We wish to thank the reviewers for their useful comments. We have revised the manuscript according to their comments. Point by point replies to all reviewers' and editorial comments can be found below.

Editorial comments:

1. Please take this opportunity to thoroughly proofread the manuscript to ensure that there are no spelling or grammar issues.
It was checked.
2. Please provide an institutional email address for each author.
Claudia Neves Correa: claudia.neves@unesp.br
Louise Oliveira Fiametti: louise.fiametti@unesp.br
Maria Eduarda Mazzi Esquinca: eduarda.mazzi@unesp.br
Leandro Mantovani de Castro: leandro.mantovani@unesp.br
3. Please revise the following lines to avoid previously published work: 22-23, 73, 81-85, 108-109, 224-225, 228-238, 242-246, 271-275, 277-278, 356-359, 373-375, 382-385. Please refer to the iThenticate report attached.
The lines were checked and changed.
4. Please revise the text to avoid the use of any personal pronouns (e.g., "we", "you", "our" etc.).It was checked and corrected.
5. JoVE cannot publish manuscripts containing commercial language. This includes trademark symbols (™), registered symbols (®), and company names before an instrument or reagent. Please remove all commercial language from your manuscript and use generic terms instead. All commercial products should be sufficiently referenced in the Table of Materials
For example: LoBind, LTQ-Orbitrap Velos, n Easy-nLC II nanoHPLC, Thermo Fisher Scientific, Phenomenex, Jupiter®, Xcalibur, PEAKS, etc..It was checked and corrected.
6. Please adjust the numbering of the Protocol to follow the JoVE Instructions for Authors. For example, 1 should be followed by 1.1 and then 1.1.1 and 1.1.2 if necessary. Please refrain from using bullets or dashes.It was checked and corrected.
7. For SI units, please use standard abbreviations when the unit is preceded by a numeral throughout the protocol. Abbreviate liters to L to avoid confusion. Examples: 10 mL, 8 µL, 7 cm2. It was checked and corrected.
8. For time units, please use abbreviated forms for durations of less than one day when the unit is preceded by a numeral throughout the protocol. Do not abbreviate day, week, month, and year. Examples: 5 h, 10 min, 100 s, 8 days, 10 weeks It was checked and corrected.
9. Please note that your protocol will be used to generate the script for the video and must contain everything that you would like shown in the video. Please add more details to your protocol steps. Please ensure you answer the "how" question, i.e., how is the step performed? Alternatively, add references to published material specifying how to perform the protocol action. Please add more specific details (e.g. button clicks for software actions, numerical values for settings, etc) to your protocol steps. There should be enough detail in each step to supplement the actions seen in the video so that viewers can easily replicate the protocol.It was checked and corrected.
10. Line 95-97: Please specify the type of cells grown. Are the cells purchased or isolated? Please mention. Please specify the conditions of incubation to grow cells
It was checked and the information added.

11. Line 101-103: Cell lysate? Does it mean the contents of the tube? Is there any centrifugation step?
12. Line 105-106: Please specify the ages/ sex, etc. of the zebra fish used in this study.
It was checked and the information added.
13. Line 112: Please specify what tissue is dissected. Please specify the organs.
It was checked and the information added.
14. Line 113-114: Please specify the volume of water. How is the tissue sonicated and homogenized? Please specify the conditions for sonication. Was it probe sonication or bath sonication? It was checked and the information added.
15. Line 115: Maximum speed? Please be specific or provide a range.It was checked and the information added.
16. Line 118-120: Please specify the volume of acid added. Vortexing for how long? It was checked and the information added.
17. Line 129-135: Please correct the step numbers.
It was checked and corrected.
18. Line 137/214: Please specify if there are any specific settings used in the vacuum centrifuge.
It was checked and the information added. line 140
19. Line 166-167: Homogenize on a spectrofluorometer? Please rephrase the sentence to add clarity. “Adjust the readings” – what does this mean? What is it adjusted to? It was checked and the information added. line173-174
20. Line 180-189: Please add the statement of caution.It was checked and the information added.
21. Line 265-280: Please ensure that the Protocol section consists of numbered steps. We cannot have non-numbered paragraphs/steps/headings/subheadings.It was checked and the information added.
22. Please include a one-line space between each protocol step and then highlight up to 3 pages of the Protocol (including headings and spacing) that identifies the essential steps of the protocol for the video, i.e., the steps that should be visualized to tell the most cohesive story of the Protocol. Remember that non-highlighted Protocol steps will remain in the manuscript, and therefore will still be available to the reader.It was checked and the protocol steps for the video were highlighted.
23. Please ensure that the references appear as the following: [LastName, F.I., LastName, F.I., LastName, F.I. Article Title. Source. Volume (Issue), FirstPage – LastPage (YEAR).] For more than 6 authors, list only the first author then et al.
It was checked and corrected.
24. Figure 4: Please include the unit of peak intensity average in the Y-axis.
It is just a numeric value.
25. Table 1: Please revise “µl” to “µL”.
It was checked and corrected.

Reviewers' comments:

Reviewer #1:

Manuscript Summary:

This manuscript described a sample preparation method for the quantification of peptides. A heating treatment was involved to minimize the protease activity, this might be the only novelty in this work. The resultant peptides were labeled with isotope formaldehyde and sodium cyanoborohydride, and analyzed by LC-HRMS. Since there are lots of reported works using multi-plex dimethyl labeling for proteomic research, some important references should be included. Moreover, this manuscript was not well organized. I'm afraid a major revision is needed before considering acceptance in JoVE.

Major Concerns:

1. Remove some generation descriptions of proteomics in Abstract and Introduction.

Answer: Thank you for the comment. We have removed generation descriptions of proteomics in Abstract and Introduction.

2. The results between with and without preheating treatment should be presented.

Answer: Thank you for the comment. This manuscript does not intend to bring novelty to the field of peptidomics, because protocols for rapid inactivation of proteases by heating or microwave radiation, as well as those for labeling by reductive dimethylation, have already been established and used in many studies, mainly for the characterization of peptidomes, as cited throughout this manuscript. However, most of these previous studies used isotope labeling with TMAB tags, which present a complicated synthesis. Our objective in organizing this manuscript was to show the step-by-step preparation of a peptide extract and its subsequent isotopic labeling by RMA, which has some advantages such as stability, it is commercially available and relatively cheap.

3. The figures of the mass spectrum should be redone in Origin/Excel, and present in sufficient resolution.

Answer: Thank you for the comment. MS images are extracted directly from the chromatograms as is. It is very difficult to improve resolution and size. However, we removed from the images some information not necessary.

4. Some figures, such as Fig 6, could be moved to supporting Information.

Answer: We would like to keep the figures in the Representative Results section for the reader to better visualize the results described.

5. The format/layout of the manuscript should follow the authors' guide.

Answer: Thank you for the comment. We follow the format/layout of the manuscript described in the authors' guide.

We would like to thank you for your comments. We hope to have reached the expectations.

Reviewer #2:

Manuscript Summary:
The focus of the paper is interesting in the sense that it addressing the problem of digestion of protein samples prior to sample mass spectrometry insertion. Where the major modification of the structure and biochemical properties caused by such digestion are known to be minimal to affect the analysis, but the possibilities of it could cause inaccurate analysis shouldn't be negated.
As an alternative to protease digestion, the authors suggested heat-inactivation instead coupled with cost-saving RMA labelling.

Major Concerns:
Line 51-".....which occur mainly in this tissue already 1 min postmortem"....the sentence doesn't make sense, maybe should be written as "....which occur mainly in the tissue after 1 min postmortem."????
Answer: Thank you for the comment. The paragraph was modified.

Line 53 and 70 please italic the in vivo
Answer: Thank you. The expression in vivo was changed to italic.

Methodology: Need to rewrite the whole methodology section into past tense, and also must be in paragraph format, not in points form.
Answer: We follow the format/layout of the manuscript described in the authors' guide.

Figure 2 B, replace the figure to include the last peak i.e. 437.7229
Answer: Thank you.The figure was replaced and included the last peak 437.7229.

line 268 Tashima and Fricker ....please add in the year in bracket i.e. Tashima and Fricker (XXXX)
Answer: Thank you for the comment. Missing information has been added.

We would like to thank the reviewer for your comments. We hope to have reached the expectations of improving your major question.