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Proteomic Analysis of Duck Fatty Liver during Post-Mortem Storage Related to the Variability of Fat Loss during Cooking of "Foie Gras"

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ABSTRACT: Fat loss during cooking of duck "foie gras" is the main problem for both manufacturers and consumers. Despite the efforts of the processing industry to control fat loss, the variability of fatty liver cooking yields remains high and uncontrolled. To understand the biochemical effects of postslaughter processing on fat loss during cooking, this study characterizes for the first time the protein expression of fatty liver during chilling using a proteomic approach. For this purpose the proteins were separated according to their solubility: the protein fraction soluble in a buffer of low ionic strength (S) and the protein fraction insoluble in the same buffer (IS). Two-dimensional electrophoresis was used to analyze the S fraction and mass spectrometry for the identification of spots of interest. This analysis revealed 36 (21 identified proteins) and 34 (26 identified proteins) spots of interests in the low-fat-loss and high-fat-loss groups, respectively. The expression of proteins was lower after chilling, which revealed a suppressive effect of chilling on biological processes. The shot-gun strategy was used to analyze the IS fraction, with the identification of all the proteins by mass spectrometry. This allowed identification of 554 and 562 proteins in the low-fat-loss and high-fat-loss groups, respectively. Among these proteins, only the proteins that were up-regulated in the high-fat-loss group were significant (p value = 3.17×10^{-3}) and corresponded to protein from the cytoskeleton and its associated proteins. Taken together, these results suggest that the variability of technological yield observed in processing plants could be explained by different aging states of fatty livers during chilling, most likely associated with different proteolytic patterns.

KEYWORDS: *proteomic, two-dimensional electrophoresis, mass spectrometry, shot-gun, fatty liver, duck, foie gras quality, technological yield*

INTRODUCTION

France is the main producer (73%) of "foie gras" (fatty liver) in the world. French foie gras is a traditional product, a coveted dish with a strong added value. The technological yield is the principal quality trait, and it is evaluated by the loss of fat during cooking. This fat loss constitutes a recurring problem for the industry and consumers because it influences the uniformity and profitability of the finished product. A maximum value of 30% of fat loss during cooking is imposed by market regulations.[1] There is a strong interindividual variability in the processing ability of fatty liver. Under industrial production, standard practice is to process only livers within a fresh weight range of 500−600 g to reduce the variability in fat loss. Indeed, there is a clear relationship between liver weight and technological yield:[2] the higher the liver weight, the less the technological yield. Even under experimental conditions, the coefficient of variation of fat loss is usually around 50%. Despite the maximum environmental control during breeding, overfeeding, and slaughtering procedures, the variability in the technological quality is still high.

Until now, research on the technological yield of foie gras has focused on lipid composition. These studies dealt with lipid storage[3,4] and membrane lipids.[5] They failed to show a clear relationship between the biochemistry of lipids and the fat loss during cooking of duck foie gras. We made a first proteomic study dealing with the comparison of protein expression early post mortem in low-fat-loss and high-fat-loss fatty livers.[6] The results suggested that at the time of slaughter, livers with low fat loss during cooking were still undergoing anabolic processes with regard to energy metabolism and protein synthesis, whereas livers with high fat loss during cooking developed cell protection mechanisms.[6] In the present study we propose to complete this earlier finding by studying the evolution of protein expression during chilling (between 20 min and 6 h post mortem) on the same experimental model and to compare this evolution between two groups of duck foie gras showing

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different rates of fat loss during cooking. We hypothesized the role of proteins in the phenomenon of melting lipid cooking and focused on the involvement of proteins of the metabolism, structure proteins, or membrane proteins. These different types of proteins belong to two fractions with different solubility properties. This is why we chose to work from a differential extraction of proteins according to their solubility. Then, we adapted the method of separation to each fraction. The protein fraction soluble at low ionic strength (S) was analyzed by two-dimensional electrophoresis coupled to mass spectrometry, both MALDI-TOF and LC-MS/MS, to identify proteins of interest. The protein fraction insoluble at low ionic strength (IS) has been studied using the shot-gun method, which consists in the separation of the proteins according to their molecular weight on sodium dodecyl sulfate−polyacrylamide gel electrophoresis (SDS-PAGE) before identification of all the proteins present in the tracks by a nano-LC-MS/MS system coupled to an Orbitrap-XL. The post mortem changes in the expression of proteins related to the technological yield for both fractions was studied to highlight differences contributing to the phenomenon of fat loss during cooking.

MATERIALS AND METHODS

Animals, Breeding, Overfeeding, and Slaughter. Proteomic analysis was performed on a first set of animals, and validation by dot-blot was performed on a second set of animals. All of the animals were bred, overfed, and slaughtered according to the following procedure. Male mule ducks (*Cairina moschata* × *Anas platyrhynchos*) were reared with access to free range until the age of 13 weeks in a poultry house under natural conditions of light and temperature at the Agricultural College of Périgueux (EPLEFPA, 24, France) following standard practices.[5] The animals were then overfed in individual cages for 12 days with a soaked-corn mixture (42% corn grain−58% corn flour) twice a day. The amount of soaked-corn mixture increased until reaching a plateau on the sixth day of overfeeding. Ducks were slaughtered in the experimental slaughterhouse of the Agricultural College of Périgueux 10 h after the last meal. The poultry house and the slaughter house were located in the same place. Birds were crated in transport modules of 4 birds each and transported (5 min) to the slaughter point in groups of 20 (5 crates). The 1st bird was slaughtered immediately after arrival and the 20th about 40 min later (slaughter rate = 1 bird every 2 min). Birds were electrically stunned head-only using scissor tongs (90 V, 50 Hz ac, 5 s) and bled by ventral cutting of neck vessels within 10 s after stunning. After a 5 min bleeding, the carcasses were scalded and plucked.

The experiments described here fully comply with legislation on research involving animal subjects according to the European Communities Council Directive of November 24, 1986 (86/609/EEC). Investigators were certificated by the French governmental authority for carrying out these experiments (agreement 31-11 43 501).

Fatty Liver Processing. At the end of the slaughter process, 20 min after bleeding, the livers were removed from the carcass and weighed. They were chilled on ice for 6 h and carefully trimmed of their main blood vessels. Each fatty liver was then transversely divided into three parts including the two lobes. In the middle part of each lobe, a slice of approximately 200 g was excised and put into a glass can. Salt (12g/kg) and pepper (2g/kg) were added, and the cans were cooked for 1 h in water in an autoclave ("Brouillon process", Sainte Bazeille, France) at 85 °C under a pressure of 0.8 bar. Temperature was controlled in the water and in two control cans equipped with temperature sensors. After 30 min of chilling (circulating cool water), the cans were stored at 4 °C for 2 months until being opened for the estimation of the technological yield.

Sample Collection. Fatty livers were sampled at two different times post mortem: 20 min (end of slaughtering process) and 6 h (end of chilling process). Ten grams of fatty liver was frozen in liquid nitrogen and stored at −80 °C. The samples were ground in liquid nitrogen to obtain a fine powder.

Technological Yield Estimation. The cans were opened and the superficial fat exuded during cooking was carefully removed from the liver. The technological yield was defined as the cooked liver weight as a percentage of initial liver weight:

$$\text{technological yield} = (\text{cooked liver wt trimmed of all visible fat}/\text{raw liver wt}) \times 100$$

Biochemical Analysis. All biochemical analyses were performed in duplicate on fatty livers sampled at 6 h post mortem, after chilling. Total lipids were extracted from the raw fatty livers by homogenization in chloroform/methanol 2:1 (v/v) and measured gravimetrically according to the method of Folch et al.[7] Total nitrogen content of fatty livers was determined using a LECO analyzer (FP 428 model) after total combustion (proteins = N × 6.25). All of the results were reported as the mean value ± standard deviation.

Protein Extraction. Nine fatty livers were selected in the group showing low technological yield (<74%) and nine others in the group showing high technological yield (>83%). The same livers were analyzed at 20 min and 6 h post mortem. The extraction method was adapted from that of Sayd et al.[8] The samples were homogenized using a glass bead agitator MM2 (Retsch, Haan, Germany) in a low ionic strength buffer (S), 40 mM Tris HCl (pH 7.4) at 4 °C at a ratio of 1:4 (w/v). The homogenate was centrifuged at 4 °C for 10 min at 10000*g*. The fat cake was removed, and the homogenization was repeated. After centrifugation, the supernatant, forming the protein fraction soluble in low ionic strength buffer, was stored at −80 °C. The pellet was washed three times with this buffer to obtain only protein insoluble in it. After the last centrifugation, the supernatant was removed and the pellet was homogenized in the following buffer (IS): 7 M urea, 2 M thiourea, 4% CHAPS (w/v), at 4 °C at the same ratio as the first step. The homogenate was centrifuged at 4 °C for 10 min at 10000*g*. The supernatant, forming the protein fraction not soluble at low ionic strength, was stored at −80 °C. The protein concentration of both fractions was determined by using the Bradford assay (Sigma-Aldrich).

Electrophoresis of the Soluble Fraction at S. First, 300 μg of proteins of the S fraction was incorporated in a buffer containing 7 M urea, 2 M thiourea, 2% CHAPS (w/v), 0.4% carrier ampholyte (v/v), 1% DTT (w/v), and bromophenol blue. Samples were loaded onto immobilized pH-gradient strips (pH 5−8, 17 cm, Bio-Rad), and isoelectric focusing was performed using a Protean IEF cell system (Bio-Rad). Gels were passively rehydrated for 16 h. Rapid voltage ramping was subsequently applied to reach a total of 86 kVh. The equilibration buffer contained 6 M urea, 30% glycerol, 2% SDS, and 50 mM Tris-Cl, pH 8.8. First, strips were incubated in an equilibration buffer containing 1% DTT. Strips were then incubated in a second equilibration buffer containing 2.5% iodoacetamide and bromophenol blue. After strip equilibration, proteins were resolved on 12% SDS-PAGE gels using a Protean II XL system (Bio-Rad) for the second dimension. Gels were stained with Coomassie Blue (colloidal blue) as previously described by Morzel et al.[9] Only one 2-D gel was performed on each fatty liver.

Image Analysis. For each time post mortem, nine gels were acquired in the low-fat-loss group and nine in the high-fat-loss group. All of the gels were analyzed with the Image Master 2D Platinum software (GE Healthcare, Uppsala, Sweden) to point out spots of interest. Per gel, each detected and matched spot was normalized by expressing its intensity relative to the total intensity of all valid spots. Spots of interest were determined by using the procedure of Meunier et al.,[10] which uses fold change ratio.

Identification of the Spots of Interest of the Soluble Fraction S by Mass Spectrometry. Coomassie Blue-stained spots of interest were manually excised using pipet tips. The spots were then destained with 100 μL of 25 mM NH_4HCO_3 with acetonitrile 95:5 (v/v) for 30 min, followed by two washes in 100 μL of 25 mM NH_4HCO_3 with acetonitrile 50:50 (v/v) and then dehydrated in 100% acetonitrile.[11] Gel spots were completely dried using a Speed Vac

before trypsin digestion at 37 °C for 5 h with 15 μL of trypsin (10 ng/μL; V5111, Promega) in 25 mM NH_4HCO_3. Peptide extraction was optimized by adding 8 μL of acetronitile followed by 10 min of sonication.

For LC-MS/MS analysis, peptide mixtures were analyzed by online nanoflow liquid chromatography using the Ultimate 3000 RSLC (Dionex, Voisins le Bretonneux, France) with nanocapillary columns of 15 cm length × 75 μm i.d. (Acclaim Pep Map RSLC, Dionex). The solvent gradient increased linearly from 4 to 50% ACN in 0.5% formic acid at a flow rate of 300 nL/min for 30 min. The elute was then electrosprayed in a LTQ-VELOS mass spectrometer (Thermo Fisher Scientific, Courtaboeuf, France) through a nanoelectrospray ion source.

Thermo Proteome Discoverer v1.3 was used for raw data file processing. For protein identification, the Uniprot Taxonomy Aves (12-09-28 - 141469 sequences) protein database was combined with sequences of human keratin contaminants. The following parameters were considered for the searches: peptide mass tolerance was set to 1.5 Da, fragment mass tolerance was set to 0.8 Da, and a maximum of two missed cleavages was allowed. Variable modifications were methionine oxidation (M) and carbamidomethylation (C) of cysteine. A protein was considered to be valid when a minimum of two unique peptides originating from one protein showed statistically significant ($p < 0.05$) Mascot scores (http://www.matrixscience.com). For several identification results of one protein spot, we selected the one with the highest score.

Analysis of the Protein Fraction Insoluble in Low Ionic Strength Buffer. *Protein Separation.* Protein preparations from the low-fat-loss group ($n = 9$) and the high-fat-loss group ($n = 9$) were reduced with 20 mM DTT and then alkylated in 60 mM chloroacetamide. Samples containing 2% (w/v) SDS, 5% β-mercaptoethanol, 10% glycerol, and 62 mM Tris-HCl, pH 6.8, at a ratio 1:1 v/v[12] were heated at 95 °C for 5 min. SDS-PAGE (12% acrylamide) was performed following the method described by Laemmli[12] using a Mini-Protean II electrophoresis unit. Samples were loaded at 50 μg of protein per lane. Gels were run at 35 mA/gel, constant current, until the dye front reached the bottom of the gel. Gels were stained overnight in Coomassie Brilliant Blue G-250 (PageBlue Protein Staining Solution, Fermentas).

Protein Digestion. Each lane (one for each fat loss group) was systematically cut into 10 bands of similar volume for MS/MS protein identification. Each band was incubated in 25 mM ammonium bicarbonate and 50% ACN until destaining. Gel pieces were dried in a vacuum SpeedVac (45 °C), further rehydrated with 30 μL of a trypsin solution (10 ng/L in 50 mM NH_4HCO_3), and finally incubated overnight at 37 °C. The resulting peptides were extracted from the gel as described previously.[13] Trypsin digests were dried in a vacuum SpeedVac and stored at −20 °C before LC-MS/MS analysis.

Nano-LC-MS/MS Analysis. The trypsin digests were separated and analyzed by nano-LC-MS/MS using an Ultimate 3000 system (Dionex, Amsterdam, The Netherlands) coupled to an LTQ-Orbitrap mass spectrometer (Thermo Fisher Scientific, Bremen, Germany). The peptide mixture was loaded on a C18 precolumn (300 μm i.d., 15 cm PepMap C18, Dionex) equilibrated in 95% solvent A (5% acetonitrile and 0.2% formic acid) and 5% solvent B (80% acetonitrile and 0.2% formic acid). Peptides were eluted using a 5−50% gradient of solvent B for 80 min at a 300 nL/min flow rate. The LTQ-Orbitrap was operated in data-dependent acquisition mode with Xcalibur software (version 2.0.6, Thermo Fisher Scientific). Survey scan MS spectra were acquired in the Orbitrap over the m/z 300−2000 range with the resolution set to a value of 60000. The five most intense ions per survey scan were selected for collision-induced dissociation (CID) fragmentation, and the resulting fragments were analyzed in the linear trap (LTQ). Dynamic exclusion was used within 60 s to prevent repetitive selection of the same peptide. To automatically extract peak lists from Xcalibur raw files, the ExtractMSN macro provided with Xcalibur was used through the Mascot Daemon interface (version 2.3.2, Matrix Science, London, UK). The following parameters were set for creation of the peak lists: parent ions in the mass range 400−4500, no grouping of MS/MS scans, and threshold at 1000. A peak list was created for each fraction analyzed (i.e., gel slice), and individual Mascot searches were performed for each fraction.

Database Search. MS/MS spectra were processed by Mascot software against the *Gallus gallus* (SwissProt-TrEmbl) and *Cairina moschata*−*Anas platyrhynchos* (NCBI) databases. The following search parameters were applied: trypsin as cleaving enzyme, "ESI-Trap" as instrument, peptide mass tolerance of 10 ppm, MS/MS tolerance of 0.8 Da, and one missed cleavage allowed. Methionine oxidation was chosen as variable modifications.

Bioinformatic Analysis. *Validation and Semiquantitation.* MFPaQ software[14] was used to validate the results (1.3% FDR) and to analyze the data. This software is a Web application that allows fast and user-friendly verification of Mascot result files as well as data quantification. In particular, the spectral counts corresponding to each identified protein were extracted from each analysis.[15,16] Taking into account the molecular weight of proteins, we define an abundance index (PAI): PAI = spectral counts/molecular wt × 2500. This index allows the proteins inside each sample to be classified in a semiquantitative way.

Functional Analysis. The proteins validated by the software MFPaQ were entered into the Protein Center software (Proxeon, Odense, Denmark, http://www.proteincenter.proxeon.com) where they were clustered with a criterion of 60% homology. Analysis of the functional distribution of groups is then done, taking into account the classification established by "Genome Ontology".

Dot Blot of β-Enolase. To validate the results obtained on the IS fractions, dot blots of β-enolase were made. The analysis was done in triplicate for each sample: 11 in the high-fat-loss group and 13 in the low-fat-loss group. For the 24 samples, 20 μg of proteins was loaded onto a nitrocellulose membrane (Hybond ECL, Amersham), which was blocked with milk buffer containing 3% w/v milk powder in Tris buffer saline (TBS, pH 7.5) at room temperature for 1 h. Following blocking, the membrane was probed for specific proteins using primary antibody (1:500 mouse anti-β-enolase; sc-100811 Santa Cruz Biotechnologies, Inc.) at room temperature for 2 h. The membrane was then washed three times with TBS and incubated with a secondary antibody (1:10000 rabbit anti-mouse IgG-HRPsc-2005, Santa Cruz Biotechnology, Inc.) at room temperature for 1 h. Following this incubation, the membrane was washed three times, and the chemiluminescent substrate (Super Signal West Pico, Pierce) was then used to detect the reactivity of the primary antibody with its antigen. The film (Amersham HyperfilmMP, GE Healthcare) was then developed by steeping in 20% revelation solution (AL4, Kodak), distilled water, and 20% fixation solution (LX24, Kodak). The amount of 20 μg of pooled samples was used as a reference amount. Spot intensity of samples was then measured by using Image Analysis (ImageMaster 2D Platinum 6.0, GE Healthcare). It was expressed as a percentage of reference protein, and the results were reported as the mean value ± standard error of the mean.

Statistical Analysis. The Student t test was used to determine the differences between the two groups of fat loss for biochemical analysis and dot blot. Differences were declared to be significant at $p < 0.05$.

RESULTS AND DISCUSSION

The aim of this proteomic study was to analyze the changes in protein expression during chilling according to fat loss during cooking of duck fatty livers. For each protein fraction, we focused on proteins for which expression increased or decreased between 20 min and 6 h post mortem, that is, during chilling (at the time livers are usually processed under industrial conditions). The liver weight and the lipid and protein contents are known to affect the technological yield.[2] This is why the comparison was made on two groups showing significant differences only for the yield ($p < 0.001$) but similar liver weights and lipid and protein contents (Table 1).

Protein Fraction Soluble at Low Ionic Strength. The image analysis performed on 2D gels allowed the matching of 187 spots (Figure 1). By comparing the proteomic maps at 20

Table 1. Technological and Biochemical Characteristics of the Fatty Livers, According to Fat Loss during Cooking[a]

	low fat loss	high fat loss	significance
fatty liver weight (g)	570 ± 44	566 ± 42	NS
technological yield (%)	88.8 ± 4.3	68.2 ± 6.1	$p < 0.001$
lipid content (%)	57.6 ± 2.0	58.6 ± 1.8	NS
protein content (%)	7.6 ± 0.8	7.0 ± 0.5	NS

[a]For each fat loss group (both $n = 9$), results are reported as the mean value ± standard deviation. NS, non significant.

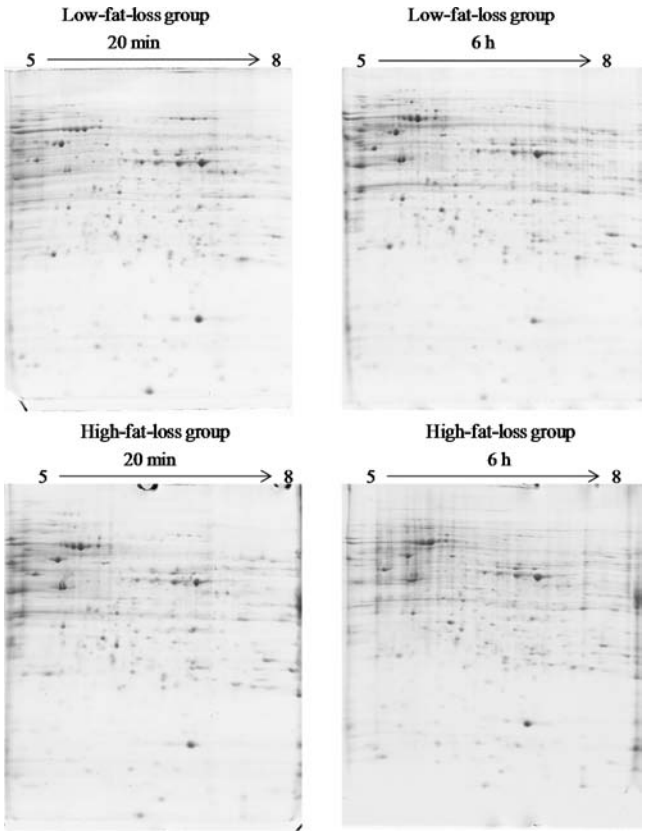

Figure 1. Representative two-dimensional gel electrophoresis map of duck fatty liver. The four gels represent the four experimental groups (low-fat-loss group collected at 20 min post mortem, low-fat-loss group collected at 6 h post mortem, high-fat-loss group collected at 20 min post mortem, and high-fat-loss group collected at 6 h post mortem) . The first dimension was performed between pH 5 and 8. The second-dimension gel contains 12% acrylamide. Three hundred micrograms of proteins is loaded.

min and 6 h post mortem in the low-fat-loss group, the statistical analysis revealed 36 spots of interest. The use of both MALDI-TOF and LC-MS/MS mass spectrometry allowed the identification of 21 proteins among these 36 spots (Table 2). The sequences were identified by comparison with the Aves database. The 21 proteins identified can be classified according to their biological functions: 5 are involved in oxidoreduction processes, 4 in metabolism, and 5 in proteolytic activity; 3 have a chaperone activity; and 4 are miscellaneous proteins. By comparison of the proteomic maps at 20 min and 6 h post mortem in the high-fat-loss group, the statistical analysis revealed 34 spots of interest. The use of both MALDI-TOF and LC-MS/MS mass spectrometry allowed the identification of 26 proteins among these 34 spots (Table 3) by comparison of sequences with the Aves database. The 26 proteins identified can be classified according to their biological functions: 5 are involved in oxidoreduction process, 7 in metabolism, and 3 in proteolytic activity; 2 have a chaperone activity; and 9 are miscellaneous proteins.

Oxidoreduction Process. Among the 21 proteins identified in the S protein fraction of livers showing low fat loss during cooking, 5 are involved in oxidoreduction processes. The aldehyde dehydrogenase 2 family (spots 79, 85, and 86) and the superoxide dismutase 1 (spot 194) were detected only before chilling; the catalase (spot 61) expression was higher after chilling. Aldehyde dehydrogenase 2 is involved in redox regulation of the cell. SOD-1 is a peroxisomal free radical-scavenging enzyme that dismutates reactive oxygen species to hydrogen peroxide and molecular oxygen. Removal of superoxide radicals by SOD prevents formation of very active hydroxyl radicals. Catalase catalyzes the decomposition of hydrogen peroxide to water and oxygen. Five spots identified in the S protein fraction of fatty livers with high fat loss during cooking were related to the oxidoreduction process. DJ-1 protein (spot 219) and malate dehydrogenase (spot 135) were detected only before chilling. Aldehyde dehydrogenase 9 (spot 76) expression was higher before chilling, and Prdx3 protein (spots 196 and 198) expressions were higher after chilling. DJ-1 protein and aldehyde dehydrogenase 9 protect cells against oxidative stress. Peroxiredoxins are very effective enzymes in scavenging peroxides in the cell. In the low-fat-loss group, the absence of these four spots identified as proteins involved in oxidoreduction processes from the S protein fraction after chilling indicated a stabilization of oxidoreduction processes by the temperature. Indeed, post mortem evolution of muscle includes a decrease in the antioxidant defense system in beef[17] and in turkey[18] muscles. In the high-fat-loss group, the detection after chilling of the aldehyde dehydrogenase (spot 76) and the increase of Prdx3 (spots 196 and 198) expression during chilling may reflect a less effective antioxidant protection of chilling in the fatty liver tissue. Furthermore, we have already shown on the same experimental material that there was an overexpression of proteins involved in antioxidant processes early post mortem in fatty livers showing a high fat loss during cooking.[6]

Metabolism. In the low-fat-loss group, four spots were identified as proteins belonging to metabolism processes. Enolase 1 was found to be down-regulated (spot 101) and up-regulated (spot 100) during chilling. Triosephosphate isomerase 1 (spot 211) expression was higher after chilling. Fatty acid binding protein 4 (spot 304) was detected only after chilling. Triosephosphate isomerase 1 and α-enolase are glycolytic enzymes, responsible for the fifth and ninth steps of glycolysis, respectively. Fatty acid binding protein 4 is involved in carbohydrate biosynthesis. Among the 26 proteins identified in the S protein fraction of livers showing high fat loss during cooking, 7 are involved in metabolic processes. Glutamine synthase (spot 117), phosphoglycerate mutase 1 (spot 157), and fatty acid synthase (spot 154) expressions were down-regulated after chilling. Triosephosphate isomerase 1 (spot 302) was detected only after chilling. The glutamine synthase is involved in the metabolism of amino acids by catalyzing the condensation of glutamate and ammonia to form glutamine. The fatty acid synthase is involved in lipid synthesis by catalyzing the formation of long-chain fatty acids from acetyl-CoA, malonyl-CoA, and NADPH. Phosphoglycerate mutase 1 is an enzyme that catalyzes step 8 of the glycolysis. Enolase (spot 129) and glutathione 5-transferase μ 4 (spot 163) were

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Table 2. Identification of Spots from the Soluble Protein Fraction, the Expression of Which during Post Mortem Chilling Was Linked to Fat Loss during Cooking in the Low-Fat-Loss Group

sequence[a]	spot	before chilling	after chilling	protein name − taxonomy[a]	score Mascot[b]	sequence cororage[c] (%)	no. of peptides matched[d]	theor MW[e] (kDa)	theor p*I*[e]
				Oxidoreduction Process					
E1BT93	79	0.57 ± 0.66	ND[f]	aldehyde dehydrogenase, mitochondrial isoform 2 − *Gallus gallu*	4237	27	12	56.8	7.80
E1BT93	85	1.46 ± 0.66	ND	aldehyde dehydrogenase, mitochondrial isoform 2 − *Gallus gallu*	5841	25	10	56.8	7.80
E1BT93	86	0.17 ± 0.18	ND	aldehyde dehydrogenase, mitochondrial isoform 2 − *Gallus gallu*	902	14	7	56.8	7.80
B2M0J9	194	0.06 ± 0.06	ND	Mn superoxide −*Anas platyrhynchos*	4008	32	6	32.3	8.52
F1NGJ7	61	0.04 ± 0.03	0.09 ± 0.03	catalase − *Gallus gallus*	126	8	4	59.8	7.84
				Metabolism					
P19140	100	0.31 ± 0.18	0.40 ± 0.08	α-enolase −*Anas platyrhynchos*	13117	44	14	47.2	6.80
P19140	101	0.61 ± 0.57	0.33 ± 0.27	α-enolase −*Anas platyrhynchos*	19619	58	18	47.2	6.80
Q70I43	211	0.16 ± 0.19	0.51 ± 0.97	triosephosphate isomerase (fragment) − *Gallus gallus*	11949	73	11	22.6	6.64
F85KC8	304	ND	0.10 ± 0.09	adipocyte fatty acid binding protein − *Anas platyrhynchos*	29464	70	9	14.9	7.14
				Proteolytic Activity					
Q05744	180	0.33 ± 0.15	0.13 ± 0.06	cathepsin D − *Gallus gallus*	4261	53	7	43.3	6.32
Q05744	222	0.36 ± 0.42	0.10 ± 0.14	cathepsin D − *Gallus gallus*	6665	20	6	43.3	6.32
Q05744	234	0.08 ± 0.10	0.02 ± 0.03	cathepsin D − *Gallus gallus*	1719	20	6	43.3	6.32
Q05744	188	0.26 ± 0.25	0.05 ± 0.06	cathepsin D − *Gallus gallus*	5095	10	3	43.3	6.32
G1NIQ7	207	0.34 ± 0.35	0.13 ± 0.08	proteasome subunit α type − *Meleagns gallopavo*	2784	38	8	27.5	6.55
				Chaperone					
I3QII5	293	0.33 ± 0.12	ND	heat shock protein 60 kDa − *Anas platyrhynchos*	21489	68	25	60.5	5.64
AOPA16	41	1.16 ± 0.77	ND	heat shock protein 70 kDa − *Coturnix japonica*	15745	49	22	70.8	5.52
AOPA16	303	0.33 ± 0.10	ND	heat shock protein 70 kDa − *Coturnix japonica*	18139	47	26	70.8	5.52
				Miscellaneous					
Q5ZLC5	95	0.31 ± 0. 18	ND	ATP synthase subunit β, mitochondrial − *Gallus gallus*	25608	69	23	56.6	5.87
Q9I839	182	0.24 ± 0 32	0.06	protein kinase C inhibitor − *Anas platyrhynchos*	1420	60	6	13.7	6.04
Q9I923	256	0.40 ± 0.38	0.14 ± 0.20	regucalcin − *Gallus gallus*	1209	34	13	43.7	8.18
P56410	19	0.11 ± 0.38	ND	ovotransferrin − *Anas platyrhynchos*	344	8	5	75.6	6.60

[a]Protein name and sequence reference were derived from Swiss-Prot database and EST database. [b]MASCOT protein score. [c]Percent of coverage of the entire amino acid sequence. [d]Number of matched peptides in the database search. [e]Molecular weight (MW) and p*I* theoretical (recorded in UniProtKB/Swiss-Prot databases for MALDI-TOF and NCBI database mammalian taxonomy for LC-MS/MS). [f]ND, not detected.

detected only before chilling. Fatty acid binding protein (spot 180) expression was higher after chilling. Glutathione S-transferase μ 4 is involved in the conjugation of reduced glutathione to a wide number of exogenous and endogenous hydrophobic electrophiles.

Overall, in the low-fat-loss group, among the proteins involved in metabolic processes, three were up-regulated during chilling, whereas in the high-fat-loss group, among the seven proteins, only two were up-regulated after chilling. In the livers that were to have a low fat loss during cooking, the anabolic processes seemed to be effective for longer during chilling than in the high-fat-loss group. These results are in accordance with a previous proteomic study of duck fatty liver made early post mortem on the same experimental model. The results revealed a higher anabolic activity in livers that will exhibit a lower fat loss during cooking.[6]

Proteolytic Activity. The five spots identified as proteins involved in proteolytic activity are down-regulated after chilling in the low-fat-loss groups. Cathepsin D (spots 180, 188, 222, and 234) expressions were higher before chilling. Cathepsin D is a lysosomal aspartic protease. Cathepsin D is a major lysosomal enzyme involved in protein degradation. In the S protein fraction of livers showing a high fat loss during cooking, three spot were identified as proteins having a proteolytic activity. Cathepsin D was detected only before chilling (spot 222) and up-regulated during chilling (spot 230). Proteasome subunit α type (spot 207) was also up-regulated during chilling. Cathepsins are thought to be involved in post mortem proteolysis in meat and meat products[19,20] and therefore in meat tenderization. Because in the low-fat-loss group five spots were down-regulated and only one in the high-fat-loss group, we can hypothesize that less proteolytic activity occurred during chilling in livers with a low fat loss during cooking. Cytoskeleton protein proteolysis is involved in the determination of water-holding capacity in pork;[21] it can occur from 45 min to 6 h post mortem.[22] In our case, proteolysis could make the tissue more fragile and/or facilitate the fusions of lipid droplets, a phenomenon that was previously described as being involved in fat loss during cooking of duck foie gras.[23]

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Table 3. Identification of Spots from the Soluble Protein Fraction, the Expression of Which during Post Mortem Chilling Was Linked to Fat Loss during Cooking in the High-Fat-Loss Group

sequence	spot	before chilling	after chilling	protein name − taxonomy[a]	score Mascot[b]	sequence coverage[c] (%)	no. of peptides matched[d]	theor MW[e] (kDa)	theor p*I*[e]
				Oxidoreduction Activity					
F1NMN7	76	0.32 ± 0.35	0.16 ± 0.14	aldehyde dehydrogenase 9 family, member A1 − *Gallus gallus*	5067	18	9	60.2	8.62
B5G179	135	0.66 ± 0.45	ND[g]	malate dehydrogenase − *Taemopygia guttata*	4686	46	15	36.5	6.58
E1BR10	196	0.27 ± 0.22	0.44 ± 0.48	Prdx 3 protsn − *Gallus gallus*	1310	8	2	30.9	8.40
B5G3Z6	198	0.18 ± 0.07	0.41 ± 0.22	Prdx 3 protsn - *Gallus gallus*	5317	29	5	20.0	6.79
Q8UW59	219	0.20 ± 0.15	ND	DJ-1 protein − *Gallus gallus*	383	16	3	19.9	6.33
				Metabolism					
G1MWS3	117	1.16 ± 0.68	0.26 ± 0.25	glutamine synthetase −*Meleagris gattopavo*	9229	40	13	42.2	6.98
Q7OI43	302	ND	0.96 ± 0.95	triosephosphate isornerase − *Anser anser*	44844	77	14	22.6	6.64
Q6LDK3	129	0.31 ± 0.13	ND	enolase − *Anas plaprhynchos*	341	11	3	40.8	6.70
Q5ZLN1	157	1.78 ± 0.43	0.92 ± 0.63	phosphoglycerate mutase l − *Gallus gallus*	7832	56	12	28.9	7.49
F8TTE4	163	0.15 ± 0.23	ND	glutathione 5-transferase μ 4 − *Meleagris gallopavo*	1456	17	3	25.8	7.49
Q5QFH8	154	0.68 ± 0.77	0.27 ± 0.34	fatty acid synthase −*Anas platyrhynchos*	1310	4	2	10.1	5.48
Q05423	180	0.25 ± 0.17	0.71 ± 0.43	fatty acid binding protein − *Gallus gallus*	4261	53	7	14.9	5.91
				Proteolytic Activity					
QO5744	222	0.51 ± 0.32	ND	cathepsin D −*Gallus gallus*	6666	20	6	43.3	6.32
QO5744	230	0.25 ± 0.08	0.52 ± 0.25	cathepsin D − *Gallus gallus*	259	8	2	43.3	6.32
G1NIQ7	207	0.26 ± 0.09	0.62 ± 0.22	proteasome subunit α type − *Meleagris gallopavo*	2784	38	8	27.5	6.55
				Chaperone					
I3QII5	68	0.81 ± 0.74	ND	heat shock protein 60 kDa − *Anas plaprhynchos*	676	17	9	92.5	8.46
AOPA16	41	0.98 ± 0.89	ND	heat shock protein 70 kDa − *Coturnix coturnix japonica*	15745	49	22	70.8	5.52
				Miscellaneous					
G1NCR2	294	0.35 ± 0.19	ND	serum albumin − *Gallus gallus*	5976	25	17	69.9	6.43
P56410	16	0.28 ± 0.17	ND	ovotransferrin − *Anas platyrhynchos*	311	14	8	75.6	6.60
P56410	19	ND	0.27 ± 0.20	ovotransferrin − *Anas platyrhynchos*	344	8	5	75.6	6.60
P56410	20	0.20 ± 0.17	ND	ovotransferrin − *Anas platyrhynchos*	4457	34	19	75.6	6.60
Q5ZMU9	11	0.20 ± 0.14	ND	valosin-containing protein − *Gallus gallus*	6655	40	27	89.3	5.26
Q5ZLCS	95	ND	055 ± 0.91	ATP synthase subunit β, mitochondnril − *Gallus gallus*	25608	69	23	56.6	5.87
D7RMT6	88	0.28 ± 0.23	009 ± 0.09	fibrinogen γ chain − *Anser anser*	1382	51	5	15.1	5.57
F1NFSO	126	0.06 ± 0.08	ND	eukaryotic translation elongation factor 2	308	8	6	95.3	6.74
B5FXT6	154	0.88 ± 0.35	046 ± 0.32	40S ribosomal protein SA − *Taeniopygia guttata*	1228	20	5	33.0	4.87

[a]Protein name and sequence reference were derived from Swiss-Prot database and EST database. [b]MASCOT protein score. [c]Percent of coverage of the entire amino acid sequence. [d]Number of matched peptides in the database search. [e]Molecular weight (MW) and p*I* theoretical (recorded in UniProtKB/Swiss-Prot databases for MALDI-TOF and NCBI database mammalian taxonomy for LC-MS/MS). [g]ND, not detected.

Chaperone. In both groups of fat loss during cooking, the spots identified as chaperone proteins were detected only before chilling. In the S protein fraction of livers showing a low fat loss during cooking, two proteins were found to have a chaperone activity: heat shock protein 60 kDa (HSP60) (spot 293) and heat shock protein 70 kDa (HSP70) (spots 41 and 303). In the S protein fraction of livers showing a high fat loss during cooking, two proteins were identified as chaperones: heat shock protein 70B (HSP70, spot 41) and heat shock protein 60 kDa (HSP60, spot 68). In the same way that the chilling process may protect tissue from oxidation reactions, it seems that the storage of livers at 4 °C for 6 h leads to a stabilization of protective mechanisms, regardless of the level of fat loss during cooking.

Miscellaneous. In the low-fat-loss group, four proteins were identified with miscellaneous functions. The ATP synthase subunit β (spot 95) and ovotransferrin (spot 19) were detected only before chilling. Protein kinase C inhibitor (spot 182) and regucalcin (spot 256) expressions were down-regulated after chilling. Among the nine miscellaneous proteins identified in the S protein fraction of livers showing a high fat loss during cooking, five were detected only before chilling: albumin (spot 294), ovotransferrin (spots 16 and 20), valosin-containing protein (spot 11), and eukaryotic translation elongation factor 2 (spot 126). Albumin is synthesized in the liver and is the major plasma protein circulating in the bloodstream. Ovotransferrin is an iron-binding transport protein that can bind two Fe^{3+} ions in association with the binding of an anion, usually bicarbonate. Valosin-containing protein is an ATP-binding protein that belongs to the AAA ATPase family and is involved in several cellular functions. The fibrinogen γ chain (spot 88) and 40S ribosomal protein SA (spot 154) expressions were down-regulated during chilling. Fibrinogen γ chain is a glycoprotein that can be cleaved to form fibrin, which is the

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Table 4. Identification of Proteins Changing Post Mortem in the Low-Fat-Loss Group in the IS Protein Fraction[a]

sequence ref	protein name − taxonomy	CC	BP	MF	PAI ratio
P09207	tubulin β-6 chain − *Gallus gallus*	Mb	Coa	CA	6.2
Q9W6H0	mimecan − *Gallus gallus*	Mb	RS	CA	5.5
Q805C1	glycogen synthase (fragment) − *Gallus gallus*		MP	CA	4.0
Q5F4B5	putative uncharacterized protein − *Gallus gallus*	Cy	MP	CA	4.0
Q5F3Q3	putative uncharacterized protein − *Gallus gallus*		RBP	CA	4.0
Q5ZK84	alcohol dehydrogenase [NADP$^+$] − *Gallus gallus*	Mb	MP	CA	3.5
Q90WF0	CgABP260 − *Gallus gallus*			PB	3.4
Q90WF1	filamin − *Gallus gallus*	Hb		PB	3.1
Q9IAY5	protein syndesmos − *Gallus gallus*	Mb		CA	3.0
Q8JGM8	BH3-interacting domain death agonist − *Gallus gallus*	Cy	CDi	CA	3.0
Q5ZLD4	transmembrane protein 11 − *Gallus gallus*	Mb		PB	3.0
Q5F470	Ras-related protein Rab-8A − *Gallus gallus*	Mb	COa	CA	3.0
Q5F3B4	putative uncharacterized protein − *Gallus gallus*			CA	3.0
Q5KTT9	eukaryotic translation initiation factor 4 γ 2 isoform 2 − *Gallus gallus*	Cy	RBP	TA	2.8
Q197 × 2	apolipoprotein B −*Gallus gallus*	Mb	COa	CA	2.8
Q5ZK08	putative uncharacterized protein − *Gallus gallus*	Cy	MP	CA	2.7
Q5ZLF0	Hsc70-interacting protein − *Gallus gallus*	Cy	MP	PB	2.7
Q8UWG6	extracellular signal-regulated kinase 2 − *Gallus gallus*	Cy	RBP	CA	2.4
Q91005	α-tropomyosin of brain − *Gallus gallus*	Cy	CDi	Str	2.3
Q5F428	eukaryotic translation initiation factor 3 subunit L − *Gallus gallus*	Cy	COa	TA	2.3
P23991	alcohol dehydrogenase 1 − *Gallus gallus*	Cy	MP	CA	2.3
Q05705	β-tropomyosin − *Gallus gallus*	Cy		PB	2.1
Q9PW08	aminopeptidase (fragment) − *Gallus gallus*	Cy	MP	CA	2.0
Q90706	CLE7 − *Gallus gallus*			PB	2.0
Q5ZMB0	putative uncharacterized protein − *Gallus gallus*		MP	CA	2.0
Q5ZLR4	epithelial splicing regulatory protein 2 − *Gallus gallus*	Nu	RBP	CA	2.0
Q5ZKG5	low molecular weight phosphotyrosine protein phosphatase − *Gallus gallus*	Cy	MP	CA	2.0
Q5ZI80	putative uncharacterized protein − *Gallus gallus*	Cy	RBP	TA	2.0
Q5ZI14	putative uncharacterized protein − *Gallus gallus*	Pr	RBP	CA	2.0
Q05744	cathepsin D − *Gallus gallus*	Cy	COa	CA	2.0
P53449	fructose-bisphosphate aldolase C (fragment) − *Gallus gallus*	Cy	MP	CA	2.0
P23228	hydroxymethylglutaryl-CoA synthase, cytoplasmic − *Gallus gallus*	Mb	MP	CA	2.0
Q9PW24	RNA-binding protein HuA − *Gallus gallus*	Cy	RBP	NB	0.1
Q98TF8	60S ribosomal protein L22 − *Gallus gallus*	Ri	CDi	Str	0.2
Q8AYP7	hexokinase 2 − *Gallus gallus*		MP	CA	0.3
Q6YJI5	tRNAguanosine-2′-*O*-methyltransferase TRM11 homologue − *Gallus gallus*		RBP	CA	0.3
Q6PVZ4	type II α-keratin IIB − *Gallus gallus*	Mb	COa	Str	0.3
Q5ZMU3	glucose-6-phosphate isomerase − *Gallus gallus*	Cy	MP	CA	0.3
Q5ZLC7	microtubule-associated protein RP/EB family member 1 − *Gallus gallus*	Mb	RBP	Sig	0.3
Q5ZL25	putative uncharacterized protein − *Gallus gallus*	Cy	RBP	TA	0.3
Q5ZJZ5	D-β-hydroxybutyrate dehydrogenase, mitochondrial − *Gallus gallus*	Mb	MP	CA	0.3
Q5ZKW4	putative uncharacterized protein − *Gallus gallus*		MP	CA	0.3
Q5ZJD6	putative uncharacterized protein − *Gallus gallus*	Cy		CA	0.4
Q5ZJA5	putative uncharacterized protein − *Gallus gallus*	Ri	DR	CA	0.4
Q5ZJ60	3-hydroxyisobutyryl-CoA hydrolase, mitochondrial − *Gallus gallus*	Mb	COa	CA	0.5
Q5ZJ21	putative uncharacterized protein − *Gallus gallus*	Mb	DR	CA	0.5
Q5ZIZ5	putative uncharacterized protein − *Gallus gallus*		RBP	NB	0.5
Q5ZIP1	putative uncharacterized protein − *Gallus gallus*	Cy	MP	CA	0.5
Q5ZI23	isoform 1 of hydroxyacylglutathione hydrolase, mitochondrial − *Gallus gallus*	Cy	MP	CA	0.5
P98157	isoform 1 of low-density lipoprotein receptor-related protein 1 − *Gallus gallus*	Mb	COa	MB	0.5
P23668	16 kDa β-galactoside-binding lectin − *Gallus gallus*				0.5
P28337	aminomethyltransferase, mitochondrial − *Gallus gallus*	Cy	MP	CA	0.5
057391	γ-enolase − *Gallus gallus*	Cy	MP	CA	0.5
Q5W4S3	fatty acid coenzyme A ligase, long chain 6 (fragment) − *Gallus gallus*	Cy	CDi	CA	0.5
P09203	tubulin β-1 chain − *Gallus gallus*	Cy	COa	CA	0.5
D0VX30	mitochondrial ubiquinol-cytochrome *c* reductase 14 kDa protein − *Gallus gallus*		MP	CA	0.5
P00378	dihydrofolate reductase − *Gallus gallus*	Csk	COa	CA	0.5
P70080	tryptophan 5-hydroxylase 1 −*Gallus gallus*		RBP	CA	0.5
P02127	hemoglobin subunit ρ −*Gallus gallus*	Cy	T	MB	0.5
Q5ZHM0	putative uncharacterized protein − *Gallus gallus*		MP	CA	0.5
Q5F3V1	putative uncharacterized protein − *Gallus gallus*	EC	MP	CA	0.5

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Table 4. continued

sequence ref	protein name − taxonomy	CC	BP	MF	PAI ratio
Q5F418	26S proteasome non-ATPase regulatory subunit 1 − *Gallus gallus*	Pr	RBP	E	0.5
P00523	isoform 1 of proto-oncogene tyrosine-protein kinase Src − *Gallus gallus*	Cy	RBP	CA	0.5
Q5F419	putative uncharacterized protein − *Gallus gallus*			Str	0.5
Q5H7M6	DRG1 (fragment) − *Gallus gallus*	Cy		NB	0.5
042388	ubiquitin-ribosomal protein fusion protein −*Gallus gallus*	Ri	MP	Str	0.5
P17790	isoform 1 of basigin − *Gallus gallus*	Mb			0.5

[a]The main cellular component (CC), biological process (BP), and molecular function (MF) are presented for each protein. The peptide count corresponds to peptides with a FDR < 1.3%. The protein abundance index (PAI) is calculated as indicated under Materials and Methods. Cellular component abbreviations: Cy, cytoplasm; Csk, cytoskeleton; EC, extracellular; Go, Golgi apparatus; Mb, membrane; Nu, nucleus; Pr, proteasome; Ri, ribosome; Sp, spliceosome. Biological process abbreviations: CDi, cell differentiation; CM, cell motility; CO, cell organization; COa, coagulation; DR, defense response; MP, metabolic process; RBP, regulation of biological process; RS, response to stimulus; T, transport. Molecular function abbreviations: CA, catalytic activity; E, enzyme regulator activity; MB, metal ion binding; NB, nucleotide binding; PB, protein binding; Sig, signal transducer activity; Str, structural molecule activity; T, transporter activity; TA, translation regulator activity.

most abundant component of blood clots. This protein is involved in platelet activation and protein polymerization. 40S ribosomal protein is involved in translation. ATPase subunit β (spot 95) and ovotransferrin (spot 19) were detected only after chilling. Overall, only two spots identified in the S protein fraction of livers from the high-fat-loss group were up-regulated after chilling (i.e., spots 95 and 209). This confirms the reduction in various enzymatic processes after chilling, that is, transport activity corresponding to the falling expression of ovotransferrin (spots 16 and 20). Because of the wide spectrum of activities performed by these proteins, it is difficult to link the phenomenon of fat loss during cooking of duck foie gras to the expression of these proteins.

To our knowledge, our study is the first to report on the proteome evolution of fatty liver during chilling related to fat loss during cooking, so our hypothesis cannot be corroborated by existing literature. Overall, the protein expression in the S fraction showed a stabilization effect of the tissue by chilling. However, it was more pronounced in the low-fat-loss fatty livers. This phenomenon has previously been indirectly demonstrated in goose fatty liver, where fat loss during cooking was decreased by removing the livers from the carcass early post mortem and chilling them until cooking the same day, instead of removing the livers after the chilling of the carcass at 24 h post mortem.[24] This allows the chilling to be accelerated and the storage duration to be shortened from 24 h to <10 h and, probably, the enzyme activity in the livers to be reduced.

Protein Fraction Insoluble at Low Ionic Strength. The shot-gun method applied to the protein fraction insoluble in the low ionic strength buffer allowed the detection and identification before and after chilling of 554 and 562 proteins in livers from the low-fat-loss and high-fat-loss groups, respectively. Within each group of fat loss during cooking, we analyzed the changes in the expression of proteins before and after chilling. Initially, the results showed that in the low-fat-loss group, 32 proteins were up-regulated and 35 were down-regulated during chilling (Table 4). In the high-fat-loss group, 45 proteins were up-regulated and 13 down-regulated during chilling (Table 5).

The data were then compared with the Gene Ontology database to determine the significance of the results in terms of "cellular component", "biological process", and "molecular function". The comparison of these groups of proteins with the Gene Ontology database revealed significant results only for the proteins of which expressions were up-regulated during chilling in the livers that were to show a high fat loss during cooking. The principal cellular component was the cytoskeleton and associated proteins (FDR p value = 3.17×10^{-3}). The proteins matching with this result were isoform 1 of F-actin-capping protein subunit β isoforms 1 and 2, gephyrin, actin cytoplasmic type 5, SMC1 protein cohesion subunit, ezrin, radixin, nuclear migration protein NudC, type II α-keratin IIB, 14-3-3 protein β/α, myosin Ic, a fragment of DRG1 protein, and two putative uncharacterized proteins. One of the two molecular functions corresponding to this analysis was "lyase activity", and this group was constituted by isoform 1 of F-actin-capping protein subunit β isoforms 1 and 2, cytosolic purine 5′-nucleotidase, serum paraoxonase, β-enolase, histidine ammonia-lyase, and a putative uncharacterized protein. The other molecular function showing a significant result in this analysis was "actin binding" and corresponded to radixin, isoform 1 of F-actin-capping protein subunit β isoforms 1 and 2, gephyrin, actin cytoplasmic type 5, ezrin, myosin Ic, and a putative uncharacterized protein. To validate this result, we chose to study more specifically one protein, β-enolase. We did a dot-blot analysis on a second set of samples as a validation on a biological replicate and analyzed the expression of β-enolase during chilling in each fat loss group (Figure 2). The results showed a higher expression after chilling in both groups of fat loss during cooking, which is consistent with results obtained by the shot-gun approach. β-Enolase could thus be a good marker for the changes in fatty liver protein expression during chilling.

The main result of all this analysis is the overexpression after chilling of proteins from cytoskeleton and associated proteins in the IS protein fraction of fatty livers that will show a high fat loss during cooking. During post mortem storage of pig liver at 37 °C, the proteosynthesis gradually falls and stops at about 4 h post mortem.[25] In our case, the livers were chilled to reach a temperature of 4 °C, but this overexpression is probably not the result of protein synthesis. During muscle post mortem maturation, proteolysis is a major factor that increases the tenderness of meat.[26,27] In the S fraction, the results from proteins with a proteolytic activity did not clearly reveal a higher proteolysis in the livers from the high-fat-loss group, but we make a hypothesis about the proteolytic pattern. Taken together, these results from the study on the changes in protein expression during chilling of fatty livers have established a link between post mortem proteolysis and fat loss during cooking of duck foie gras. We believe that this overexpression in the high-fat-loss group can be the result of the detection at 6 h post mortem of protein fragments. The variability in fat loss observed in processing plants could thus be partly explained by a different stage of proteolysis in fatty livers during chilling.

Table 5. Identification of Proteins in Evolution Post Mortem in the High-Fat-Loss Group in the IS Protein Fraction[a]

sequence ref	protein name − taxonomy	CC	BP	MF	PAI ratio
Q5ZLA6	myosin-Ic − *Gallus gallus*	Mb	T	CA	3.0
Q5ZJ22	outative uncharacterized protein − *Gallus gallus*	Cy		Sig	2.0
Q9YGW6	ezrin − *Gallus gallus*	Mb		Str	2.1
Q9PU45	radixin − *Gallus gallus*	Mb	RBP	Str	2.0
Q802A0	histidine ammonia-lyase − *Gallus gallus*	Cy	MP	CA	2.7
Q5ZKJ9	putative uncharacterized protein − *Gallus gallus*	Mb	MP	CA	5.0
Q5ZIN1	nuclear migration protein nudC − *Gallus gallus*	Cy	CDi	PB	2.2
Q8AWB7	SMC1 protein cohesin subunit − *Gallus gallus*	Mb	Coa	CA	2.7
Q5ZME3	putative uncharacterized protein − *Gallus gallus*		MP	CA	4.0
P07322	β-enolase − *Gallus gallus*	Cy	MP	CA	2.4
P50147	guanine nucleotide-binding protein G(i) subunit α-2 − *Gallus gallus*		CO	NB	2.0
Q5H7M6	DRG1 (fragment) − *Gallus gallus*	Cy		NB	3.0
012945	vitronectin − *Gallus gallus*	EC	RS	CA	3.3
Q5F354	putative uncharacterized protein − *Gallus gallus*	Mb	Coa	Str	2.2
093601	apolipoprotein AIV − *Gallus gallus*	EC	MP	CA	2.3
Q5ZMD2	ankyrin repeat and MYND domain-containing protein 2 − *Gallus gallus*		MP	CA	2.0
Q90952	serum paraoxonase/arylesterase 2 − *Gallus gallus*	Mb	MP	CA	4.5
Q5ZLR4	epithelial splicing regulatory protein 2 − *Gallus gallus*	Nu	RBP	CA	2.0
P62801	histone H4 − *Gallus gallus*	Nu	RBP	CA	2.4
Q5ZIZ5	putative uncharacterized protein − *Gallus gallus*		RBP	NB	2.0
Q5ZLH7	putative uncharacterized protein − *Gallus gallus*			NB	2.0
Q5ZIZ4	cytosolic purine 5′-nucleotidase − *Gallus gallus*	Cy	MP	CA	2.6
Q5ZKQ2	putative uncharacterized protein − *Gallus gallus*	Cy	MP	CA	3.5
Q8QH01	flavin-containing monooxygenase 3 − *Gallus gallus*	Mb	MP	CA	2.5
Q5ZHS2	putative uncharacterized protein − *Gallus gallus*	Mb	MP	CA	2.0
Q9PW38	gephyrin − *Gallus gallus*	Mb	CDi	CA	2.7
Q9I9B8	noncanonical ubiquitin conjugating enzyme 1 − *Gallus gallus*		RBP	CA	3.0
Q5ZM11	arginyl-tRNA synthetase, cytoplasmic − *Gallus gallus*	Cy	MP	CA	2.0
Q5ZJQ5	putative uncharacterized protein − *Gallus gallus*	Cy	MP	CA	2.5
Q5F3C0	putative uncharacterized protein − *Gallus gallus*	Cy	MP	CA	2.2
Q5ZMI5	putative uncharacterized protein − *Gallus gallus*	Mb	COa	CA	2.0
Q5ZIC3	putative uncharacterized protein − *Gallus gallus*		MP	CA	2.0
Q5ZHW5	proteasome subunit α type − *Gallus gallus*	Pr	MP	CA	2.0
Q5ZJK3	putative uncharacterized protein − *Gallus gallus*	Cy	RBP	CA	6.0
P53478	actin, cytoplasmic type 5 − *Gallus gallus*	Cy	CDi	NB	2.4
Q6PVZ4	type II α-keratin IIB − *Gallus gallus*	Mb	COa	Str	2.0
Q5ZL92	putative uncharacterized protein − *Gallus gallus*	Mb	CDi	CA	3.0
Q6U7I1	isoform 1 of ubiquitin carboxyl-terminal hydrolase 7 − *Gallus gallus*	Nu	MP	CA	2.2
Q5ZLI9	putative uncharacterized protein − *Gallus gallus*	Cy	MP	CA	2.0
Q5ZJD6	putative uncharacterized protein − *Gallus gallus*	Cy		CA	2.0
Q5ZI60	putative uncharacterized protein − *Gallus gallus*			CA	3.7
Q5F3B4	putative uncharacterized protein − *Gallus gallus*			CA	2.0
Q5ZLQ6	14-3-3 protein β/α − *Gallus gallus*	Cy	RBP	PB	2.9
Q5ZK94	putative uncharacterized protein − *Gallus gallus*	Nu		PB	2.3
Q5ZIR0	putative uncharacterized protein − *Gallus gallus*	Mb			2.0
C7G537	ADF actin binding protein − *Gallus gallus*			PB	0.5
Q5ZJ60	3-hydroxyisobutyryl-CoA hydrolase, mitochondrial − *Gallus gallus*	Mb	COa	CA	0.4
057535	nucleoside diphosphate kinase − *Gallus gallus*	Mb	CDi	CA	0.4
Q5ZID4	putative uncharacterized protein − *Gallus gallus*	Nu	RBP	CA	0.3
Q5ZLC4	oxysterol-binding protein − *Gallus gallus*		MP		0.5
Q5ZL34	cleavage and polyadenylation specificity factor subunit 6 − *Gallus gallus*	Nu	MP	NB	0.5
Q5ZJ68	putative uncharacterized protein − *Gallus gallus*	Cy	MP	CA	0.4
Q5ZIK6	BSD domain-containing protein 1 − *Gallus gallus*				0.3
Q5F428	eukaryotic translation initiation factor 3 subunit L − *Gallus gallus*	Cy	COa	TA	0.3
Q5ZMB0	putative uncharacterized protein − *Gallus gallus*		MP	CA	0.5
Q5MAJ0	NOGO isoform A2 − *Gallus gallus*	Mb	RBP	PB	0.3
Q5ZLV0	putative uncharacterized protein − *Gallus gallus*	Mb	T	PB	0.5
Q5ZL90	putative uncharacterized protein − *Gallus gallus*	Cy		PB	0.3

[a]The main cellular component (CC), biological process (BP), and molecular function (MF) are presented for each protein. The peptide count corresponds to peptides with a FDR < 1.3%. The protein abundance index (PAI) is calculated as indicated under Materials and Methods. Cellular component abbreviations: Cy, cytoplasml; Csk, cytoskeleton; EC, extracellular; Go, Golgi apparatus; Mb, membrane; Nu, nucleus; Pr, proteasome;

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Table 5. continued

Ri, ribosome; Sp, spliceosome. Biological process abbreviations: CDi, cell differentiation; CM, cell motility; CO, cell organization; COa, coagulation; DR, defense response; MP, metabolic process; RBP, regulation of biological process; RS, response to stimulus; T, transport. Molecular function abbreviations: CA, catalytic activity; E, enzyme regulator activity; MB, metal ion binding; NB, nucleotide binding; PB, protein binding; Sig, signal transducer activity; Str, structural molecule activity; T, transporter activity; TA, translation regulator activity.

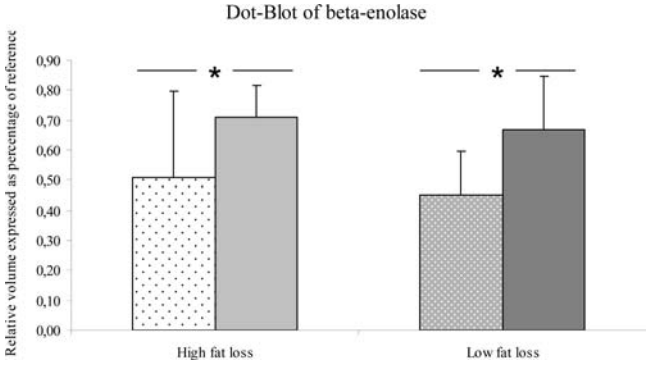

Figure 2. Relative amounts of β-enolase protein obtained by using dot blots from IS protein fraction at 20 min (with dots) and at 6 h (full color) post mortem (mean ± standard deviation, n = 13 and 11 for low and high fat loss, respectively).

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