

2.2. Measurement of meat qualities

2.2.1. Drip loss

A 50 g sample of meat (5 cm × 5 cm × 1 cm) cut perpendicularly to muscle fibres was taken 0 h during postmortem aging, suspended in a polyamide/polyethylene bag (55.86 cm^3/m^2/24 h oxygen transmission rate, 4.40 g/m^2/24 h moisture transmission rate) at 4 °C. After 48 h, the sample was taken out of the bag, dried on absorbent paper, and reweighed. The percent change in weight over the subsequent 48 h was taken as the drip loss, as described by Honikel (1998).

2.2.2. pH measurement

pH was recorded from 45 min (pH_{45}) up to 168 h during postmortem aging with a portable pH meter (SenvenGo, Mettler-Toledo, Switzerland), which was adjusted for each measurement with standard buffer before being inserted into muscles.

2.2.3. Color measurement

The surface color of LL was measured at 0 d (exposed to air directly for 30 min at 4 °C), 1 d and 7 d by using a Minolta Colorimeter (Model CR-400; 8 mm diameter aperture, Illuminant D65, 0° observer; Konica Minolta, Osaka, Japan). L^*, a^* and b^* values were recorded, which represent lightness, redness and yellowness, respectively.

2.2.4. Warner-Bratzler shear force

Warner-Bratzler shear force (WBSF) was measured on cooked meat (2.54 cm thick) per muscle according to the protocol of Wheeler, Shackelford, and Koohmaraie (1996). A transversal section of the LL muscle for each animal was cooked to a core temperature of 70 °C in a pre-heated water bath, subsequently cooled in running water for 30 min to reach a core temperature below 30 °C. Cores (1.27 cm, parallel to longitudinal orientation of fibres) were then taken from each sample, and peak force was determined using a V-shaped shear blade with a cross-head speed of 400 mm/min.

2.2.5. Protein solubility

Sarcoplasmic protein solubility was determined by homogenizing duplicate 1 g muscle samples in 10 ml of cold 25 mM potassium phosphate buffer (pH 7.2). Homogenates were centrifuged at 8000 ×*g* for 20 min at 4 °C and protein concentration in the supernatants was determined by the Biuret method (Joo, Kauffman, Kim, & Park, 1999). Total protein solubility was similarly determined in 0.1 M potassium phosphate (pH 7.2) buffer with 1.1 M potassium iodide. The calculation of myofibrillar protein solubility was based on the difference between total and sarcoplasmic protein solubility.

2.3. Extraction of muscle proteins

Frozen muscle tissue (50 mg) was ground to a fine powder in liquid nitrogen by a mortar and pestle. Then the fine powder was homogenized in 1 ml of 8 M urea, 2 M thiourea, 1% (*w*/*v*) dithiothreitol (DTT), 2% (*w*/*v*) CHAPS and 2% (*v*/*v*) Bio-lyte at pH 3–10 (Kim et al., 2008). The homogenate was centrifuged at 10,000 ×*g* for 30 min at 4 °C. After centrifugation the supernatant was aliquoted and frozen at −80 °C. The protein quantification was performed according to the method developed by Bradford (Bradford, 1976).

2.4. Two-dimensional gel electrophoresis and image analysis

Samples of approximately 300 μg for analytical gels were applied to 17 cm immobilized pH gradient (IPG) strips (pH 3–10, linear, Bio-Rad). After rehydrating the IPG strips at 50 V for 14 h, isoelectric focusing (IEF) was performed as follows. 1 h at 500 V, 1 h at 1000 V by applying a rapid voltage increase, followed by 6 h at 9000 V by a linear voltage ramping, and then a rapid voltage ramping up to 80 kVh, and finally 500 V for <24 h. Then a final rapid voltage ramping to reach a total of 80 kVh, and then kept at 500 V for <24 h. IEF was performed using a Protean IEF cell (Bio-Rad, Hercules, CA, USA). The current limit was adjusted to 50 mA per strip, and the run was carried out at 20 °C. After IEF, the strips were equilibrated by equilibration buffer I (7 M urea, 50 mM Tris–HCl, pH 8.8, 30% (*v*/*v*) glycerol, 2% (*w*/*v*) SDS and 2% (*w*/*v*) DTT) for 15 min. After removal from the DTT solution, the strips were equilibrated by

Table 1
Changes in quality characteristics in LDrip and HDrip groups of yak LL muscle during postmortem aging at 4 °C (d 0, 1, & 7).

Parameter	Category	Postmortem aging days		
		0 d	1 d	7 d
pH	LDrip	7.03 ± 0.17^{ax}	5.56 ± 0.39^{cx}	6.12 ± 0.11^{bx}
	HDrip	6.65 ± 0.29^{ay}	5.49 ± 0.42^{cy}	5.95 ± 0.08^{by}
L^* value	LDrip	37.82 ± 1.38^{ax}	35.67 ± 1.40^{ax}	37.02 ± 1.68^{ax}
	HDrip	43.95 ± 2.32^{ax}	42.62 ± 2.33^{ax}	43.62 ± 2.33^{ax}
a^* value	LDrip	18.17 ± 0.18^{ax}	17.40 ± 0.25^{ax}	17.01 ± 0.39^{ax}
	HDrip	15.60 ± 1.65^{ax}	14.76 ± 1.56^{ax}	15.51 ± 1.17^{ax}
b^* value	LDrip	9.42 ± 0.32^{ay}	10.68 ± 0.51^{ay}	8.13 ± 0.90^{ay}
	HDrip	10.76 ± 0.16^{cx}	12.29 ± 0.29^{bx}	13.22 ± 0.07^{ax}
WBSF/N	LDrip	66.05 ± 3.56^{ax}	50.57 ± 5.72^{ax}	69.76 ± 4.10^{ax}
	HDrip	74.09 ± 2.08^{abx}	65.66 ± 3.79^{bx}	80.16 ± 2.24^{ax}
Solubility-T	LDrip	0.39 ± 0.01^{ax}	0.41 ± 0.01^{ax}	0.39 ± 0.01^{ax}
	HDrip	0.39 ± 0.01^{ax}	0.40 ± 0.01^{ax}	0.41 ± 0.01^{ax}
Solubility-M	LDrip	0.23 ± 0.01^{ax}	0.24 ± 0.01^{ax}	0.20 ± 0.01^{ax}
	HDrip	0.25 ± 0.02^{ax}	0.23 ± 0.01^{ax}	0.18 ± 0.02^{ax}
Solubility-S	LDrip	0.15 ± 0.01^{bx}	0.19 ± 0.01^{bx}	0.29 ± 0.03^{ax}
	HDrip	0.13 ± 0.01^{bx}	0.15 ± 0.01^{by}	0.19 ± 0.02^{ay}

[a–c] Means without common superscripts in a row within are different ($P < 0.05$).
[x–y] Means without common superscripts in a column within a parameter are different ($P < 0.05$).
[d] Results ($n = 8$) are expressed as the mean ± standard error.
[e–g] Solubility-T, Solubility-M, and Solubility-S mean total protein solubility, myofibrillar protein solubility and sarcoplasmic protein solubility, respectively.