**A Straightforward Gel-Free Proteomics Pipeline Assisted by Liquid
Isoelectric Focusing (OFFGEL) and Mass Spectrometry Analysis to Study
Bovine Meat Proteome**

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Abstract

Bovine sarcoplasmic sub-proteome was studied through a straightforward gel-free pipeline supported by liquid isoelectric focusing protein fractionation (OFFGEL) coupled to LC-MS analysis. Full-MS and data-dependent MS/MS analyses were simultaneously performed by a conventional three-dimensional ion-trap (3D-IT) addressing targeted quantitative and untargeted qualitative research, respectively. There were unambiguously identified 47 proteins distributed along twelve OFFGEL fractions assayed. Regarding intermediate- and high-abundant peptides, bulky quantitative data processing performed by MZmine 2 freeware yielded a satisfactory linearity and coefficient of variation with r^2 in the 0.95-0.99 range and about 25%, respectively. Up to 41 peptides from 20 identified proteins were relatively quantified throughout OFFGEL fractions.

This reliable, flexible and affordable gel-free proteomic approach could be readily implemented by industry to improve quality assessment of protein-based food products.

Keywords: Proteomics; food proteins, quality assessment; chromatography analysis.

INTRODUCTION

Continuous innovations in “omic” technologies move researchers towards the generation of voluminous data that needs to be appropriately refined to achieve concise conclusions (Lin et al., 2011). In the particular case of LC-based proteomics and metabolomics in “top” basic research, their current success strongly depends on the use of modern MS detectors merging, totally or partially, features such as high-mass resolution (HRMS), multiplexing capacity, dynamic range, scan speed and sensitivity (Griffiths and Wang, 2009). Uncertainties from conventional low resolution (LRMS) devices were surpassed by actual LC-HRMS hardware that greatly facilitated the design of simple analytical procedures that can also skip the chromatographic separation step through direct infusion mass spectrometry (DIMS) approaches (Southam et al., 2017; Lu et al., 2019). Furthermore, bundles mating modern LC-HRMS detectors and licensed software packages constitute a powerful budget-dependent technology that is not commonly implemented in food industry. Generation of valuable results using conventional, and affordable, LC-LRMS instruments demands special attention regarding sample preparation (i. e. enrichment/purification), chromatographic resolution and optimization of MS settings of analysis to overcome their inherent technical limitations such as low scan rate, sensitivity, signal stability and mass resolution. Data processing also deserves special attention to ameliorate such drawbacks that greatly affect qualitative and quantitative analyses (Bridgewater 2014; Mann and Kelleher, 2008). From this, food industry calls for straightforward, affordable, flexible, robust and reliable high-output “omic” pipelines of analysis supported by conventional LC-LRMS detection (Bu et al., 2016).

Actions addressing sample enrichment greatly help in the betterment of LC-MS sensitivity that, in the particular case of protein analysis, was traditionally guided by gel-based approaches. However, gels have intrinsic disadvantages such as the use of hazardous chemicals (i. e. polyacrylamide), higher susceptibility to contamination, lack of reproducibility, experimental costs and manual work demanding (Monteoliva and Albar, 2004). Recent studies proposed liquid isoelectric focusing (OFFGEL) as a preliminary enrichment/fractionation step followed by SDS-PAGE and LC-MS approaches for biomarkers hunting of meat

tenderness (Beldarrain et al., 2018; Fuente-García et al., 2019). Interestingly, authors emphasized the good reproducibility of the achieved OFFGEL protein profiles in addition to the simplicity of the methodology, suggesting its potential use for gel-free proteomics. To date, there are no studies dealing with the advantages of direct hyphenation of OFFGEL protein fractionation with LC-MS analysis in meat quality research (i. e. discovery of peptide biomarkers related to tenderness or detection of defective Pale Soft Exudative, PSE, and Dark Firm Dry, DFD, meats).

Unlike qualitative analysis, automation in LC-MS quantitative proteomics appeared over the last decade. It was traditionally carried out manually or using inaccessible and intricate in-house programming approaches that hindered their externalization (Wang et al., 2003). Recent popularization of open source programming languages (i. e. R environment) led to release of flexible alternatives for automated quantification (Kohlbacher et al., 2009; Taverner et al., 2012), but their implementation is limited by the need to incorporate skilled programmers into research units. In this line, instability of the signal response of former MS detectors decreases efficiency of algorithms embedded by most of the currently available data processing solutions. All those limitations together with uncertainties from LC-LRMS detection restricted former quantitative proteomic assays to some manually assisted label-free determinations (Sentandreu et al., 2010). Thus, there is a necessity for robust, easy, affordable and reliable alternatives for automated qualitative/quantitative protein research supported by LC-LRMS approaches. The simplest strategy to carry out the automated processing of MS data is given by the classical metabolomics workflow. Large data sets are uploaded into the considered computational application loading an in-house/on-line library listing m/z and retention times of target analytes. Main constraints for positive assignments are mass and retention times tolerances but in the particular case of right peak picking MS¹ filtered peptides, uncertainties from LRMS detection result less harmful than in metabolomics research since target masses use to be above m/z 500. Then, time-aligned identified peaks are integrated and results finally merged in an exportable report. Main differences between programs are the algorithms

considered for peak picking/integration of signals and the possibility of operating as search engines using on-line spectral libraries such as UniprotKB or NCBIprot in the case of proteomic approaches. However, uncertainties from LC-LRMS analysis combined with the impossibility of considering retention time of signals as a constraint negatively affect automated quantification of peptides supported by on-line databases. On the contrary, it is not critical for automated identification of proteins by search engines interrogating MS/MS breakdown patterns of their enzymatically released peptides. In any case, the number of studies addressing qualitative/quantitative gel-free meat proteomics combining OFFGEL fractionation, untargeted/targeted LC-LRMS analysis and automated data processing by open source solutions is negligible.

This research aims at studying sarcoplasmic proteins in detail through a flexible and straightforward pipeline research. OFFGEL protein fractionation was coupled to LC-LRMS determination of tryptic peptides as an easy and flexible gel-free strategy for bulky analysis. Complementary untargeted qualitative and targeted quantitative workflow analysis combining full-MS and data dependent MS/MS (dd-MS²) experiments featured by a conventional three-dimensional ion-trap (3D-IT) was exhaustively evaluated.

This affordable gel-free methodology can be of great interest for food industry to study and enhance protein quality of traded products.

MATERIALS AND METHODS

Chemicals

LC-MS grade ACN, TFA, formic acid (FA), EDTA, Tris, glycerol, urea, thiourea and DTT were from Scharlab (Scharlab S. L., Barcelona, Spain). Ultrapure grade water was from Millipore (EMD Millipore Co., Billerica, MA, USA). Sucrose, protease inhibitor cocktail (P8340) and ammonium bicarbonate were from Sigma-Aldrich (Sigma-Aldrich Co., St. Louis, MO, USA). Bradford Protein Assay Kit was from Bio-Rad (Bio-Rad, Hercules, CA, USA). Immobilized pH gradient gel (IPG) strips and IPG buffer pH 3-10 were from GE healthcare (GE healthcare, Uppsala, Sweden). Modified trypsin was from Promega (Promega, Madison, WI, USA).

Preparation of the internal standards (IS) solution

Wild almond was from a local market (Valencia, Spain) and protein extract was obtained as proposed by Mirzapour et al. (2016) finding a resultant protein content of 10 mg/mL determined by Bradford protein assay (Bradford, 1976). Extracts were tryptically digested (3 h, 37 °C and pH 7.8) at an enzyme/substrate ratio of 1.0% (w/w) and centrifuged at 4000 g for 10 min at 4 °C using an Avanti J-26S XP Beckman Coulter centrifuge (Brea, CA, USA). Supernatants were 1:46 diluted with aqueous 0.1% TFA and aliquots stored at -20 °C until spiked as internal standards (IS) into meat protein samples.

Sample selection

Longissimus Thoracis (LT) muscle was obtained from a 18 months old Limousine veal carcass (Industrias Cárnicas Corella S.L. Venta del Aire, Teruel, Spain) immediately after slaughter and kept at 4 °C. The epimysium was carefully dissected and, at 24 h post-mortem, samples were vacuum packed in 50 g portions and stored at -80 °C until processed for protein extraction.

Extraction of sarcoplasmic proteins

Half a gram of meat was homogenized in 4 mL of extraction buffer (10 mM Tris pH 7.6, 1 mM EDTA, 0.25 M Sucrose) containing 25 µL of inhibitors cocktail using an Ultra-Turrax Yellow Line Di 25 (IKA-Werke GmbH, Staufen, Germany). The homogenate was centrifuged at 20,000 g for 20 min at 4 °C using a Beckman Coulter centrifuge (Brea, CA, USA), being the obtained supernatant filtered through a 45 µm PVDF filter and stored at -80°C until fractionation. Total protein quantification of the extract was assessed by the aforementioned assay.

Gel- free OFFGEL fractionation/enrichment

Sarcoplasmic proteins were separated according to their isoelectric point (pI) in liquid phase along 12 fractions using 13 cm immobilized pH gradient gel (IPG) strips with a linear pH gradient in the range 3-10. A volume containing 1 mg of total protein of the sarcoplasmic extract was considered for protein fractionation, bringing it up to a final volume of 2 mL with 1.25X protein OFFGEL stock

solution (6% v/v glycerol, 7 M urea, 2 M thiourea, 65 mM DTT and IPG buffer pH 3-10) in order to load 150 μ L of this solution on each well. Fractionation was carried out using an Agilent 3100 OFFGEL fractionator (Agilent Tech., Palo Alto, CA, USA) at 20 °C with a constant current of 50 μ A to reach 20 kVh during 20 hours following the manufacturer's instructions. Fractions were separately collected by pipetting, cleaned up using OMIX 100 μ L C18 pipette tips (Agilent Tech. S. L., Madrid, Spain) and recovered acetonitrile eluates completely evaporated by a SPD121P Speed Vac concentrator (Thermo Scientific, San Jose, CA, USA) previously to tryptic digestion.

In-solution trypsin digestion of the OFFGEL protein fractions

Dried fractions were rediluted in 15 μ L of a sequencing grade modified trypsin solution at a 12.5 μ g/mL concentration and mixed with an equal volume of 50 mM ammonium bicarbonate (pH 8.5). The mix was then incubated overnight at 37 °C with continuous gently shaking allowing digestion of proteins by trypsin. After incubation, each fraction was completely evaporated as indicated above and resuspended in 70 μ L of a 0.1% TFA aqueous solution. Fifty μ L of each fraction were separately mixed with 15 μ L of the IS solution in Eppendorf tubes, stirred and centrifuged at 22000 *g* for 3 min prior to be poured into LC-MS vials, constituting the OFFGEL (OFGL) sarcoplasmic fractions batch. The remaining 20 μ L of each fraction were collected in the same Eppendorf tube, giving rise to a final pooled volume of 240 μ L. Seventy μ L of this pool were mixed with 20 μ L of the IS solution in a separate Eppendorf tube, stirred, centrifuged (22000 *g* for 3 min) and poured into a LC-MS vial, constituting the quality control (QC) batch. The remaining 170 μ L of the pooled mix were used to construct the calibration curves of targeted peptides as follows: five different volumes of the mix ranged from 10 to 50 μ L (in 10 μ L steps) were isolately mixed in Eppendorf tubes with 10 μ L of the IS solution and a variable volume of aqueous 0.1% TFA solution to reach a final volume of 60 μ L. Tubes were stirred, centrifuged (22000 *g* for 3 min) and poured into LC-MS vials constituting the calibration (CAL) batch.

LC-MS analysis

Chromatographic analysis was carried out in a Thermo Surveyor LC system with a cooled autosampler, quaternary pump and vacuum degasser coupled to a Thermo LCQ Advantage ion-trap analyzer loading an ESI probe operating in positive ionization mode (Thermo Scientific, San Jose, CA, USA). Peptides separation was achieved by a reverse-phase 150 x 0.5 mm, 4 μ m particle size Jupiter Proteo column from Phenomenex (Phenomenex Inc., Torrance, CA, USA). Mobile phases were solvent A containing 0.1% FA (v/v) in ultrapure water and solvent B containing 0.1% FA (v/v) in ACN with the following separation conditions: initially 0% B, held for 15 min, 0-20% B in 2 min, held for 4 min, 20-40% B in 1 min, held for 8 min, 40-100% B in 1 min, washing with 100% B for 8 min and column equilibration at initial conditions for 55 min. Injection volume was 10 μ L for quality control (QC) and 25 μ L for CAL and OFGL batches. Autosampler and column temperatures were 10 °C and 23 °C, respectively. Flow-rate was set to 50 μ L/min.

Operating conditions of the MS device were: capillary temperature, 250 °C; normalized collision (CID) energy for MS/MS analysis, 35%; spray voltage, 4.0 kV; capillary voltage, 15.0 V. Injection method merged two scan events: full-MS analysis of intact peptide masses in the 400-2000 m/z range followed by a dd-MS² experiment of most intense ions from full-MS scan with +1 to +4 charges with the following dynamic parameters: minimum MS/MS ion intensity threshold, 1×10^5 ; exclusion list, 25 masses including those from spiked almond ISs and background noise from a blank injection; exclusion time, 3 min; exclusion mass width, 3 amu; repeat count for MS/MS of most intense ion, 3; repeat count duration, 0.5 min. Regarding sensitivity and scan speed of the mass analyzer the number of microscans-maximum injection time were 1-200 ms and 1-300 ms for full-MS and dd-MS², respectively. Control of the LC-MS system and manual data processing was featured by a PC loading the Thermo Xcalibur v2.04 software (Thermo Sci., San Jose, CA, USA).

The LC-MS device continuously operated until completing the sample list analysis (about 40 hrs) to test robustness of determinations. Then, QC vial was injected at the beginning, middle and end (n=3) of the OFGL batch sequence (n=12), finalizing the sample list with the CAL batch analysis (n=5).

Untargeted protein identification analysis (protein mapping)

Sarcoplasmic protein were identified interrogating dd-MS² data from OFGL batch analysis using a licensed copy of Mascot v2.3 search engine (www.matrixscience.com) loading the UniprotKB (www.uniprot.org) and NCBIprot protein databases with the following settings: enzyme, trypsin; no fixed or variable modifications but enabled the “Error tolerant” option; mass accuracy; 1.2 and 0.8 Da for full MS and MS/MS scans, respectively; taxonomy restriction parameter, Mammalia. The decoy option was used to estimate the false positive rates by means of False Discovery Rate (FDR) threshold 1%. Only those identifications with a protein score derived from individual ion scores indicating identity or extensive homology ($p < 0.05$) were considered as true protein identifications.

Tryptic almond peptides of the IS solution were partially characterized as mentioned above but using “green plants” as a taxonomy restriction parameter.

Targeted peptide quantification analysis

Full-MS data from OFGL batch analysis was manually processed using Xcalibur to study peak symmetry of signals corresponding to peptides that positively covered previously identified proteins. Mass tolerance considered was 0.5 Da and assignments were manually inspected by matching experimental MS/MS breakdown patterns with those detailed in Mascot search. Thus, an in-house library (.csv format, **Table S1**) was built listing intact masses and respective retention times of identified sarcoplasmic peptides with acceptable peak shape. Database also listed 16 known/unknown almond peptides (IS) used to normalize quantitative results. Since peptides at m/z 903.1 and 949.2 were shared by alpha and beta enolases, their automated determination will address the achievement of a unique alpha/beta enolase assignment.

Proteomic research assisted by LC-LRMS detection conventionally addressed qualitative analysis and then, only MS/MS data used to be relevant independently of symmetry and retention time reproducibility of chromatographic peaks achieved by Full-scan analyses (i.e. Full-MS, Multiple Reaction Monitoring (MRM) and All Ion Fragmentation (AIF)). In contrast, success in automated quantitation strongly depends on chromatographic-

related features affecting peak area integration and alignment. As an example, **Figure S1** illustrates acceptable (**S1A**) and non-acceptable (**S1B**) Full-MS signals (in gray) of ions at m/z 1045.5 and 927.7 corresponding to peptides of hemoglobin subunit beta and glutathione-S-transferase found in OFGL fraction 9, respectively. Peak asymmetry constrained quantitative analysis since many signals were tentatively identified (see **Figure S1C** for Mascot search of ion at m/z 927.7) but no further quantified as consequence of their poor peak shape (not considered in **Table S1**). Similarly, a minimum number of scans across the chromatographic peak is needed to achieve correct area integrations of Full-scan signals. Low scan rate of affordable analyzers that can be of interest for food industry greatly compromise quality of quantifications. Such setback must be handled through the appropriate optimization of the scanning acquisition according to the width of chromatographic peaks and the number of multiplexed scan events, thus reaching a good compromise between qualitative and quantitative approaches. In our case, peak width of peptides listed in **Table S1** was rather wide (1 to 2 min) as consequence of the diffusion effects from injecting large volumes into a 0.5 mm i. d. chromatographic column. Under MS conditions assayed the Full-MS scan rate was 1 scan/1.8 sec, giving 35-60 scans/peak that was translated into the obtaining of irregularly shaped signals as consequence of loading former unstable ESI probes.

Considering the above, there was performed a preliminary screening of freely available computational solutions addressing the automated quantitative analysis of batches studied. Freeware MZmine 2 v.2.53 (<https://github.com/mzmine/mzmine2/releases>) was finally selected since it exhibited important advantages such as its user-friendly interface, processing of Thermo RAW data files without a preliminary format conversion (i. e. mzXML, mzML etc.), wide mass and retention time tolerances, low hardware requirements and algorithm of analysis yielding comparable results than those from manual Xcalibur processing. In this line, other licensed and open-source alternatives such as Thermo Sieve 2.2.58 SP2 and Maven_682, respectively, achieved very unsatisfactory results (data not shown). The well-formatted customized library loaded was **Table S1** whereas automated workflow and optimized settings used are summarized in **Table S2**.

RESULTS AND DISCUSSION

Supplementary **Figure S2** displays a graphical summary of the workflow followed in this research to ease understanding of the proposed proteomic pipeline.

Untargeted qualitative analysis

Coverage of the exploratory proteome research of OFGL samples yielded the unambiguous identification of 47 sarcoplasmic proteins. **Table S3** shows protein distribution across the 12 OFFGEL fractions sorted by their molecular (Mr) weight and pH range assayed. In contrast to previously stated regarding **Table S1**, alpha and beta enolases were differentiated by Mascot analysis since MS/MS patterns from discriminant peptides were considered. Qualitative analysis addressed majority of identifications to *Bos taurus* species with only six exceptions (bolded in **Table S3**) since protein databases loaded did not list sequences of such proteins from bovine species. Moreover, efficiency of fractionation was evidenced when highest Mascot scores (in brackets at ID cells in **Table S3**) were generally achieved in fractions whose experimental pH range met, or was close to, the theoretical pI of positive protein assignments. Apparition of proteins in fractions with a far or neighboring pH range from their theoretical pI (i. e. glyceraldehyde-3-phosphate dehydrogenase and myoglobin, respectively) can be explained by post-translational modifications such as phosphorylations or acetylations (Franco et al., 2015; Meisrimler and Lühje, 2012).

Targeted quantitative analysis of peptides

As stated above, comparable peak area integrations of peptides were reached by MZmine and Xcalibur in a preliminary screening to optimize the scan rate of the MS system. After that, it was carried out a more detailed validation assessment of quantitative analysis by processing QC data.

Firstly, we must consider that customized library (**Table S1**) was built from the analysis of the OFGL batch (25 μ L as injection volume), finding shorter retention times for assignments from QC injections (10 μ L of injection volume). Then,

automated processing of QC batch loaded such database but listing reduced retention times (-0.5 and -1.5 min for assignments up to the first 10 min of run time and from 55 min onwards, respectively). Secondly, integrated peak areas of positive assignments (sarcolemmic peptides and ISs) from QC, CAL and OFGL analyses were normalized in this work by the closest IS eluted (analyte/IS ratio) to relatively quantify samples. Differences in the election of IS normalizers between batches can occur since averaged retention times of assignments varied by different injection volumes assayed.

Figure S3 illustrates manual (A to C) and automated (D to F) peak area integration of low- (m/z at 1012.3, **Figures S3A and S3D**), medium- (m/z at 748.3, **Figures S3B and S3E**) and high- (m/z at 1222.3, **Figures S3C and S3F**) abundant peptides found in QC2 run (center of the injection sequence of OFGL batch, see section 2.7) from phosphoglycerate kinase 1, myoglobin and unknown IS proteins, respectively. Comparable results were reached by Xcalibur and MZmine data processing, finding similar results in most assignments throughout QC samples (Xcalibur vs MZmine, **Table 1**). Automated results were IS normalized and ratios achieved led to evaluate reproducibility among QC injections (3 technical replicates). From this, there was established a confidence threshold whereby peak areas automatically integrated with values above 7×10^6 were generally comparable with those from manual processing and yielded reproducible results with acceptable deviations (up to 25% of coefficient of variation, CV, bolded in **Table 1**). Such threshold was influenced by inherent sensitivity limitations of 3D-ITs aggravated by interferences from using low-mass resolution detection that increased its overall value. Obviously, harmfulness of such limitations was lower for signals unaffected by mass uncertainties as shown by the low-abundant phosphoglycerate kinase 1-4 peptide ~~in~~ (**Figures S3A-S3D and Table 1**), suggesting their potential use as robust peptide biomarkers in meat quality assessment. Overall, reliability of this methodology was mostly limited to medium- and high- abundant (integrated peak area values from 7×10^6 onwards) peptides under instrumental conditions assayed.

Upon defining validation boundaries, next step was to test linearity of those sarcoplasmic peptides that exhibited acceptable results in the QC experiment (15, bolded in **Table 1**). Automated quantitative analysis of CAL batch (**Table 2**) yielded 18 calibration curves of bovine peptides and included those from triosephosphate isomerase-1, actin-2 and Protein/nucleic acid deglycase DJ 1-3 that were close to reach acceptable results in the preliminary QC study (25%<CV<50%, **Table 1**) finding, as expected, that retention times of assignments met those listed in **Table S1**. Linearity achieved was rather good in all cases (r^2 ranged between 0.95 and 0.99) as shown by **Table 2** and **Figure S4**. Among these meaningful results, triosephosphate isomerase-1, actin-2 and Protein/nucleic acid deglycase DJ 1-3 peptides confirmed the relevance of reaching the confidence threshold in their peak area integrations (**Table 2**). Similarly to LC-MS-based metabolomics, peptides with lower experimental masses (about m/z <850) had the weakest results ($r^2 \approx 0.95$) since they were more prone to deviations exerted by LRMS detection.

Results from pooled samples (QC and CAL batches) may suggest feasibility of direct quantitative peptide analysis of raw (unpurified/unfractionated) protein extracts once completing the preliminary qualitative approach. Further research is needed about the potential use of this methodology for routine analysis.

The final step of the proposed proteomics pipeline was to perform the automated quantitative analysis of OFGL batch. As a constraint, integrated peak areas below 7×10^6 were discarded to ensure reliability of determinations. **Table S4** shows direct automated peak area integration and IS normalized results of 41 sarcoplasmic peptides found in OFFGEL fractions studied. Then, normalized ratios for each identified peptide were divided by the lowest value reached by same assignment across pH ranges assayed (**Table S5**) to calculate the fold change variation. This representation facilitated the elucidation of representative peptides (bolded in **Table S5**) from identified proteins according to their presence in fractions. As an example, we have creatine kinase M with three quantitative peptides (**Table S1**) but among them, peptide at m/z 893.8 was present in all detectable fractions (1, 7, 8, 9, 10, 11 and 12) addressing the identification of the parent protein. In contrast, peptides

at m/z 998.3 and 754.7 appeared in only five (7, 8, 9, 10 and 11) and three (7, 8 and 9) of those fractions, respectively. Summation of the fold change of peptides belonging to the same parent protein detailed in **Table S5** enabled visualization of the OFFGEL distribution of 20 muscle sarcoplasmic proteins, not peptides, achieved by the targeted quantitative approach (**Table 3A**). Results (sorted by molecular weight) were comparable with those regarding same proteins (**Table 3B**) previously characterized by the untargeted qualitative study and listed in **Table S3**. Interestingly, normalized protein abundances shown by **Table 3A** generally followed the same trend than Mascot scores in **Table 3B**, suggesting that higher protein abundances were translated into higher scores.

Compared to **Table S3**, number of proteins listed in **Table 3A** must be lower since many of identified peptides by Mascot analysis had a poor chromatographic response, thus hindering quantitative analysis as previously mentioned. Moreover, automated quantitative processing of OFGL data was also constrained by the integrated peak area confidence threshold considered, which evidently compromised determination of low abundant peptides (i. e. absence of myoglobin peptides in OFFGEL fraction 1 in **Table 3A**). Improving chromatographic features of signals (through narrower and better-shaped peaks) and/or using LC-HRMS approaches that greatly raise sensitivity and selectivity of analyses can surpass such restriction. In contrast, apparition of peptides in OFGL experiment not previously mentioned by the QC study (i. e. at m/z s 907.5 and 911.6 from phosphoglucosmutase-1 and histidine triad nucleotide-binding protein 1, respectively, **Table S4** and **Table 3A**) demonstrated efficiency of the OFFGEL fractionation as a protein enrichment strategy.

Independently of the different experimental conditions assayed to analyze batches, well-established ratios among ISs must remain constant all time. To test such assumption, **Table S6** summarizes overall robustness of the automated quantitative analysis since ISs in QC (3 technical replicates), CAL (5 technical replicates) and OFGL (12 technical replicates) approaches were equally normalized through pairs initially proposed in QC study (**Table 1**). In

general, averaged ratios from different batches were rather comparable and confirmed reliability of the methodology proposed as shown by global CVs achieved (up to 20% in most cases, **Table S6**) considering 20 technical replicates.

CONCLUSIONS

It was demonstrated the efficiency of the proposed straightforward gel-free pipeline analysis to perform reliable qualitative/quantitative proteomic approaches supported by conventional LC-MS analysis. Protein enrichment carried out by OFFGEL fractionation directly hyphenated to optimized LC-LRMS analysis greatly facilitated sampling procedure and improved sensitivity in the detection of tryptic peptides derived from muscle sarcoplasmic proteins.

Implementation of this robust, flexible, reliable and affordable LC-MS proteomic-based methodology is extremely easy and can promote the creation of new insights in proteome research of foodstuffs by industry to obtain higher quality products.

ACKNOWLEDGEMENTS

The Department of Economic Development & Competitiveness of the Basque Government is acknowledged for the doctoral fellowship of C. Fuente-García. This work was funded by the Spanish Instituto Nacional de Investigación y Tecnología Agraria y Alimentaria (RTA2014-00034-C04).

CONFLICT OF INTEREST

Authors declare no conflict of interest.

ACCESSIBILITY OF RESEARCH MATERIAL

Authors declare that any underlying research materials related to this research can be accessed.

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FIGURE CAPTIONS

Figure S1. Full-MS signals with acceptable (A) and non-acceptable (B) peak symmetry for automated quantification corresponding to peptides at m/z 1045.5 and 927.7 from hemoglobin subunit beta and glutathione-S-transferase proteins, respectively, found in OFGL fraction 9. Positive identification of glutathione-S-transferase through MS/MS Mascot analysis of peptide at m/z 927.7 (C).

Figure S2. Summary of the proposed gel-free LC-MS proteomic workflow.

Figure S3. Manual (A to C, shown as AA) and automated (D to F, shown as Area) peak area integration of m/z values at 1012.3 (A and D), 748.3 (B and E) and 1222.3 (C and F) corresponding to phosphoglycerate kinase 1-4, myoglobin-3 and IS almond (unknown)-7 peptides, respectively.

Figure S4. Linearity achieved in CAL batch analysis by peptides with coefficients of variation (CV) up to 50% in the preliminary QC validation assessment (**Table 1**).

Table 1. Reproducibility of automated quality control (QC) batch processing.

m/z [M+H] ⁺	Rt (min)	row identity (main ID)	^a QC1 Xcalibur	^a QC1 MZmine	^a QC2 Xcalibur	^a QC2 MZmine	^a QC3 Xcalibur	^a QC3 MZmine	^b IS normalizer	^c QC 1 normalized	^c QC 2 normalized	^c QC 3 normalized	Norm. mean	Norm. std dev	^d CV (%)
519.2	1.96	IS, Almond (unknown)-1	2.66E+07	2.47E+07	2.42E+07	2.16E+07	2.40E+07	2.33E+07	IS, Almond (unknown)-2	1.0007	0.9595	1.2313	1.0638	0.1465	13.8
941.4	1.99	beta enolase-1	1.15E+07	1.24E+07	1.45E+07	1.49E+07	1.67E+07	1.57E+07	IS, Almond (unknown)-1	0.5027	0.6896	0.6730	0.6218	0.1034	16.6
968.2	2.02	Galectin 1-1	1.90E+06	2.61E+05	1.96E+06	8.80E+05	1.98E+06	4.74E+05	IS, Almond (unknown)-1	0.0106	0.0406	0.0203	0.0239	0.0153	64.3
629.2	2.03	Myoglobin- 2	6.24E+06	5.41E+06	2.01E+06	1.54E+06	4.47E+06	2.44E+06	IS, Almond (unknown)-1	0.2195	0.0713	0.1047	0.1319	0.0777	59.0
978.6	2.35	Actin-1	1.51E+06	8.66E+05	6.27E+06	2.35E+06	6.66E+06	5.12E+06	IS, Pru 2 partial-3	0.0144	0.0425	0.0997	0.0522	0.0435	83.4
1002.4	2.39	Serum albumin	2.24E+06	5.00E+05	4.72E+06	1.75E+06	2.10E+06	6.40E+05	IS, Pru 2 partial-3	0.0083	0.0317	0.0125	0.0175	0.0125	71.4
727.5	2.46	Protein/nucleic acid deglycase DJ 1-1	9.36E+05	6.27E+05	2.32E+06	1.26E+06	9.62E+05	3.65E+05	IS, Pru 2 partial-3	0.0104	0.0229	0.0071	0.0135	0.0083	61.9
955.7	2.51	IS, Pru 2 partial-3	6.03E+07	6.03E+07	5.45E+07	5.52E+07	5.25E+07	5.14E+07	IS, Almond (unknown)-1	2.4452	2.5520	2.2038	2.4003	0.1784	7.4
748.3	2.77	Myoglobin-3	8.20E+06	8.03E+06	1.07E+07	1.15E+07	9.19E+06	8.63E+06	IS, Almond (unknown)-2	0.3259	0.5097	0.4358	0.4238	0.0925	21.8
954.4	2.95	Triosephosphate isomerase-1	4.81E+06	5.05E+06	7.62E+06	6.48E+06	8.40E+06	7.47E+06	IS, Almond (unknown)-2	0.2048	0.2874	0.3774	0.2899	0.0863	29.8
593.1	2.96	IS, Almond (unknown)-2	2.53E+07	2.46E+07	2.34E+07	2.26E+07	1.98E+07	1.98E+07	IS, Prunin 1 precursor-3	0.7904	0.7047	0.6302	0.7084	0.0802	11.3
1162.8	3.13	IS, Prunin 1 precursor-3	2.87E+07	3.12E+07	2.90E+07	3.20E+07	2.91E+07	3.00E+07	IS, Pru 2 partial-3	0.5171	0.5795	0.5848	0.5605	0.0377	6.7
1055.2	3.78	IS, Pru 2 partial-1	9.35E+07	9.60E+07	9.29E+07	9.77E+07	9.43E+07	9.47E+07	IS, Almond (unknown)-3	2.8764	2.7249	2.8472	2.8162	0.0803	2.9
734.3	4.00	Phosphoglycerate kinase 1-1	3.53E+06	1.45E+06	2.19E+06	9.26E+05	6.25E+06	2.86E+06	IS, Almond (unknown)-1	0.0588	0.0428	0.1229	0.0748	0.0424	56.6
1245.4	4.35	IS, Almond (unknown)-3	3.39E+07	3.34E+07	3.63E+07	3.59E+07	3.43E+07	3.33E+07	IS, Prunin 1 precursor-3	1.0712	1.1197	1.1074	1.0994	0.0252	2.3
718.6	4.71	IS, Prunin 1 precursor-1	1.06E+07	1.00E+07	1.08E+07	1.10E+07	8.91E+06	9.42E+06	IS, Almond (unknown)-3	0.3003	0.3079	0.2833	0.2972	0.0126	4.2
1026.3	5.19	IS, Almond (unknown)-4	2.72E+07	2.76E+07	3.11E+07	3.08E+07	3.01E+07	2.96E+07	IS, Prunin 1 precursor-1	2.7556	2.7877	3.1383	2.8939	0.2123	7.3
770.6	5.68	Triosephosphate isomerase-2	1.19E+07	1.17E+07	1.19E+07	1.18E+07	1.24E+07	1.22E+07	IS, Almond (unknown)-4	0.4234	0.3830	0.4139	0.4067	0.0211	5.2
595.0	63.04	IS, Prunin 2 precursor partial-2	7.30E+06	7.26E+06	8.05E+06	7.81E+06	8.53E+06	8.72E+06	IS, Prunin 2 precursor partial-1	0.2921	0.2612	0.3871	0.3135	0.0656	20.9
687.0	63.09	IS, Prunin 2 precursor, partial-1	2.43E+07	2.49E+07	2.86E+07	2.99E+07	2.11E+07	2.25E+07	IS, Almond (unknown)-8	1.0541	1.1547	0.9195	1.0428	0.1180	11.3
893.6	63.10	Creatine kinase M- 3	1.21E+07	1.27E+07	1.46E+07	1.49E+07	1.20E+07	1.25E+07	IS, Prunin 2 precursor partial-1	0.5096	0.4991	0.5526	0.5205	0.0283	5.4
1056.4	63.39	Phosphoglycerate kinase 1-2	9.25E+06	9.21E+06	1.00E+07	1.01E+07	8.76E+06	8.81E+06	IS, Prunin 2 precursor partial-1	0.3707	0.3375	0.3908	0.3663	0.0269	7.3
1123.8	63.50	Actin-2	4.97E+06	3.08E+06	3.42E+06	2.80E+06	5.52E+06	5.30E+06	IS, Prunin 2 precursor partial-1	0.1238	0.0938	0.2350	0.1509	0.0744	49.3
876.6	64.51	Malate deshydrogenase-1	3.52E+06	1.49E+06	3.84E+06	3.69E+05	2.86E+06	1.91E+06	IS, Pru 2 partial-2	0.0804	0.0199	0.1132	0.0712	0.0474	66.5

992.2	64.53	Phosphoglycerate kinase 1-3	7.05E+06	7.48E+06	6.97E+06	6.24E+06	7.07E+06	6.31E+06	IS, Pru 2 partial-2	0.4045	0.3360	0.3733	0.3712	0.0343	9.2
879.1	64.90	IS, Pru 2 partial-2	1.87E+07	1.85E+07	1.88E+07	1.86E+07	1.68E+07	1.69E+07	IS, Prunin 2 precursor partial-1	0.7444	0.6217	0.7498	0.7053	0.0725	10.3
1317.9	64.95	IS, Almond (unknown)-8	2.21E+07	2.36E+07	2.45E+07	2.59E+07	2.34E+07	2.45E+07	IS, Pru 2 partial-2	1.2744	1.3930	1.4505	1.3727	0.0898	6.5
903.1	65.15	alpha/beta enolase-3	1.78E+07	1.71E+07	1.73E+07	1.69E+07	1.61E+07	1.71E+07	IS, Pru 2 partial-2	0.9249	0.9092	1.0128	0.9490	0.0558	5.9
1012.3	65.57	Phosphoglycerate kinase 1-4	4.41E+06	4.06E+06	4.88E+06	4.20E+06	5.58E+06	4.65E+06	IS, Pru 2 partial-2	0.2192	0.2259	0.2755	0.2402	0.0307	12.8
743.3	65.73	Triosephosphate isomerase-3	3.79E+06	3.01E+06	3.12E+06	3.42E+06	2.71E+06	2.60E+06	IS, Pru 2 partial-2	0.1630	0.1839	0.1549	0.1673	0.0150	9.0
1292.9	66.98	Protein/nucleic acid deglycase DJ 1-3	2.76E+06	2.81E+06	5.08E+06	4.88E+06	4.45E+06	2.08E+06	IS, Almond (unknown)-5	0.1649	0.2518	0.1089	0.1752	0.0720	41.1
754.7	67.05	Creatine kinase M-2	4.20E+06	3.15E+06	5.10E+06	4.68E+06	3.45E+06	2.78E+06	IS, Almond (unknown)-5	0.1846	0.2412	0.1456	0.1905	0.0481	25.3
802.1	67.08	Triosephosphate isomerase-4	1.03E+07	1.04E+07	1.06E+07	1.00E+07	1.10E+07	1.03E+07	IS, Almond (unknown)-5	0.6098	0.5159	0.5403	0.5553	0.0488	8.8
1045.8	68.16	Hemoglobin subunit beta	3.57E+06	3.98E+06	5.13E+06	4.73E+06	3.91E+06	3.30E+06	IS, Almond (unknown)-5	0.2333	0.2443	0.1729	0.2168	0.0384	17.7
1275.5	68.70	Heat shock prot beta6-2	5.40E+06	6.96E+06	6.31E+06	5.43E+06	4.84E+06	1.46E+06	IS, Almond (unknown)-5	0.4080	0.2801	0.0765	0.2549	0.1672	65.6
1288.4	68.72	IS, Almond (unknown) 5	1.63E+07	1.71E+07	1.70E+07	1.94E+07	1.67E+07	1.91E+07	IS, Almond (unknown)-6	0.1861	0.2053	0.1997	0.1970	0.0099	5.0
850.8	68.81	Heat shock prot beta6-1	3.88E+06	3.51E+06	3.19E+06	2.66E+06	4.59E+06	3.33E+06	IS, Almond (unknown)-5	0.2058	0.1373	0.1744	0.1725	0.0343	19.9
1088.8	69.23	IS, Prunin 1 precursor 2	3.00E+07	2.95E+07	3.24E+07	3.42E+07	2.98E+07	3.07E+07	IS, Almond (unknown)-5	1.7287	1.7668	1.6055	1.7003	0.0843	5.0
863.2	69.31	Galectin 1-2	6.25E+06	5.21E+06	6.27E+06	5.69E+06	6.01E+06	6.03E+06	IS, Prunin 1 precursor-2	0.1766	0.1663	0.1967	0.1799	0.0155	8.6
1236.4	70.28	IS, Almond (unknown)-6	9.19E+07	9.16E+07	9.32E+07	9.44E+07	9.62E+07	9.57E+07	IS, Prunin 1 precursor-2	3.1079	2.7570	3.1191	2.9947	0.2059	6.9
1139.3	72.39	Glyceraldehyde-3-phosphate dehydrogenase	2.83E+07	3.13E+07	3.32E+07	3.38E+07	3.22E+07	3.55E+07	IS, Almond (unknown)-7	0.6787	0.6301	0.7209	0.6766	0.0454	6.7
1222.3	73.22	IS, Almond (unknown)-7	4.45E+07	4.61E+07	5.39E+07	5.36E+07	4.85E+07	4.93E+07	IS, Almond (unknown)-6	0.5027	0.5677	0.5152	0.5285	0.0345	6.5

In bold: Assignments with averaged normalized results exhibiting CVs up to 25%.

^aPeak area integration from manual (Xcalibur) and automated (MZmine) processing of full MS data (scan event 1, see LC-MS analysis section for full description).

^bNormalization of automated data through the closest internal standard (IS) eluted to the considered analyte (analyte/IS).

^cNormalized automated results from ratios defined in b.

^dCV: coefficient of variation expressed as %.

Table 2. ^aRobustness of automated relative quantitation of calibration (CAL) batch analysis and linearity of ^bpeptides assayed.

[M+H] ⁺ m/z	Rt (min)	Peptide	^c A Peak area	^c B Peak area	^c C Peak area	^c D Peak area	^c E Peak area	^d IS normalizer	^e A norm	^e B norm	^e C norm	^e D norm	^e E norm	^f R ²
519.1	2.4	IS, Almond (unknown)-1	6.75E+07	7.92E+07	8.34E+07	7.28E+07	7.32E+07	IS, Almond (unknown)-2	1.1340	1.1852	1.0015	1.2291	1.1544	
941.4	2.8	Beta enolase-1	5.20E+06	1.70E+07	2.23E+07	3.30E+07	3.73E+07	IS, Pru 2 partial-3	0.0330	0.1063	0.1430	0.1932	0.2164	0.9820
955.6	2.9	IS, Pru 2, partial-3	1.57E+08	1.60E+08	1.56E+08	1.71E+08	1.72E+08	IS, Almond (unknown)-1	2.3320	2.0173	1.8697	2.3463	2.3559	
748.3	3.3	Myoglobin-3	6.68E+06	1.73E+07	4.01E+07	3.67E+07	5.40E+07	IS, Almond (unknown)-2	0.1122	0.2586	0.4817	0.6198	0.8524	0.9823
593.1	3.4	IS, Almond (unknown)-2	5.95E+07	6.69E+07	8.33E+07	5.92E+07	6.34E+07	IS, Prunin 1 precursor-3	0.9594	0.8044	0.8647	0.5948	0.6267	
954.2	3.5	Triosephosphate isomerase-1	4.94E+06	1.07E+07	2.03E+07	3.06E+07	3.51E+07	IS, Prunin 1 precursor-3	0.0796	0.1287	0.2106	0.3073	0.3469	0.9906
1162.8	3.6	IS, Prunin 1 precursor-3	6.20E+07	8.31E+07	9.63E+07	9.96E+07	1.01E+08	IS, Pru 2 partial-3	0.3941	0.5200	0.6176	0.5830	0.5867	
1055.2	4.3	IS, Pru 2, partial-1	2.27E+08	2.91E+08	3.21E+08	3.19E+08	3.40E+08	IS, Almond (unknown)-3	4.1006	3.9728	4.0688	3.9871	4.1140	
1245.3	4.9	IS, Almond (unknown)-3	5.55E+07	7.31E+07	7.90E+07	8.01E+07	8.27E+07	IS, Prunin 1 precursor-3	0.8938	0.8798	0.8198	0.8047	0.8174	
718.5	5.6	IS, Prunin 1 precursor-1	3.12E+07	4.02E+07	3.94E+07	3.93E+07	3.84E+07	IS, Almond (unknown)-3	0.5624	0.5494	0.4985	0.4909	0.4651	
1026.2	5.6	IS, Almond (unknown)-4	5.95E+07	7.02E+07	8.08E+07	8.18E+07	1.09E+08	IS, Prunin 1 precursor-1	1.9069	1.7469	2.0521	2.0807	2.8311	
770.5	5.9	Triosephosphate isomerase-2	1.34E+07	2.37E+07	4.41E+07	5.60E+07	6.88E+07	IS, Prunin 1 precursor-1	0.4311	0.5895	1.1196	1.4237	1.7895	0.9896
595.0	64.3	IS, Prunin 2 precursor, partial-2	1.47E+07	2.12E+07	2.75E+07	2.45E+07	2.94E+07	IS, Prunin 2 precursor partial-1	0.1897	0.2457	0.2619	0.2620	0.3112	
686.9	64.4	IS, Prunin 2 precursor, partial-1	7.73E+07	8.62E+07	1.05E+08	9.34E+07	9.44E+07	IS, Almond (unknown)-7	1.6241	1.5153	1.5734	1.4898	1.5323	
893.6	64.4	Creatine kinase M-3	1.13E+07	2.43E+07	4.16E+07	4.66E+07	6.91E+07	IS, Prunin 2 precursor partial-1	0.1466	0.2813	0.3960	0.4989	0.7324	0.9852
1123.7	64.7	Actin-2	4.08E+06	9.68E+06	2.36E+07	2.65E+07	3.75E+07	IS, Prunin 2 precursor partial-1	0.0527	0.1123	0.2242	0.2838	0.3968	0.9769
1056.3	65.0	Phosphoglycerate kinase 1-2	4.68E+06	1.75E+07	2.93E+07	3.86E+07	4.28E+07	IS, Prunin 2 precursor partial-1	0.0605	0.2025	0.2794	0.4130	0.4534	0.9826
992.1	65.8	Phosphoglycerate kinase 1-3	5.04E+06	1.53E+07	2.27E+07	2.83E+07	3.63E+07	IS, Almond (unknown)-8	0.1057	0.2691	0.3406	0.4512	0.5893	0.9925
1318.0	66.0	IS, Almond (unknown)-8	4.76E+07	5.69E+07	6.68E+07	6.27E+07	6.16E+07	IS, Pru 2, partial-2	1.1985	1.0910	1.1096	1.1273	1.4162	
879.1	66.0	IS, Pru 2, partial-2	3.97E+07	5.22E+07	6.02E+07	5.56E+07	4.35E+07	IS, Prunin 2 precursor partial-1	0.5137	0.6049	0.5728	0.5954	0.4608	
903.1	66.4	Alpha/Beta-enolase-3	1.63E+07	2.69E+07	6.11E+07	8.23E+07	1.02E+08	IS, Almond (unknown)-8	0.3423	0.4723	0.9156	1.3126	1.6509	0.9825
1012.2	66.7	Phosphoglycerate kinase 1-4	5.39E+06	8.86E+06	1.85E+07	2.35E+07	2.69E+07	IS, Almond (unknown)-8	0.1132	0.1558	0.2769	0.3748	0.4375	0.9888
743.0	67.1	Triosephosphate isomerase-3	2.82E+06	6.06E+06	1.40E+07	1.54E+07	2.06E+07	IS, Almond (unknown)-8	0.0593	0.1065	0.2095	0.2460	0.3351	0.9862
1292.8	68.2	Protein/nucleic acid deglycase DJ 1-3	2.37E+06	5.77E+06	1.20E+07	1.78E+07	2.34E+07	IS, Almond (unknown) 5	0.0710	0.1251	0.1908	0.2957	0.3306	0.9885

802.1	68.2	Triosephosphate isomerase-4	1.26E+07	1.88E+07	4.19E+07	4.55E+07	7.92E+07	IS, Almond (unknown)-5	0.3761	0.4067	0.6689	0.7560	1.1202	0.9456
754.6	68.3	Creatine kinase M-2	2.99E+06	8.92E+06	1.47E+07	1.77E+07	2.37E+07	IS, Almond (unknown)-5	0.0894	0.1934	0.2352	0.2938	0.3352	0.9543
1045.7	69.5	Hemoglobin subunit beta	1.53E+06	4.34E+06	8.66E+06	1.23E+07	1.90E+07	IS, Almond (unknown)-5	0.0457	0.0941	0.1383	0.2038	0.2687	0.9901
1288.3	69.9	IS, Almond (unknown)-5	3.34E+07	4.61E+07	6.27E+07	6.02E+07	7.07E+07	IS, Almond (unknown)-6	0.2006	0.1897	0.2097	0.2006	0.2329	
850.7	70.0	Heat shock protein beta 6-1	2.95E+06	4.80E+06	1.40E+07	1.59E+07	2.64E+07	IS, Almond (unknown)-5	0.0882	0.1040	0.2240	0.2645	0.3734	0.9723
1088.8	70.3	IS, Prunin 1 precursor-2	5.48E+07	8.78E+07	1.02E+08	1.06E+08	1.32E+08	IS, Almond (unknown)-5	1.6396	1.9033	1.6344	1.7638	1.8723	
863.1	70.4	Galectin-1-2	3.02E+06	8.76E+06	2.13E+07	2.13E+07	3.61E+07	IS, Prunin 1 precursor-2	0.0552	0.0997	0.2080	0.2009	0.2727	0.9574
1236.3	71.6	IS, Almond (unknown)-6	1.66E+08	2.43E+08	2.99E+08	3.00E+08	3.04E+08	IS, Prunin 1 precursor-2	3.0399	2.7693	2.9182	2.8258	2.2935	
1139.2	72.7	Glyceraldehyde-3-phosphate dehydrogenase	1.67E+07	3.78E+07	7.35E+07	9.03E+07	1.25E+08	IS, Almond (unknown)-6	0.1002	0.1556	0.2461	0.3009	0.4106	0.9913
1222.2	74.7	IS, Almond (unknown)-7	6.98E+07	1.13E+08	1.46E+08	1.47E+08	1.79E+08	IS, Almond (unknown)-6	0.4195	0.4641	0.4892	0.4909	0.5894	

^aFrom reproducibility of ISs response thorough samples.

^bPeptides from quality control (QC) analysis with coefficients of variation (CVs) up to 50% (**Table 1**)

^cResults from automated peak area integration. Pooled volumes (μL) assayed: A, 10; B, 20; C, 30; D, 40; E, 50.

^dNormalization of data by the closest internal standard (IS) eluted to the considered analyte (analyte/IS). Ratios proposed for ISs normalization were from QC analysis (**Table 1**).

^eNormalized results from ratios defined in b.

^fLinear correlation coefficient from y=mx representation (full graphic details illustrated by **Figure S4**).

Table 3. OFFGEL muscle sarcoplasmic protein distribution achieved by targeted MZmine quantitative (A) a

¹ Targeted MZmine relative quantitative distribution		
Mr (Da)	Protein characterized	Fraction 1
71196	Heat shock cognate 71 kDa	
69248	Serum albumin	
61551	Phosphoglucomutase	
47066	Alpha/beta-enolase	1,0
44509	Phosphoglycerate kinase 1	
42962	Creatine kinase M	10,7
42024	Actin	
36415	Malate deshydrogenase	
35845	Glyceraldehyde 3 phosphate dehydrogenase	
28667	Phosphoglycerate mutase	
26673	Triosephosphate isomerase	
25051	Peroxi redoxin 6	
22379	Heat shock protein beta 1	
21650	Adenylate kinase isoenzyme	
20023	Protein/nucleic acid deglycase DJ 1	
17458	Heat shock protein beta 6	
17067	Myoglobin	
15944	Hemoglobin subunit beta	
14734	Galectin 1	
13770	Histidine triad nucleotide binding protein	

² Untargeted MASCOT qualitative score distribution		
Mr (Da)	Protein characterized	Fraction 1
71196	Heat shock cognate 71 kDa	
69248	Serum albumin	
61551	Phosphoglucomutase	
47066	³ Alpha/beta-enolase	b 67
44509	Phosphoglycerate kinase 1	
42962	Creatine kinase M	66
42024	Actin	
36415	Malate deshydrogenase	
35845	Glyceraldehyde 3 phosphate dehydrogenase	49
28667	Phosphoglycerate mutase	
26673	Triosephosphate isomerase	
25051	Peroxi redoxin 6	
22379	Heat shock protein beta 1	
21650	Adenylate kinase isoenzyme	
20023	Protein/nucleic acid deglycase DJ 1	
17458	Heat shock protein beta 6	
17067	Myoglobin	39
15944	Hemoglobin subunit beta	
14734	Galectin 1	
13770	Histidine triad nucleotide binding protein	

¹Results from summation of the fold change of peptides belonging to the same parent protein

²Results from Mascot scores detailed in **Table S3**.

³Alpha (a) and beta (b) enolases fully distinguished by the untargeted qualitative analysis (**Table S3**)

nd untargeted Mascot qualitative (B) approaches.

Fraction 2	Fraction 3	Fraction 4	Fraction 5	Fraction 6
		2,0	2,7	1,0
				1,4
	9,6	31,9	1,0	1,0
				11,0
				1,4
				4,4
				3,9
				1,0
			7,0	3,1
				34,8
1,0	21,3	3,0		
Fraction 2	Fraction 3	Fraction 4	Fraction 5	Fraction 6
		67	175	69
				a 61
	198	196	30	213
				64
				276
				142
		53	31	50
				36
			28	44
137	137	157		

1 detailed in **Table S5**.

ble S3).

Fraction 7	Fraction 8	Fraction 9	Fraction 10	Fraction 11	Fraction 12
1,0					
3,8	15,8	37,9	26,3	17,7	14,0
		2,2	2,0	8,4	7,0
3,0	18,8	41,4	13,4	9,3	7,1
				2,7	
3,6					
1,0	2,2	4,5	20,1	32,9	32,9
			1,0	2,0	6,8
12,4	53,2	6,9	2,0		
1,0					
1,0			2,4	2,9	2,6
3,4	1,0				
8,8	1,0				
2,0	18,6	22,7	20,8	6,9	1,9
		3,6	4,0	1,9	1,0
1,0					

Fraction 7	Fraction 8	Fraction 9	Fraction 10	Fraction 11	Fraction 12
42,0					
a 164 + b 150	b 228	b 351	b 219	b 225	b 342
			98	258	253
104	328	417	184	176	42
33				36	
89					
74	44	56	371	285	356
				34	95
278	524	396	89	31	
38					
37			118	66	156
72	37				
59	119	628	421	78	154
	43	194	129	120	63
117					

A

B

Table S1. In-house library^a of peptides used for automated MZmine quantitative analysis.

^b Peptide mass (<i>m/z</i>)	Rt (min)	^c Protein ID
978,6	2,8	Actin-1
1123,5	65	Actin-2
1031,2	70,8	Adenylate kinase isoenzyme-1
903,1	66	alpha/beta-enolase-3
949,2	77,5	alpha/beta-enolase-4
941,4	3	beta enolase-1
1296,3	4,1	beta enolase-2
998,2	7,2	Creatine kinase M-1
754,6	67,7	Creatine kinase M-2
893,5	64,4	Creatine kinase M-3
968,5	2,6	Galectin 1-1
863,2	71,2	Galectin 1-2
1139,5	72,5	Glyceraldehyde 3 phosphate dehydrogenase
809,1	65,1	Heat shock cognate 71 kDa-1
1387,7	67,4	Heat shock cognate 71 kDa-2
1597,6	71,1	Heat shock prot beta 1
850,6	69,2	Heat shock prot beta 6-1
1275,4	69,4	Heat shock prot beta 6-2
1045,7	69,3	Hemoglobin subunit beta
911,8	69,9	Histidine triad nucleotide binding protein 1
519,1	2,4	IS, Almond (unknown)-1 ^d
593,1	3,5	IS, Almond (unknown)-2 ^d
1245,4	5,1	IS, Almond (unknown)-3 ^d
1026,3	5,9	IS, Almond (unknown)-4 ^d
1288,4	70	IS, Almond (unknown)-5 ^d
1236,4	71,8	IS, Almond (unknown)-6 ^d
1222,2	75,1	IS, Almond (unknown)-7 ^d
1317,8	66,4	IS, Almond (unknown)-8 ^d
1055,3	4,6	IS, Pru 2 partial-1
879,1	66,4	IS, Pru 2 partial-2
955,7	3	IS, Pru 2 partial-3
718,5	5,8	IS, Prunin 1 precursor-1
1088,8	70,75	IS, Prunin 1 precursor-2
1162,7	3,8	IS, Prunin 1 precursor-3
686,9	65	IS, Prunin 2 precursor partial-1
595,1	65,1	IS, Prunin 2 precursor partial-2
876,5	65,5	Malate deshydrogenase-1
893	70	Malate deshydrogenase-2
797	2,3	Myoglobin-1
629,4	2,4	Myoglobin-2
748,4	3,5	Myoglobin-3
761,7	4	Myoglobin-4
943,5	64,2	Peroxi redoxin-6
907,5	3,2	Phosphoglucomutase
734,3	4,4	Phosphoglycerate kinase 1-1
1056,4	65	Phosphoglycerate kinase 1-2

992,1	65,7 Phosphoglycerate kinase 1-3
1012,2	66,4 Phosphoglycerate kinase 1-4
821,5	69,1 Phosphoglycerate mutase 2
727,5	2,6 Protein/nucleic acid deglycase DJ 1-1
838,6	64,5 Protein/nucleic acid deglycase DJ 1-2
1293	68 Protein/nucleic acid deglycase DJ 1-3
1002,4	3,1 Serum albumin
954,4	3,7 Triosephosphate isomerase-1
770,5	6,7 Triosephosphate isomerase-2
743,1	67,3 Triosephosphate isomerase-3
802,2	68,4 Triosephosphate isomerase-4
1010,9	75 Triosephosphate isomerase-5

^aLibrary must be loaded into MZmine application in .csv format.

^bAnnotation expressed as $[M+H]^+$.

^cNumbered dash means different peptides belonging to the same parent protein.

^dUnknown=almond peptides not assigned to a protein. Internal standard, IS.

Nomenclature used: IS, peptide spiked as internal standard.

Table S2. MZmine settings for automated data processing addressing targeted quantitative analysis of batches assayed.

Workflow step	Main menu option	Sub-folders	Settings
1	Raw data methods	*Raw data import	Upload Thermo .RAW files
2	Raw data methods	Peak detection (Targeted peak detection)	MS level: 1 ^a Peak list file: TableS1.csv ^b Ignore first line: checked ^c Intensity tolerance: 100% ^d Noise level: 1e ⁵ ^e m/z tolerance: 0.5 (absolute)-520 ppm (relative) ^f Retention time tolerance: 1.5 absolute (min)
3	Peak list methods	^g Peak detection (Peak extender)	m/z tolerance: 0.5 (absolute)-520 ppm (relative) Minimum height: 1e ⁴
4	Peak list methods	^h Peak alignment (Join alignment)	m/z tolerance: 0.5 Da (absolute)-520 ppm (relative) Weight for m/z: 100 Retention time tolerance: 1.5 absolute (min) Weight for RT: 100 Require same ID: checked
5	Peak list methods	Export/Import (Export of CSV file)	Selection of filename, destination and Info columns

*Supported files formats: mzML, mzXML, mzData, NetCDF, Thermo native RAW, Waters native RAW.

^aOnly CSV files with columns distribution as detailed in **Table S1** are allowed.

^bFirst line corresponds to columns header of loaded CSV library.

^cValues can vary from symmetric (30) to asymmetric (100) peaks.

^dOnly intensities above the selected level are considered for positive assignments interrogating the loaded library.

^eFinal tolerance range is automatically calculated using maximum of absolute and relative mass shifts for positive assignments. Relative tolerance was calculated for an absolute mass deviation of 0.5 Da and the averaged media of masses (m/z 960) listed in **Table S1**.

^fMaximum deviation allowed between retention times listed in the loaded library and experimental values to achieve positive assignments.

^gExtension of chromatographic peaks of only positive assignments from step 2 by adding surrounding scans matching mass and minimum ion intensities selected.

^hPeak alignment of same positive assignments found in samples processed matching settings selected.

Table S3. Tentative identification of sarcoplasmic proteins distributed across the 12 OFFGEL fractions assayed.

	Protein Mr (Da)	Fraction 1 (3.59 - 4.08)	Fraction 2 (4.08 - 4.56)	Fraction 3 (4.56 - 5.05)
	3 813 652			Titin =Homo sapiens (48) pI 6.02
	772 428		Nebulin=Homo sapiens (40) pI 9.11	
	97 232			
	71 196			
	70 215			
	69 248			
	61 551			
	57 981			
	47 296			
	47 066	Beta-enolase =Bos taurus (67) pI 7.6		
	44 509			
	42 962	Creatine kinase M-type =Bos taurus (66) pI 6.63		
	42 024			Actin, alpha skeletal muscle =Bos taurus (198) pI 5.23
	39 502			
	39 327			
	36 744			
	36 574			
	36 415			
	35 845	Glyceraldehyde-3-phosphate dehydrog =Bos taurus (49) pI 8.50		
	32 817		Tropomyosin beta chain =Bos taurus (51) 4.66	
	32 675		Tropomyosin alpha-1 chain =Bos taurus (66) pI 4.69	

	29 352			
	28 667			
	28 235			
	26 673			
	26 165		Ubiquitin carboxyl-terminal hydrolase isozyme L3 =Bos taurus (39) pI 4.84	
	25 802			
	25 051			
	23 598			
	23 168	Alpha-1-acid glycoprotein =Bos taurus (60) pI 5.62		
	22 379			
	21 650			
	20 919		Myosin light chain 1/3, skeletal muscle isoform =Bos taurus (72) pI 4.96	
	20 024			
	20 023			
	19 000		Myosin regulatory light chain 2, skeletal muscle isoform =Bos taurus (37) pI 4.91	Myosin regulatory light chain 2, skeletal muscle isoform =Bos taurus (103) pI 4.91
	18 964		Protein CutA =Bos taurus (84) pI 8.64	
	18 725			
	18 013	Troponin C, skeletal muscle = Sus scrofa (43) pI 4.06		
	17 953			
	17 458			

	17 067	Myoglobin =Bos taurus (39) pI 6.90		
	16 827	Calmodulin =Bos taurus (49) pI 4.07		
	15 944			
	15 048			
	14 734		Galectin-1 =Bos taurus (137) pI 5.37	Galectin-1 =Bos taurus (137) pI 5.37
	13 770			
Identification (grayed) cells nomenclature: Protein assignment*=Taxonomy (Mascot score) theoretical pI.				
In bold (grayed cells): Taxonomies outside Bos Taurus specie.				
*Protein assignment procedure detailed in main text.				

OFFGEL fractions (pH range ass			
Fraction 4 (5.05 - 5.53)	Fraction 5 (5.53 - 6.02)	Fraction 6 (6.02 - 6.50)	Fraction 7 (6.50 - 6.98)
Titin =Homo sapiens (38) pI 6.02			
Heat shock cognate 71 kDa protein =Bos taurus (67) pI 5.37			
	Heat shock 70 kDa protein 1A =Bos taurus (38) pI 5.68		
	Serum albumin =Bos taurus (175) pI 5.82	Serum albumin =Bos taurus (69) pI 5.82	
			Phosphoglucosmutase-1 =Bos taurus (42) pI 6.36
		Alpha-enolase =Bos taurus (61) pI 6.37	Alpha-enolase =Bos taurus (164) pI 6.37
			Beta-enolase =Bos taurus (150) pI 7.6
			Creatine kinase M-type =Bos taurus (104) pI 6.63
Actin, alpha skeletal muscle =Bos taurus (196) pI 5.23			Actin, alpha skeletal muscle =Bos taurus (33) pI 5.23
	Malate dehydrogenase, cytopl =Bos taurus (30) pI 6.16	Malate dehydrogenase, cytopl =Bos taurus (213) pI 6.16	Malate dehydrogenase, cytopl =Bos taurus (89) pI 6.16
		Glyceraldehyde-3-phosphate dehydrog =Bos taurus (64) pI 8.50	Glyceraldehyde-3-phosphate dehydrog =Bos taurus (74) pI 8.50

	14-3-3 protein gamma =Bos taurus (42) pI 4.80		
		Triosephosphate isomerase =Bos taurus (276) pI 6.45	Triosephosphate isomerase =Bos taurus (278) pI 6.45
			Mitochondrial peptide methionine sulfoxide reductase =Bos taurus (41) pI 7.15
		Peroxiredoxin-6 =Bos taurus (142) pI 6.00.	Peroxiredoxin-6 =Bos taurus (38) pI 6.00.
Heat shock protein beta-1 =Bos taurus (53) pI 5.98	Heat shock protein beta-1 =Bos taurus (31) pI 5.98	Heat shock protein beta-1 =Bos taurus (50) pI 5.98	
			Adenylate kinase isoenzyme 1 =Bos taurus (37) pI 8.40
		Protein/nucleic acid deglycase DJ-1 =Bos taurus (36) pI 6.84	Protein/nucleic acid deglycase DJ-1 =Bos taurus (72) pI 6.84
	Heat shock protein beta-6 =Bos taurus (28) pI 5.95	Heat shock protein beta-6 =Bos taurus (44) pI 5.95	

			Myoglobin =Bos taurus (59) pI 6.90
Galectin-1 taurus (157) pI 5.37	=Bos		
			Histidine triad nucleotide-binding protein 1 =Bos taurus (117) pI 6.28

sayed)		
Fraction 8 (6.98 - 7.47)	Fraction 9 (7.47 - 7.95)	Fraction 10 (7.95 - 8.44)
	Pyruvate kinase PKM =Pongo abelii (72) pl 7.98	
Beta-enolase =Bos taurus (228) pl 7.6	Beta-enolase =Bos taurus (351) pl 7.6	Beta-enolase =Bos taurus (219) pl 7.6
		Phosphoglycerate kinase 1 =Bos taurus (98) pl 8.48
Creatine kinase M-type =Bos taurus (328) pl 6.63	Creatine kinase M-type =Bos taurus (417) pl 6.63	Creatine kinase M-type =Bos taurus (184) pl 6.63
		Fructose-bisphosphate aldolase C =Pan troglodytes (88) pl 6.21
	Fructose-bisphosphate aldolase A =Rattus norvegicus (37) pl 8.31	Fructose-bisphosphate aldolase A =Rattus norvegicus (90) pl 8.31
	L-lactate dehydrogenase A chain =Bos taurus (33) pl 8.12	L-lactate dehydrogenase A chain =Bos taurus (147) pl 8.12
Glyceraldehyde-3-phosphate dehydrog =Bos taurus (44) pl 8.50	Glyceraldehyde-3-phosphate dehydrog =Bos taurus (56) pl 8.50	Glyceraldehyde-3-phosphate dehydrog =Bos taurus (371) pl 8.50

Triosephosphate isomerase =Bos taurus (524) pI 6.45	Triosephosphate isomerase =Bos taurus (396) pI 6.45	Triosephosphate isomerase =Bos taurus (89) pI 6.45
	Glutathione S-transferase P =Bos taurus (94) pI 6.89	
		Adenylate kinase isoenzyme 1 =Bos taurus (118) pI 8.40
	Alpha-crystallin B chain =Bos taurus (31) pI 6.76	
Protein/nucleic acid deglycase DJ-1 =Bos taurus (37) pI 6.84		
Cofilin-2 =Bos taurus (100) pI 7.66		
Ubiquitin-40S ribosomal protein S27a =Bos taurus (129) pI 9.68		

Myoglobin =Bos taurus (119) pI 6.90	Myoglobin =Bos taurus (628) pI 6.90	Myoglobin =Bos taurus (421) pI 6.90
Hemoglobin subunit beta =Bos taurus (43) 7.01	Hemoglobin subunit beta =Bos taurus (194) 7.01	Hemoglobin subunit beta =Bos taurus (129) 7.01
		Profilin-1 =Bos taurus (63) pI 8.46

Fraction 11 (8.44 - 8.92)	Fraction 12 (8.92 - 9.41)
Glycogen phosphorylase, muscle form =Bos taurus (42) pI 6.65	
	Pyruvate kinase PKM =Pongo abelii (57) pI 7.98
Beta-enolase =Bos taurus (225) pI 7.6	Beta-enolase =Bos taurus (342) pI 7.6
Phosphoglycerate kinase 1 =Bos taurus (258) pI 8.48	Phosphoglycerate kinase 1 =Bos taurus (253) pI 8.48
Creatine kinase M-type =Bos taurus (176) pI 6.63	Creatine kinase M-type =Bos taurus (42) pI 6.63
Actin, alpha skeletal muscle =Bos taurus (36) pI 5.23	
Fructose-bisphosphate aldolase C =Pan troglodytes (96) pI 6.21	
Fructose-bisphosphate aldolase A = Rattus norvegicus (177) pI 8.31	Fructose-bisphosphate aldolase A = Rattus norvegicus (77) pI 8.31
Fructose-1,6-bisphosphatase isozyme 2 =Bos taurus (41) pI 7.52	
L-lactate dehydrogenase A chain =Bos taurus (148) pI 8.12	L-lactate dehydrogenase A chain =Bos taurus (60) pI 8.12
Glyceraldehyde-3-phosphate dehydrog =Bos taurus (285) pI 8.50	Glyceraldehyde-3-phosphate dehydrog =Bos taurus (356) pI 8.50

[illegible]

[illegible]

Table S4. Direct automated peak area integration and IS normalized results of peptides found in OFFGE

m/z	Retention time (min)	Peptide identity
941,5	2,27	beta enolase-1
629,3	2,39	Myoglobin-2
519,1	2,40	IS, Almond (unknown)-1
968,3	2,43	Galectin 1-1
1002,1	2,54	Serum albumin
727,6	2,54	Protein/nucleic acid deglycase DJ 1-1
978,6	2,82	Actin-1
955,6	2,91	IS, Pru 2 partial-3
796,8	2,96	Myoglobin-1
748,4	3,03	Myoglobin-3
1296,3	3,08	beta enolase-2
907,4	3,44	Phosphoglucomutase
593,2	3,48	IS, Almond (unknown)-2
1162,8	3,64	IS, Prunin 1 precursor-3
954,4	3,65	Triosephosphate isomerase-1
734,5	4,10	Phosphoglycerate kinase 1-1
1055,2	4,41	IS, Pru 2 partial-1
1245,4	4,86	IS, Almond (unknown)-3
718,6	5,38	IS, Prunin 1 precursor-1
1026,3	5,62	IS, Almond (unknown)-4
770,5	6,53	Triosephosphate isomerase-2
998,3	7,20	Creatine kinase M-1
838,7	63,27	Protein/nucleic acid deglycase DJ 1-2
687,0	64,14	IS, Prunin 2 precursor partial-1
595,0	64,29	IS, Prunin 2 precursor partial-2
893,8	64,40	Creatine kinase M-3
992,1	64,51	Phosphoglycerate kinase 1-3
1123,6	64,58	Actin-2
1056,3	64,97	Phosphoglycerate kinase 1-2
943,5	65,29	Peroxi redoxin-6
876,5	65,29	Malate deshydrogenase-1
1012,0	65,43	Phosphoglycerate kinase 1-4
1318,0	65,67	IS, Almond (unknown)-8
879,1	65,69	IS, Pru 2 partial-2
903,2	65,95	alpha/beta-enolase-3
809,2	66,11	Heat shock cognate 71 kDa-1
743,0	66,57	Triosephosphate isomerase-3
802,1	68,07	Triosephosphate isomerase-4
1045,6	68,12	Hemoglobin subunit beta
754,7	68,32	Creatine kinase M-2
1387,5	68,74	Heat shock cognate 71 kDa-2
1292,7	68,85	Protein/nucleic acid deglycase DJ 1-3
911,6	68,88	Histidine triad nucleotide binding protein 1
821,4	69,14	Phosphoglycerate mutase-2
1288,4	69,52	IS, Almond (unknown)-5
850,7	69,60	Heat shock protein beta 6-1
1088,8	69,87	IS, Prunin 1 precursor-2

1275,5	69,97 Heat shock protein beta 6-2
1031,1	70,03 Adenylate kinase isoenzyme-1
863,1	70,33 Galectin 1-2
892,8	70,51 Malate deshydrogenase-2
1597,6	70,84 Heat shock protein beta 1
1236,4	71,14 IS, Almond (unknown)-6
1139,3	72,11 Glyceraldehyde 3 phosphate dehydrogenase
1010,9	74,08 Triosephosphate isomerase-5
1222,3	74,17 IS, Almond (unknown)-7
948,9	76,09 alpha/beta-enolase-4

*Automated peak area integration from MZmine analysis.

** Normalization by closest IS eluted (IS normalizer defined in Q column).

∴L fractions studied.

*Fraction 1 R/	*Fraction 2 R/	*Fraction 3 R/	*Fraction 4 R/	*Fraction 5 R/	*Fraction 6 R/	*Fraction 7 R/
						8,04E+06
8,20E+07	1,01E+08	7,47E+07 5,18E+07	8,48E+07 7,00E+06	5,95E+07 7,61E+07	7,44E+07 3,50E+07 2,58E+07 1,04E+07	7,87E+07 4,05E+07
1,69E+08	2,05E+08	3,39E+07 1,67E+08	1,03E+08 1,87E+08	1,49E+08	2,05E+08	2,08E+08 1,76E+07 1,43E+07
						3,00E+07
7,05E+07 7,28E+07	7,99E+07 9,18E+07	7,24E+07 8,85E+07	8,06E+07 8,16E+07	6,90E+07 9,72E+07	7,29E+07 1,18E+08	7,61E+07 9,57E+07 6,09E+07
2,67E+08 1,03E+08 2,89E+07 8,70E+07	3,30E+08 1,29E+08 3,71E+07 1,04E+08	3,12E+08 1,14E+08 3,14E+07 8,84E+07	3,11E+08 1,15E+08 3,24E+07 9,33E+07	2,57E+08 1,08E+08 2,56E+07 9,21E+07	3,35E+08 1,16E+08 3,13E+07 9,47E+07 2,81E+07	3,26E+08 1,23E+08 3,39E+07 9,58E+07 9,09E+07 1,75E+07
					1,21E+07	2,32E+07
8,72E+07 2,03E+07 1,00E+08	9,18E+07 2,46E+07	9,97E+07 2,36E+07	9,97E+07 2,78E+07	9,56E+07 2,64E+07	1,00E+08 3,02E+07	9,88E+07 3,33E+07 1,53E+07
		3,31E+07	1,47E+08			
					3,07E+07 8,15E+07	9,62E+06 3,27E+07
				9,27E+06		
5,05E+07 3,67E+07 7,81E+06	6,58E+07 4,41E+07	7,39E+07 5,58E+07	8,41E+07 6,35E+07 8,85E+06	6,86E+07 4,70E+07	7,63E+07 5,53E+07 1,68E+07	9,26E+07 7,43E+07 2,98E+07
					7,01E+06 2,49E+07	2,09E+07 7,35E+07
						7,82E+06
			2,34E+07		3,95E+07	3,12E+07
5,07E+07 7,29E+07	5,75E+07 8,12E+07	6,11E+07 1,01E+08	6,34E+07 1,04E+08	5,06E+07 1,36E+07 8,71E+07	5,60E+07 8,77E+07 9,31E+07	7,60E+07 1,91E+07 1,16E+08

				2,64E+07	1,37E+08	4,23E+07 1,10E+07
	9,03E+06	1,46E+08	2,25E+07		6,87E+07 2,32E+07	2,74E+07
2,58E+08	3,04E+08	3,24E+08	3,46E+08	2,88E+08	3,12E+08 1,74E+07	4,09E+08 1,60E+07
1,15E+08	1,40E+08	1,65E+08	1,66E+08	1,39E+08	1,42E+08	1,93E+08

*Fraction 8 R/ *Fraction 9 R/ *Fraction 10 F *Fraction 11 F *Fraction 12 RAW Peak area

8,03E+07	1,01E+08	8,63E+07	1,97E+07	2,59E+07
	5,93E+07	4,34E+07	2,84E+07	
6,78E+07	5,78E+07	5,02E+07	6,00E+07	7,13E+07

			1,19E+07	
2,05E+08	1,56E+08	1,37E+08	1,42E+08	1,45E+08
2,91E+08	9,39E+07	6,50E+07	2,00E+07	
2,61E+07	1,44E+08	1,25E+08	4,13E+07	1,88E+07
	3,44E+07	1,04E+07	3,70E+07	2,26E+07

6,36E+07	4,58E+07	5,37E+07	6,34E+07	6,47E+07
9,25E+07	7,34E+07	6,20E+07	9,37E+07	9,80E+07
1,94E+08				

			4,97E+07	3,67E+07
3,11E+08	2,64E+08	2,22E+08	3,09E+08	3,39E+08
1,07E+08	9,84E+07	8,40E+07	1,17E+08	1,11E+08
3,02E+07	2,48E+07	2,39E+07	3,13E+07	2,94E+07
9,07E+07	9,46E+07	8,50E+07	8,86E+07	1,01E+08
3,94E+08	5,16E+07	1,57E+07		
1,14E+08	1,95E+08	4,25E+07	1,60E+07	

9,87E+07	1,07E+08	9,67E+07	8,96E+07	8,77E+07
3,83E+07	3,11E+07	2,72E+07	2,80E+07	3,04E+07
1,22E+08	2,33E+08	1,33E+08	1,07E+08	9,89E+07
		4,29E+07	1,21E+08	1,02E+08
			7,03E+06	
			1,73E+08	1,54E+08

9,55E+06				
	7,70E+07	3,10E+07	8,29E+07	9,00E+07
1,22E+08	1,15E+08	1,02E+08	1,06E+08	1,05E+08
7,77E+07	7,96E+07	7,14E+07	6,23E+07	6,64E+07
8,04E+07	2,87E+08	1,19E+08	1,69E+08	1,22E+08

9,74E+07				
3,45E+08	4,81E+07	1,42E+07		
	4,66E+07	3,51E+07	2,40E+07	1,29E+07
3,89E+07	9,07E+07			

4,92E+07

		7,84E+06	1,74E+07	6,07E+07
7,71E+07	6,44E+07	5,62E+07	6,32E+07	6,41E+07

1,41E+08	1,28E+08	9,72E+07	1,10E+08	1,23E+08
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7,24E+06

2,16E+07

3,02E+07

2,93E+07

4,15E+08

3,91E+08

2,83E+08

3,54E+08

3,62E+08

3,57E+07

6,89E+07

2,22E+08

4,55E+08

4,67E+08

1,00E+09

2,34E+08

1,96E+08

1,46E+08

1,88E+08

1,84E+08

2,05E+08

8,53E+07

IS normalizer	**Fraction 1 r	**Fraction 2 r	**Fraction 3 r	**Fraction 4 r	**Fraction 5 r
IS, Almond (unknown) 1	0,0000	0,0000	0,0000	0,0000	0,0000
IS, Almond (unknown) 1	0,0000	0,0000	0,0000	0,0000	0,0000
IS, Almond (unknown) 2	1,1624	1,2599	1,0314	1,0528	0,8623
IS, Almond (unknown) 1	0,0000	0,0000	0,6936	0,0826	0,0000
IS, Almond (unknown) 1	0,0000	0,0000	0,0000	0,0000	1,2786
IS, Almond (unknown) 1	0,0000	0,0000	0,0000	0,0000	0,0000
IS, Pru 2, partial 3	0,0000	0,0000	0,2024	0,5494	0,0000
IS, Almond (unknown) 1	2,0648	2,0397	2,2391	2,2068	2,5082
IS, Pru 2, partial 3	0,0000	0,0000	0,0000	0,0000	0,0000
IS, Pru 2, partial 3	0,0000	0,0000	0,0000	0,0000	0,0000
IS, Pru 2, partial 3	0,0000	0,0000	0,0000	0,0000	0,0000
IS, Almond (unknown) 2	0,0000	0,0000	0,0000	0,0000	0,0000
IS, Prunin 1 precursor 3	0,9694	0,8699	0,8189	0,9877	0,7103
IS, Pru 2, partial 3	0,4298	0,4473	0,5288	0,4358	0,6510
IS, Prunin 1 precursor 3	0,0000	0,0000	0,0000	0,0000	0,0000
IS, Pru 2, partial 1	0,0000	0,0000	0,0000	0,0000	0,0000
IS, Almond (unknown) 3	2,5960	2,5630	2,7417	2,7154	2,3701
IS, Prunin 1 precursor 3	1,4111	1,4032	1,2855	1,4039	1,1156
IS, Almond (unknown) 3	0,2813	0,2878	0,2762	0,2826	0,2361
IS, Prunin 1 precursor 1	3,0135	2,8074	2,8148	2,8833	3,5970
IS, Almond (unknown) 4	0,0000	0,0000	0,0000	0,0000	0,0000
IS, Almond (unknown) 4	0,0000	0,0000	0,0000	0,0000	0,0000
IS, Prunin 2 precursor, partial 1	0,0000	0,0000	0,0000	0,0000	0,0000
IS, Almond (unknown) 8	1,7259	1,3944	1,3477	1,1846	1,3936
IS, Prunin 2 precursor, partial 1	0,2326	0,2681	0,2367	0,2785	0,2760
IS, Prunin 2 precursor, partial 2	4,9374	0,0000	0,0000	0,0000	0,0000
IS, Prunin 2 precursor, partial 2	0,0000	0,0000	0,0000	0,0000	0,0000
IS, Prunin 2 precursor, partial 2	0,0000	0,0000	1,4016	5,2940	0,0000
IS, Prunin 2 precursor, partial 2	0,0000	0,0000	0,0000	0,0000	0,0000
IS, Almond (unknown) 8	0,0000	0,0000	0,0000	0,0000	0,0000
IS, Almond (unknown) 8	0,0000	0,0000	0,0000	0,0000	0,1351
IS, Almond (unknown) 8	0,0000	0,0000	0,0000	0,0000	0,0000
IS, Pru 2, partial 2	1,3748	1,4922	1,3243	1,3250	1,4599
IS, Prunin 2 precursor, partial 1	0,4214	0,4806	0,5603	0,6371	0,4915
IS, Almond (unknown) 8	0,1547	0,0000	0,0000	0,0000	0,0000
IS, Almond (unknown) 8	0,0000	0,0000	0,0000	0,1051	0,0000
IS, Almond (unknown) 8	0,0000	0,0000	0,0000	0,0000	0,0000
IS, Almond (unknown) 5	0,0000	0,0000	0,0000	0,0000	0,0000
IS, Almond (unknown) 5	0,0000	0,0000	0,0000	0,0000	0,0000
IS, Almond (unknown) 5	0,0000	0,0000	0,0000	0,0000	0,0000
IS, Almond (unknown) 5	0,0000	0,0000	0,0000	0,3699	0,0000
IS, Almond (unknown) 5	0,0000	0,0000	0,0000	0,0000	0,0000
IS, Almond (unknown) 5	0,0000	0,0000	0,0000	0,0000	0,0000
IS, Almond (unknown) 5	0,0000	0,0000	0,0000	0,0000	0,0000
IS, Almond (unknown) 5	0,0000	0,0000	0,0000	0,0000	0,0000
IS, Almond (unknown) 6	0,1962	0,1891	0,1887	0,1830	0,1754
IS, Almond (unknown) 5	0,0000	0,0000	0,0000	0,0000	0,2680
IS, Almond (unknown) 5	1,4370	1,4110	1,6597	1,6352	1,7208

IS, Prunin 1 precursor 2	0,0000	0,0000	0,0000	0,0000	0,3028
IS, Prunin 1 precursor 2	0,0000	0,0000	0,0000	0,0000	0,0000
IS, Prunin 1 precursor 2	0,0000	0,1112	1,4363	0,2175	0,0000
IS, Prunin 1 precursor 2	0,0000	0,0000	0,0000	0,0000	0,0000
IS, Almond (unknown) 6	0,0000	0,0000	0,0000	0,0000	0,0000
IS, Prunin 1 precursor 2	3,5460	3,7475	3,1932	3,3411	3,3130
IS, Almond (unknown) 6	0,0000	0,0000	0,0000	0,0000	0,0000
IS, Almond (unknown) 7	0,0000	0,0000	0,0000	0,0000	0,0000
IS, Almond (unknown) 6	0,4441	0,4601	0,5097	0,4806	0,4831
IS, Almond (unknown) 7	0,0000	0,0000	0,0000	0,0000	0,0000

**Fraction 6 r	**Fraction 7 r	**Fraction 8 r	**Fraction 9 r	**Fraction 10	**Fraction 11	**Fraction 12
0,0000	0,1022	1,1834	1,7468	1,7192	0,3281	0,3639
0,0000	0,0000	0,0000	1,0258	0,8650	0,4741	0,0000
1,0210	1,0338	1,0671	1,2615	0,9356	0,9457	1,1025
0,0000	0,0000	0,0000	0,0000	0,0000	0,0000	0,0000
0,4695	0,0000	0,0000	0,0000	0,0000	0,0000	0,0000
0,3469	0,5147	0,0000	0,0000	0,0000	0,0000	0,0000
0,0507	0,0000	0,0000	0,0000	0,0000	0,0842	0,0000
2,7591	2,6468	3,0248	2,7083	2,7288	2,3649	2,0318
0,0000	0,0847	1,4201	0,5999	0,4742	0,1407	0,0000
0,0000	0,0685	0,1273	0,9185	0,9145	0,2914	0,1301
0,0000	0,0000	0,0000	0,2196	0,0762	0,2609	0,1558
0,0000	0,3942	0,0000	0,0000	0,0000	0,0000	0,0000
0,6191	0,7953	0,6870	0,6236	0,8660	0,6770	0,6601
0,5734	0,4595	0,4509	0,4694	0,4523	0,6605	0,6763
0,0000	0,6358	2,0311	0,0000	0,0000	0,0000	0,0000
0,0000	0,0000	0,0000	0,0000	0,0000	0,1610	0,1082
2,8916	2,6530	2,9017	2,6789	2,6469	2,6345	3,0600
0,9825	1,2830	1,1589	1,3392	1,3563	1,2520	1,1314
0,2704	0,2759	0,2813	0,2520	0,2844	0,2670	0,2648
3,0254	2,8278	3,0094	3,8139	3,5547	2,8312	3,4435
0,2968	0,9492	4,3400	0,5453	0,1844	0,0000	0,0000
0,0000	0,1829	1,2580	2,0584	0,5007	0,1804	0,0000
0,1209	0,2349	0,0000	0,0000	0,0000	0,0000	0,0000
1,3161	1,0673	0,8076	0,9282	0,9433	0,8493	0,8347
0,3004	0,3374	0,3881	0,2919	0,2812	0,3126	0,3473
0,0000	0,4593	3,1826	7,5014	4,8997	3,8175	3,2492
0,0000	0,0000	0,0000	0,0000	1,5797	4,3271	3,3593
0,0000	0,0000	0,0000	0,0000	0,0000	0,2510	0,0000
0,0000	0,0000	0,0000	0,0000	0,0000	6,1650	5,0589
0,4029	0,1039	0,0000	0,0000	0,0000	0,0000	0,0000
1,0689	0,3531	0,0000	0,0000	0,0000	0,0000	0,0000
0,0000	0,0000	0,0000	0,6708	0,3028	0,7853	0,8568
1,3786	1,2467	1,5722	1,4427	1,4355	1,6952	1,5819
0,5511	0,7515	0,7876	0,7468	0,7385	0,6946	0,7574
0,2197	0,3219	0,6581	2,4977	1,1624	1,6043	1,1592
0,0000	0,0000	0,0000	0,0000	0,0000	0,0000	0,0000
0,0919	0,2263	0,7970	0,0000	0,0000	0,0000	0,0000
0,4445	0,9666	4,4706	0,7476	0,2520	0,0000	0,0000
0,0000	0,0000	0,0000	0,7235	0,6252	0,3790	0,2017
0,0000	0,1028	0,5049	1,4092	0,0000	0,0000	0,0000
0,0000	0,0000	0,0000	0,0000	0,0000	0,0000	0,0000
0,7050	0,0000	0,6384	0,0000	0,0000	0,0000	0,0000
0,0000	0,4103	0,0000	0,0000	0,0000	0,0000	0,0000
0,0000	0,0000	0,0000	0,0000	0,1395	0,2748	0,9471
0,1794	0,1859	0,1858	0,1648	0,1985	0,1788	0,1772
1,5673	0,2517	0,0000	0,0000	0,0000	0,0000	0,0000
1,6635	1,5285	1,8246	1,9815	1,7292	1,7341	1,9240

1,4665	0,3640	0,0514	0,0000	0,0000	0,0000	0,0000
0,0000	0,0948	0,0000	0,0000	0,2228	0,2752	0,2378
0,0000	0,0000	0,0000	0,0000	0,0000	0,0000	0,0000
0,7384	0,2361	0,0000	0,0000	0,0000	0,0000	0,0000
0,0742	0,0000	0,0000	0,0000	0,0000	0,0000	0,0000
3,3518	3,5196	2,9503	3,0625	2,9131	3,2259	2,9330
0,0558	0,0392	0,0861	0,1763	0,7858	1,2867	1,2899
0,0000	0,0000	0,0000	5,1052	0,0000	0,0000	0,0000
0,4537	0,4725	0,5626	0,5019	0,5173	0,5308	0,5074
0,0000	0,0000	0,0000	1,0455	0,5822	0,0000	0,0000

normalized

Table S5. Fold change variation of sarcoplasmic peptides across the 12 OFFGEL fractions assayed.

m/z	Retention time	Peptide identity	*Fraction 1	*Fraction 2
978,6	2,82	Actin-1	0,0	0,0
1123,6	64,58	Actin-2	0,0	0,0
1031,1	70,03	Adenylate kinase isoenzyme-1	0,0	0,0
903,2	65,95	alpha/beta enolase-3	1,0	0,0
948,9	76,09	alpha/beta enolase-4	0,0	0,0
941,5	2,27	beta enolase-1	0,0	0,0
1296,3	3,08	beta enolase-2	0,0	0,0
998,3	7,20	Creatine kinase M-1	0,0	0,0
754,7	68,32	Creatine kinase M-2	0,0	0,0
893,8	64,40	Creatine kinase M-3	10,7	0,0
968,3	2,43	Galectin 1-1	0,0	0,0
863,1	70,33	Galectin 1-2	0,0	1,0
1139,3	72,11	Glyceraldehyde 3 phosphate dehydrogenase	0,0	0,0
809,2	66,11	Heat shock cognate 71 kDa-1	0,0	0,0
1387,5	68,74	Heat shock cognate 71 kDa-2	0,0	0,0
1597,6	70,84	Heat shock prot beta 1	0,0	0,0
850,7	69,60	Heat shock prot beta 6-1	0,0	0,0
1275,5	69,97	Heat shock prot beta 6-2	0,0	0,0
1045,6	68,12	Hemoglobin subunit beta	0,0	0,0
911,6	68,88	Histidine triad nucleotide binding protein 1	0,0	0,0
876,5	65,29	Malate deshydrogenase-1	0,0	0,0
892,8	70,51	Malate deshydrogenase-2	0,0	0,0
796,8	2,96	Myoglobin-1	0,0	0,0
629,3	2,39	Myoglobin-2	0,0	0,0
748,4	3,03	Myoglobin-3	0,0	0,0
943,5	65,29	Peroxi redoxin-6	0,0	0,0
907,5	3,44	Phosphoglucomutase	0,0	0,0
734,5	4,10	Phosphoglycerate kinase 1-1	0,0	0,0
1056,3	64,97	Phosphoglycerate kinase 1-2	0,0	0,0
992,1	64,51	Phosphoglycerate kinase 1-3	0,0	0,0
1012,0	65,43	Phosphoglycerate kinase 1-4	0,0	0,0
821,4	69,14	Phosphoglycerate mutase-2	0,0	0,0
727,6	2,54	Protein/nucleic acid deglycase DJ 1-1	0,0	0,0
838,7	63,27	Protein/nucleic acid deglycase DJ 1-2	0,0	0,0
1292,7	68,85	Protein/nucleic acid deglycase DJ 1-3	0,0	0,0
1002,1	2,54	Serum albumin	0,0	0,0
954,4	3,65	Triosephosphate isomerase-1	0,0	0,0
770,5	6,53	Triosephosphate isomerase-2	0,0	0,0
743,0	66,57	Triosephosphate isomerase-3	0,0	0,0
802,1	68,07	Triosephosphate isomerase-4	0,0	0,0
1010,9	74,08	Triosephosphate isomerase-5	0,0	0,0

*Results were from per unit standardization of IS normalized findings detailed in **Table S4**.

In bold: most representative peptides from identified proteins.

*Fraction 3	*Fraction 4	*Fraction 5	*Fraction 6	*Fraction 7	*Fraction 8	*Fraction 9
4,0	10,8	0,0	1,0	0,0	0,0	0,0
5,6	21,1	0,0	0,0	0,0	0,0	0,0
0,0	0,0	0,0	0,0	1,0	0,0	0,0
0,0	0,0	0,0	1,4	2,1	4,3	16,1
0,0	0,0	0,0	0,0	0,0	0,0	1,8
0,0	0,0	0,0	0,0	1,0	11,6	17,1
0,0	0,0	0,0	0,0	0,0	0,0	2,9
0,0	0,0	0,0	0,0	1,0	7,0	11,4
0,0	0,0	0,0	0,0	1,0	4,9	13,7
0,0	0,0	0,0	0,0	1,0	6,9	16,3
8,4	1,0	0,0	0,0	0,0	0,0	0,0
12,9	2,0	0,0	0,0	0,0	0,0	0,0
0,0	0,0	0,0	1,4	1,0	2,2	4,5
0,0	1,0	0,0	0,0	0,0	0,0	0,0
0,0	1,0	0,0	0,0	0,0	0,0	0,0
0,0	0,0	0,0	1,0	0,0	0,0	0,0
0,0	0,0	1,1	6,2	1,0	0,0	0,0
0,0	0,0	5,9	28,5	7,1	1,0	0,0
0,0	0,0	0,0	0,0	0,0	0,0	3,6
0,0	0,0	0,0	0,0	1,0	0,0	0,0
0,0	0,0	1,0	7,9	2,6	0,0	0,0
0,0	0,0	0,0	3,1	1,0	0,0	0,0
0,0	0,0	0,0	0,0	1,0	16,8	7,1
0,0	0,0	0,0	0,0	0,0	0,0	2,2
0,0	0,0	0,0	0,0	1,0	1,9	13,4
0,0	0,0	0,0	3,9	1,0	0,0	0,0
0,0	0,0	0,0	0,0	1,0	0,0	0,0
0,0	0,0	0,0	0,0	0,0	0,0	0,0
0,0	0,0	0,0	0,0	0,0	0,0	0,0
0,0	0,0	0,0	0,0	0,0	0,0	0,0
0,0	0,0	0,0	0,0	0,0	0,0	2,2
0,0	0,0	0,0	0,0	0,0	0,0	0,0
0,0	0,0	0,0	1,0	1,5	0,0	0,0
0,0	0,0	0,0	1,0	1,9	0,0	0,0
0,0	0,0	0,0	1,1	0,0	1,0	0,0
0,0	0,0	2,7	1,0	0,0	0,0	0,0
0,0	0,0	0,0	0,0	1,0	3,2	0,0
0,0	0,0	0,0	1,6	5,1	23,5	3,0
0,0	0,0	0,0	1,0	2,5	8,7	0,0
0,0	0,0	0,0	1,8	3,8	17,7	3,0
0,0	0,0	0,0	0,0	0,0	0,0	1,0

*Fraction 10	*Fraction 11	*Fraction 12
0,0	1,7	0,0
0,0	1,0	0,0
2,4	2,9	2,5
7,5	10,4	7,5
1,0	0,0	0,0
16,8	3,2	3,6
1,0	3,4	2,0
2,8	1,0	0,0
0,0	0,0	0,0
10,7	8,3	7,1
0,0	0,0	0,0
0,0	0,0	0,0
20,1	32,9	32,9
0,0	0,0	0,0
0,0	0,0	0,0
0,0	0,0	0,0
0,0	0,0	0,0
0,0	0,0	0,0
3,1	1,9	1,0
0,0	0,0	0,0
0,0	0,0	0,0
0,0	0,0	0,0
5,6	1,7	0,0
1,8	1,0	0,0
13,4	4,3	1,9
0,0	0,0	0,0
0,0	0,0	0,0
0,0	1,5	1,0
0,0	1,2	1,0
1,0	2,7	2,1
1,0	2,6	2,8
1,0	2,0	6,8
0,0	0,0	0,0
0,0	0,0	0,0
0,0	0,0	0,0
0,0	0,0	0,0
0,0	0,0	0,0
0,0	0,0	0,0
1,0	0,0	0,0
0,0	0,0	0,0
1,0	0,0	0,0
0,0	0,0	0,0

Table S6. Reproducibility of LC-MS analysis and automated data processing between batches assayed.

			^a QC batch			^b CAL batch			^c OFGL batch			^d Global		
[M+H] ⁺ m/z	IS target peptide	^e IS normalizer	mean	std dev	CV (%)	mean	std dev	CV (%)	mean	std dev	CV (%)	mean	std dev	CV (%)
519.2	IS, Almond (unknown)-1	IS, Almond (unknown)-2	1.0638	0.1465	13.8	1.1408	0.0858	7.5	1.0613	0.1219	11.5	1.0887	0.0452	4.2
955.7	IS, Pru 2 partial-3	IS, Almond (unknown)-1	2.4003	0.1784	7.4	2.1842	0.2260	10.3	2.4436	0.3323	13.6	2.3427	0.1389	5.9
593.1	IS, Almond (unknown)-2	IS, Prunin 1 precursor-3	0.7084	0.0802	11.3	0.7700	0.1559	20.3	0.7737	0.1295	16.7	0.7507	0.0367	4.9
1162.8	IS, Prunin 1 precursor-3	IS, Pru 2 partial-3	0.5605	0.0377	6.7	0.5403	0.0891	16.5	0.5196	0.0955	18.4	0.5401	0.0204	3.8
1055.2	IS, Pru 2 partial-1	IS, Almond (unknown)-3	2.8162	0.0803	2.9	4.0486	0.0650	1.6	2.7044	0.1801	6.7	3.1897	0.7459	23.4
1245.4	IS, Almond (unknown)-3	IS, Prunin 1 precursor-3	1.0994	0.0252	2.3	0.8431	0.0406	4.8	1.2602	0.1367	10.8	1.0676	0.2104	19.7
718.6	IS, Prunin 1 precursor-1	IS, Almond (unknown)-3	0.2972	0.0126	4.2	0.5133	0.0411	8.0	0.2716	0.0150	5.5	0.3607	0.1328	36.8
1026.3	IS, Almond (unknown)-4	IS, Prunin 1 precursor-1	2.8939	0.2123	7.3	2.1235	0.4172	19.6	3.1352	0.3628	11.6	2.7175	0.5284	19.4
595.0	IS, Prunin 2 precursor partial-2	IS, Prunin 2 precursor partial-1	0.3135	0.0656	20.9	0.2541	0.0436	17.2	0.2959	0.0451	15.2	0.2878	0.0305	10.6
687.0	IS, Prunin 2 precursor partial-1	IS, Almond (unknown)-8	1.0428	0.1180	11.3	1.5470	0.0528	3.4	1.1494	0.2899	25.2	1.2464	0.2657	21.3
879.1	IS, Pru 2 partial-2	IS, Prunin 2 precursor partial-1	0.7053	0.0725	10.3	0.5495	0.0610	11.1	0.6349	0.1284	20.2	0.6299	0.0780	12.4
1317.9	IS, Almond (unknown)-8	IS, Pru 2 partial-2	1.3727	0.0898	6.5	1.1885	0.1336	11.2	1.4441	0.1271	8.8	1.3351	0.1319	9.9
1288.4	IS, Almond (unknown)-5	IS, Almond (unknown)-6	0.1970	0.0099	5.0	0.2067	0.0162	7.9	0.1836	0.0093	5.1	0.1958	0.0116	5.9
1088.8	IS, Prunin 1 precursor-2	IS, Almond (unknown)-5	1.7003	0.0843	5.0	1.7627	0.1259	7.1	1.6874	0.1745	10.3	1.7168	0.0402	2.3
1236.4	IS, Almond (unknown)-6	IS, Prunin 1 precursor -2	2.9947	0.2059	6.9	2.7693	0.2850	10.3	3.2581	0.2650	8.1	3.0074	0.2446	8.1
1222.3	IS, Almond (unknown)-7	IS, Almond (unknown)-6	0.5285	0.0345	6.5	0.4906	0.0623	12.7	0.4936	0.0345	7.0	0.5043	0.0211	4.2

^aResults from automated raw data processing detailed in **Table 1**. Number of technical replicates, n=3.^bResults from automated raw data processing detailed in **Table 2**. Number of technical replicates, n=5.^cResults from automated raw data processing detailed in **Table S4**. Number of technical replicates, n=12.^dResults from averaged QC, CAL and OFGL mean values. Number of technical replicates, n=20.^eNormalization ratios initially proposed in QC analysis (**Table 1**).

Extracted sarcoplasmic proteins

Liquid isoelectric focussing (OFFGEL) fractionation

Tryptic digestion

Spiking of almond internal standards

Targeted peptide quantification

Untargeted protein identification

Simultaneous full-MS and dd-MS/MS analyses(3D ion-trap)

Automated Mascot analysis of MS/MS data

Manual peak symmetry assessment of full-MS data from identified peptides

m/z	Rt {min}	Peptide ID
• 903.1	• 66	• a/b enolase
• 1222.2	• 75.1	• IS prunin 1-4

In-house peptide database listing intact masses (.csv)

OFFGEL protein distribution

Automated peptide quantification by MZmine processing of bulky Full-MS data

RT: 1.00-80.00 SM: 15G

		m/z:			RT:	
Average		Identity	Peak shape		QC2.RAW	
m/z	RT				Area	
1012.28	65.83	Phosphoglycerate kinase 1-4			D	4.2E6
748.33	2.78	Myoglobin-3			E	1.2E7
1222.25	73.27	IS, Almond (unknown)-7			F	5.4E7