Identification of dipeptides by MALDI-ToF mass spectrometry in long-processing Spanish dry-cured ham

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ABSTRACT

The processing of dry-cured ham results in the generation of small peptides by the action of endogenous enzymes on muscle proteins. Common proteomic workflows involve previous separation techniques based on liquid chromatography which are expensive and time-consuming. In this study, a convenient proteomic approach based on MALDI-ToF is proposed for the first time for the detection of dipeptides in Spanish dry-cured ham. Dipeptides AH, AL, DD, EV, and VF were identified in hams of 18 and 24 months of dry-curing. This work provides insights on the efficiency of a new peptidomic workflow for the short peptide identification from a complex food matrix and permits to evaluate the sample in terms of the presence of taste-related and bioactive dipeptides.

1. Introduction

Dry-cured ham is a high added value product world-wide consumed with an enormous economic importance for the Mediterranean meat industry. The dry-curing process of ham involves a set of many biochemical reactions which determines the organoleptic properties of the final product. Proteolysis, in conjunction with lipolysis, is the main biochemical mechanism, and a better knowledge of this phenomenon is essential to produce regular batches with the highest quality. Muscle sarcoplasmic and myofibrillar proteins undergo an intense proteolysis by endogenous muscle enzyme proteases (Toldrá et al., 2020). Specifically, the naturally occurring dipeptides are of great interest as they exert a wide range of bioactivities with high probability to remain intact after gastrointestinal digestion as well as play a key role in the organoleptic properties of the final product (Gallego et al., 2019). On the other hand, an intense proteolysis can lead to unpleasant tastes such as unwanted bitter taste. Considering there is limited information about the influence of short peptides in the final characteristics of dry-cured ham (Toldrá et al., 2018), it is necessary to advance in the research for the characterization of these valuable compounds (Sentandreu et al., 2003).

Proteomic approaches can enhance the knowledge about biochemical processes, specially concerning the evolution of proteolysis during the processing of dry-cured meats, and they can also be used in the identification of biomarkers for meat quality traits (Mora et al., 2016). Above all, peptidomics has become an important area for the characterisation of dry-cured hams in order to identify and quantitate potential biomarkers, bioactive and/or sensory peptides, but also allows to conduct studies on peptide profiling and bioavailability (Gallego, Mora, & Toldrá, 2018; López-Pedrouso, Pérez-Santaescolica, Franco, Fullaosa, & Carballo, 2018). However, the study of dipeptides show several challenges as they are frequently in the limit of some standard mass spectrometry (MS) techniques due to their small size and low abundance (Panchaud et al., 2012). Considering the wide range of combinations that can occur between amino acids, the analysis of each dipeptide in terms of profiling, structural estimation, and quantification is really challenging. The study of dipeptides in complex matrices using peptidomic approaches has difficulties due to interferences with other compounds, which very often result in signal inhibition in the mass spectrometers (Mora et al., 2017). In order to avoid these problems, complex extraction protocols and previous chromatographic separation steps are necessary to decrease the incidence of matrix effects (Gallego et al., 2018). Due to the fact that a particular dipeptide sequence can

Abbreviations: a, Ala; D, Asp; E, Glu; F, Phe; G, Gly; H, His; I, Ile; K, Lys; L, Leu; M, Met; P, Pro; R, Arg; S, Ser; V, Val; ACE-I, Angiotensin I-converting enzyme; CHCA, a-Cyano-4-hydroxycinnamic acid; DPP, dipeptidyl peptidase; PDP, peptidyl dipeptidase; ESI, electrospray ionization; HILIC, hydrophilic interaction chromatography; HPLC, high-performance liquid chromatography; LC, liquid chromatography; MS, mass spectrometry; MS/MS, mass spectrometry in tandem; MALDI, matrix-assisted laser desorption/ionization; MRM, multiple reaction monitoring; Q, quadrupole; RP-HPLC, reverse-phase-HPLC; SEC, size-exclusion chromatography;ToF, time of flight; SPE, ultra-high-throughput-solid-phase extraction; UPLC, ultra-performance liquid chromatography..

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appear in a wide range of proteins, the identification by matching the \( m/z \) spectrum with theoretical peptide sequences using databases is not feasible. Furthermore, current search algorithms are often limited to peptides containing 4 or more amino acids (Tang et al., 2014). Thus, \textit{de novo} identification of the sequences by highly-experienced personnel is frequently needed, which can be difficult and time-consuming, particularly for untargeted analysis (Mora et al., 2017). The main proteomic approach used for the identification and quantitation of small peptides is the Multiple Reaction Monitoring (MRM) with triple quadrupole mass spectrometer instruments (Panchaud et al., 2012). However, in the case of dipeptides, low quality fragmentation results in ambiguous identification when dealing with low collision energy (Paizs & Suhai, 2005). MRM also presents certain difficulties, as it needs to be previously optimized, and requires trained personnel and expensive instruments.

On the other hand, mass spectrometry approaches based on matrix-assisted laser desorption/ionization (MALDI) mass spectrometry have the advantage of convenience, speed, and accuracy, but also present good resolution, robustness, and sensitivity. Relative and absolute quantification can be performed using MALDI approaches based on mass spectrometry in tandem (MS/MS) (Wang & Giese, 2017). However, this methodology is not very popular for the identification of small peptides mainly due to the potential interferences of low-molecular-weight peptides with MALDI matrices used for ionization.

This study proposes a MALDI Time-of-Flight (ToF) approach to accomplish the rapid detection of dipeptides in dry-cured ham peptide extracts with the advantage of using very low amounts of sample, decreasing time and cost, with the goal to follow up the evolution of the dipeptides generated during the time of curing and their relation with bioactivity and/or taste quality.

2. Material and methods

2.1. Chemicals and reagents

Dipeptides Ala-His (AH), Ala-Leu (AL), Asp-Asp (DD), Glu-Val (EV), and Val-Phe (VF) were used as standards in this study. Dipeptides AH (Catalog number: 4002657), AL (Catalog number: 4005016), DD (Catalog number: 4010210), EV (Catalog number: 4001676), and VF (Catalog number: 4001995) were purchased from Bachem AG (Bubendorf, Switzerland). The peptides were diluted in bidistilled water to 6 mM, and later rediluted in acetonitrile with 0.1% trifluoroacetic acid to 3.5 mM. Finally, peptides were stored at −80 °C for further analysis. A dipeptide standard mix was also prepared in bidistilled water. The mixture was diluted in acetonitrile with 0.1% trifluoroacetic acid to 3.5 mM per peptide. α-Cyano-4-hydroxycinnamic acid (CHCA) matrix substance for MALDI-MS, >99.0% (Catalog number: 70990; Merck KGaA, Darmstadt, Germany) was used.

Reagents used in the peptide extraction were of analytical grade and purchased from Scharlab (Barcelona, Spain).

A total of 12 dry-cured hams from pigs of industrial genotypes Landrace × Large White were processed at 18 and 24 months of curing (6 for each curing period) in the factory Incarlopsa (Tarancón, Spain).

2.2. Sample deproteinization and total peptide extraction

Hams were bled and prepared following the traditional procedures, controlling temperature and humidity during the different salting and ripening-drying stages.

Once dry-cured hams were received, the extramuscular fat of Biceps femoris muscle was removed and the dry-cured muscle was processed according to Mora et al., (2009). Briefly, peptide extraction was carried out by homogenisation of 50 g of Biceps femoris muscle with 200 mL 0.01 N HCl in a Stomacher (IUL Instruments, Barcelona, Spain) for 8 min at 4 °C. After centrifugation for 20 min at 12,000g and 4 °C, the homogenate was filtered through glass wool. The proteins were precipitated by adding 3 volumes of ethanol and keeping the mixture at 4 °C during 20 h. After a second centrifugation for 20 min at 12,000g and 4 °C, ethanol was evaporated and samples lyophilized (SCANVAC CoolSafe, Labogene APS, Lyng, Denmark).

2.3. Peptide extracts ultrafiltration

A total of 100 mg of lyophilised extract was dissolved in 3 mL of bidistilled water (n = 6 per processing time). Then, samples were filtered using a 0.45 μm nylon membrane syringe filters (Teknokroma, Barcelona, Spain). Then, samples were centrifuged using 10 kDa Amicon Ultra filters (UFC501096, Merck Millipore, Billerica, Massachusetts, USA) during 15 min at 12,000xg and 4 °C. The supernatants (>10 kDa) were stored at −80 °C and the filtrates were filtered again using 3 kDa centrifugal Amicon Ultra filters (UFC506396, Merck Millipore, Billerica, Massachusetts, USA) using same conditions. Supernatants (from 3 to 10 kDa) were stored at −80 °C and the filtrates (<3 kDa) were freeze-dried. The resulting lyophilized samples were resuspended in 50 μL of bidistilled water and later diluted (1:20) for further analysis.

2.4. Analysis by matrix-assisted laser desorption/ionisation mass spectrometry (MALDI-ToF MS)

The MALDI-ToF analysis provides a \( m/z \) spectrum from which useful information about possible candidates is obtained by matching the \((\text{M}−\text{H})^{+}\) peak values with their respective from a standard \( m/z \) spectrum.

The standard peptide mix and the < 3 kDa peptide extracts were analysed by MALDI-ToF mass spectrometry, evaluating three technical replicates per sample. A volume of 0.8 μL was spotted in the MALDI plate. Droplets were air-dried at room temperature, and 0.8 μL of CHCA matrix (10 mg/mL in ACN:0.1%TFA (70:30, v/v)) were added and dried at room temperature. The analysis was done in a 5800 MALDI-ToF/ToF instrument (ABSciex, CA, USA) in automatic positive-ion reflector mode for mass analysis between 100 and 1,500 Da. Spectra were obtained from 3,000 shots in every position with a laser intensity of 3,800–4,100. The analysis of data was done using mMass – Open Source Mass Spectrometry Tool Software v.5.5.0 (http://www.mmass.org) (Strohalm et al., 2010). Plate model and acquisition method were calibrated by a peptide mass standards calibration mixture (ABSciex, CA, USA).

Dipeptide identification was performed within the \( m/z \) range from 190 to 280.

2.5. Peptide identification confirmation by mass spectrometry in tandem

5 μL of sample was analysed using an Agilent 1260 Infinity LC system (Agilent, Palo Alto, CA, USA) coupled to a triple quadrupole mass spectrometer (QQQ) 6420 Triple Quad LC/MS (Agilent, CA, USA) with an electrospray ionization source (ESI). Firstly, samples were concentrated on a SeQuant ZIC®-HILIC guard fitting PEEK column (5 μm, 14 mm × 1 mm; Merk KGaA, Darmstadt, Germany) at a flow rate of 0.02 mL/min for 5 min. Mobile phase was ACN in 10 mM ammonium acetate (90:10, v/v). The trap column was automatically switched in-line onto a SeQuant ZIC®-HILIC capillary column (5 μm, 150 mm × 0.3 mm; Merk KGaA, Darmstadt, Germany). Solvent A was 10 mM ammonium acetate, and Solvent B, ACN. The flow rate was 6 μL/min at 30 °C, and gradient conditions were used: 0–8 min, 80 % B; 8–25 min, from 80 to 30 % B; 25–28 min 30 % B; and 28–35 min, from 30 to 80 % B. The column outlet was connected to an ESI, and spectra were obtained in positive polarity mode to acquire full scan mass spectra from 70 to 500 m/z. Other MS parameters were: nitrogen gas flow, 6 L/min; gas temperature, 350 °C; nebulizer pressure, 15 psi; capillary, 3500 V; fragmentor, 100 V; scan time, 500 ms; cell accelerator, 4 V.

Standards were analysed with the same methodology at a concentration of 1 nmol/μL to get their \( m/z \) ratio and their specific retention times. This data was used to confirm the presence of the dipeptides in dry-cured ham extracts. The analysis of the samples was done using
MassHunter LC/MS Data Acquisition (version B.08.00) and the data analysis of the obtained results was done using MassHunter Qualitative Analysis software (version B.07.00) (Agilent Technologies, Inc.). The analysis of the standard dipeptides and dry-cured ham samples were done in triplicate.

3. Results and discussion

Dipeptides are the products of the enzymatic action of dipeptidyl peptidases (DPPs) and peptidyl dipeptidases (PDPs) by releasing two amino acids from the N-terminal and C-terminal location of longer peptides, respectively. DPPs have been the most studied and their substrate specificities are better known (Sentandreu & Toldrà, 2007; Toldrà et al., 2020).

As mentioned above, ultrafiltered (<3 kDa) peptide extracts from dry-cured hams of 18 and 24 months of processing were analysed by MALDI-ToF MS. A total set of 5 dipeptides were elucidated to be present over all the analysed samples, according to their molecular masses. Information about main characteristics of these peptides is shown in Table 1.

In Fig. 1, the peptide standard mix spectrum reveals the molecular masses of the singly charged ions ([M–H]–), 203.18, 227.16, 247.17, 249.14, and 265.20, corresponding with peptides AL, AH, DD, EV, and VF, respectively.

The molecular masses of these peptides match with the signals obtained from the MALDI-ToF mass spectrum of dry-cured hams of both periods of dry-curing, as it is indicated in Fig. 2. The dipeptides detection in dry-cured hams of 18 and 24 months of dry-curing is showed in detail in supplementary material (Figs. S1–S5).

In order to confirm the presence of dipeptides, dry-cured ham extracts were also analysed by LC-MS/MS. Fig. 3 and Fig. 4 show the dipeptides MS/MS spectra generated in dry-cured hams of 18 and 24 times of processing. These results confirm the sequence of the dipeptides and discard the presence of other sequences with same m/z.

In recent years, MALDI-ToF technology has been increasingly used

| Table 1 |
| Structural representation and main physicochemical characteristics attributed to the dipeptides of this study. |
| MW (g/mol) | Charge | pI | Steric hindrance | Sidebulk | Hydrophilicity | Amphipathicity |
| AH | 226.25 | 0.50 | 7.10 | 0.26 | 0.72 | 0.72 |
| AL | 202.27 | 0.00 | 5.88 | 0.53 | 0.00 | 0.00 |
| DD | 248.20 | −2.00 | 3.57 | 0.76 | 3.00 | 3.00 |
| EV | 246.28 | −1.00 | 4.00 | 0.76 | 0.75 | 1.15 |
| VF | 264.34 | 0.00 | 5.88 | 0.39 | 0.00 | 0.00 |

Peptide sequences are given as amino acid one-letter code. *Physicochemical property values obtained from ToxinPred (Gupta et al., 2013).
for detection analysis in a wide range of areas because of its suitability, cost-effectiveness and efficiency. Its use in peptidome profiling, demonstrates its applicability for diagnosis (Pusch & Kostrzewa, 2005) and therefore, it can play an important role in the rapid detection of foods compounds, being a useful tool for food authenticity and traceability (Chambery et al., 2009).

A further point is that MALDI-ToF analysis shows high potential for faster peptide identification when coupled with separation techniques. Actually, ultra-high-throughput-solid-phase extraction (SPE)-RP-ultra performance liquid chromatography (UPLC)- electrospray ionization (ESI)-MALDI-ToF was proposed as an analytical workflow to support the rapid analysis of large SPE-purified peptide libraries (Bennett et al., 2021). On the other hand, MALDI-ToF has also been used in the detection of food adulterations and characterization of food allergens (Calvano, Bianco, Losito, & Cataldi, 2021).

Unfortunately, there is still a lack of experimentation concerning the use of MALDI-ToF MS for short peptide sequences identification, especially due to potential matrix interferences. Thus, methods using MS in tandem have been the most used option for the analysis of di and tripeptides. In this way, RP-HPLC and ESI-MS/MS have been optimized using a MRM approach to quantitate chloroformate derivatized-dipeptides in complex biological matrices (Fonteh et al., 2007; Ubhi et al., 2013). Capillary electrophoresis (CE) coupled to MRM-ESI-MS/MS has been used for peptide profiling and quantitation of structural isomers, and also for the quantitation of γ-glutamyl di- and tripeptides although this approach is only applicable to a limited number of dipeptides (Ozawa et al., 2020). On the other hand, hydrophilic interaction chromatography (HILIC) coupled to ESI-MS/MS was used to create a database-search platform for the identification of some highly polar di- and tripeptides (Tang et al., 2014).

Despite the development of all these methodologies, only a few strategies have been optimised for the identification and quantitation of dipeptides in dry-cured ham. One of the most common proteomics methodologies currently used in the analysis of dry-cured ham samples...
consists of the use of two-dimensional electrophoresis as a first separation step before MALDI-ToF analysis. In this regard, Biceps femoris from Spanish dry-cured ham have been analysed determining that myosin-1, α-actin and myosin-4 proteins were the biomarkers that underwent the most intense response to proteolysis (López-Pedroso, Pérez-Santaeascolastica, Franco, Fulladosa, & Carballo, 2018).

Peptidomic workflows applied to dry-cured ham samples frequently start with the separation of the peptide mixture by using chromatographic techniques. Size-exclusion chromatography (SEC) or RP-HPLC can be used coupled to a fraction collector to isolate and purify main chromatographic peaks. The fractions are then analysed using liquid chromatography coupled to MS/MS (Mora et al., 2013). In another

![Fig. 3. ESI-QQQ spectra of the dipeptides AH, AL, DD, EV and VF identified in 18 months dry-cured ham extracts.](image-url)
study, water-soluble extracts obtained from 8 months-aged Spanish dry-cured hams were fractionated by gel filtration, and fractions with the highest peptide content were further separated using RP and cation-exchange-HPLC. Finally, sequences were determined by automated Edman degradation and dipeptides VE, IV, LE, ID, AM, GE, ER, PL, GS, DV and SK were identified (Sentandreu, Stoeva, Aristoy, Laib, Voelter, & Toldrà, 2003). Also SEC and RP-HPLC are frequently used in the separation of complex mixtures of peptides that can be later analysed by MALDI-ToF or other MS techniques such as nanoLC – nanoESI – quadrupole (Q)ToF MS (Mora, Escudero, & Toldrà, 2016; Mora, Sentandreu, & Toldrà, 2010; Wang et al., 2012; Zhu, Tian, Li, Liu, & Zhao, 2017). This strategy has been used to identify peptides from 400 to 3000

Fig. 4. ESI-QQQ spectra of the dipeptides AH, AL, DD, EV and VF identified in 24 months dry-cured ham extracts.
Regarding the dipeptide AL, in vitro DPP-IV inhibitory activity and a role as anti-inflammatory by inhibition of NO production in LPS-induced RAW 264.7 macrophages have been documented. EV, and VF have been reported to be in vitro ACE and DPP-IV inhibitors.

4. Conclusions

Traditional proteomic strategies are frequently very challenging when the objective is the analysis of very short sequences such as dipeptides, because they involve expensive and time-consuming methodologies. Thus, the optimization of different approaches by developing dynamic protocols is very useful and necessary. Here, dipeptides AH, AL, DD, EV and VF were identified in the MALDI-ToF spectra of ultrafiltrated peptide extracts (<3 kDa) from dry-cured ham samples of 18 and 24 months with a simple and fast methodology and avoiding several separation steps.

The identification of these dipeptides using a fast and simple methodology is very important to know the potential characteristics of the sample including the presence of taste-related dipeptides but also their potential content in bioactive candidates, and also evaluate the need for the necessary procedures to confirm and quantitate such dipeptides, which requires longer optimization times.

Declarations of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.jfochms.2021.100048.

References

Bennett, R., Fornone, G. F., Nowak, T., Mukherjee, D., Shchurik, V., Mapelli, C. A., Makarov, A. A. (2021). Ultra-high-throughput SPE-MALDI workflow: Blueprint for efficient purification and screening of peptide libraries. Analytica Chimica Acta, 1142, 10–18. https://doi.org/10.1016/j.aca.2020.10.045

Calvino, C. D., Bianco, M., Losito, J., & Catalli, T. R. L. (2021). Proteomic Analysis of Food Allergens by MALDI ToF/ToF Mass Spectrometry. In N. E. Labrou (Ed.), Protein Downstream processing. Methods in Molecular Biology. 375–376. New York, N.Y.: Humana. https://doi.org/10.1007/978-1-0716-0775-6_24.

Chambery, A., del Monaco, G., Di Marco, A., & Parente, A. (2009). Peptide fingerprint of high quality Campania white wines by MALDI-ToF mass spectrometry. Food Chemistry, 113(4), 1283–1289. https://doi.org/10.1016/j.foodchem.2008.08.031

Degnes, K. F., Kvitvag, H. F. N., Haslunde-Hex, H., & Aase, I. M. (2017). Changes in the Profiles of Metalloproteins Originating from Protein Degradation During Ripening of Dry Cured Ham. Food and Bioprocess Technology, 10(6), 1122–1130. https://doi.org/10.1007/s11947-017-1894-3

Fontenot, A. N., Harrington, R. J., & Harrington, M. G. (2007). Quantification of free amino acids and dipeptides using isotope dilution liquid chromatography and electrospray ionization tandem mass spectrometry. Amino Acids, 32(2), 203–212. https://doi.org/10.1007/s00726-006-0370-6

Gallego, M., Mora, L., & Toldrà, F. (2018). Perspectives in the Use of Peptidomics in Ham. Proteomics, 18(18), 1–22. https://doi.org/10.1002/pmic.201700422

Table 2

Reported taste perceptions and bioactivities attributed to the dipeptides of this study registered in BIOPEP database (Mankiewicz, Iwańska, & Darewicz, 2019).

| Dipeptide | Taste | Bioactivity |
|-----------|-------|-------------|
| AH        | umami | in vitro ACE inhibitor |
|           |       | in vitro DPP-IV inhibitor |
|           |       | in vitro antioxidant |
| AL        | –     | in vitro DPP-IV inhibitor |
| EV        | sour, umami and salty | inhibition of NO production in RAW 264.7 |
| DD        | sour, umami and salty | – |
| VF        | bitter | in vitro ACE inhibitor |
|           |       | in vitro DPP-IV inhibitor |

* Peptide sequences are given as amino acid one-letter code.
Gupta, S., Kapoor, P., Chaudhary, K., Gautam, A., Kumar, R., Raghava, G. P. S., & Gallego, M., Mora, L., Aristoy, M. C., & Toldrà, F. (2017). Separation and identification of small troponin T peptides generated in dry-cured ham. Food Chemistry, 223(3), 691–697. https://doi.org/10.1016/j.foodchem.2015.05.035

Ozawa, H., Hirayama, A., Ishikawa, T., Kudo, R., Marruyama, M., Shoji, F., … Tomita, M. (2020). Comprehensive Dipeptide Profiling and Quantitation by Capillary Electrophoresis and Liquid Chromatography Coupled with Tandem Mass Spectrometry. Analytical Chemistry, 92(14), 9799–9806. https://doi.org/10.1021/acs.analchem.0c01258

Paizs, B., & Suhai, S. (2005). Fragmentation pathways of protonated peptides. Mass Spectrometry Reviews, 24(4), 508–548. https://doi.org/10.1002/mas.20024

Panchaud, A., Affolter, M., & Kussmann, M. (2012). Mass spectrometry for nutritional peptidomics: How to analyze food bioactives and their health effects. Journal of Proteomics, 75(12), 3546–3559. https://doi.org/10.1016/j.jprot.2011.12.022

Pusch, W., & Kostrzewa, M. (2005). Application of MALDI-ToF Mass Spectrometry in Screening and Diagnostic Research. Current Pharmaceutical Design, 11(20), 2577–2591. https://doi.org/10.2174/138161205446932

Sentandrèu, M. A., Stoeva, S., Aristoy, M. C., Labk, K., Voelter, W., & Toldrà, F. (2003). Identification of Small Peptides Generated in Spanish Dry-Cured Ham. Journal of Food Science, 68(1), 64–69. https://doi.org/10.1111/j.1156-2621.2003.tb41415.x

Sentandrèu, M. A., Armenteros, M., Calvete, J. I., Ouali, A., Aristoy, M. C., & Toldrà, F. (2007). Proteomic Identification of Acan Derived Oligopeptides in Dry-Cured Ham. Journal of Agriculture and Food Chemistry, 55(9), 3613–3619. https://doi.org/10.1021/jf061911g

Sentandrèu, M. A., & Toldrà, F. (2007). Evaluation of ACE inhibitory activity of dipeptides generated by the action of porcine muscle dipeptidyl peptidases. Food Chemistry, 102(2), 511–515. https://doi.org/10.1016/j.foodchem.2006.04.018

Strohalm, M., Kavan, D., Nováčk, P., Volný, M., & Havlíček, V. (2010). mMass 3: A Cross-Platform Software Environment for Precise Analysis of Mass Spectrometric Data. Analytical Chemistry, 82(11), 4648–4651. https://doi.org/10.1021/ac100818g

Tang, Y., Li, R., Lin, G., & Li, L. (2014). PEP Search in MyCompoundID: Detection and Identification of Dipeptides and Tripeptides Using Dimethyl Labeling and Hydrophilic Interaction Liquid Chromatography Tandem Mass Spectrometry. Analytical Chemistry, 86(7), 3568–3574. https://doi.org/10.1021/ac500109y

Toldrà, F., Gallego, M., Reig, M., Aristoy, M. C., & Mora, L. (2020). Recent Progress in the Identification of Low Molecular Weight Small Troponin T Peptides Generated in Dry-Cured Ham. Trends in Food Science & Technology, 91, 1–8. https://doi.org/10.1016/j.tifs.2019.10.013

Ugli, B. K., Davenport, P. W., Welch, M., Riley, J., Griffin, J. L., & Connor, S. C. (2013). Analysis of chloroformate-derivatised amino acids, dipeptides and polyamines by LC-MS/MS. Journal of Chromatography B, 934, 79–88. https://doi.org/10.1016/j.jchromb.2013.06.026

Wang, J., Zhao, G. M., Zhang, J. W., Liu, Y. X., Li, M. Y., & Hu, D. H. (2012). Separation, purification and structural identification of small peptides from Jinhua ham. Food Science, 33(9), 16–20.

Wang, P., & Giese, R. W. (2017). Recommendations for quantitative analysis of small molecules by matrix-assisted laser desorption ionization mass spectrometry. Journal of Chromatography A, 1486, 35–41. https://doi.org/10.1016/j.jchroma.2017.01.040