

Development of a mass spectrometry immunoassay for unambiguous detection of egg allergen traces in wines

Rosa Pilolli¹ · Ravindra Chaudhari² · Francesco Palmisano³ · Linda Monaci¹

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Abstract A mass spectrometry immunoassay (MSIA) specifically designed for the detection of egg allergens in wines is described. MSIA is based on an immunoaffinity enrichment procedure combined with targeted MS/MS detection of selected egg peptide markers. Polyclonal antibodies raised against native ovalbumin, chosen as the target protein tracing for egg powder, were immobilized onto low backpressure monolithic MSIA customized disposable tips. Ovalbumin-free wine samples were fortified with standard protein at different concentrations in the low microgram-per-milliliter range. A simple protocol was devised consisting of a 1:4 dilution of the wine sample with a basic solution for pH adjustment, followed by a semi-automated purification/enrichment step on MSIA customized disposable tips fitted on a multichannel electronic pipette. Among the main figures of merit, LOD and LOQ values as low as 0.01 and 0.03 µg/mL, respectively, and within-day precision of 18% should be noticed. Noteworthy, the developed assay outperformed current MS-based methods for the detection of allergenic protein in wine matrices, thanks to the immunoaffinity enrichment. In addition, compared to other immunoassays, the present approach boasts the unquestionable advantage of providing an unambiguous identification of the target protein by simultaneous detection of three unique peptide markers each giving three specific MS/MS transitions.

Keywords Egg allergen · Wine · MSIA · Immunoassay · Mass spectrometry · Ovalbumin

Introduction

Various commercial preparations of egg and derived products are commonly included in winemaking procedures to promote wine clarifications. However, as already pointed out by the European Food Safety Agency (EFSA) [1], any allergenic protein residue in the final product could represent a risk for allergic consumers. To safeguard the health of allergic consumers, the European Commission has issued regulation no. 579/2012 (amending the Commission Regulation 607/2009) mandating the allergen labeling of wines treated with allergenic additives (or processing aids) as long as their presence can be detected in the end-product [2, 3]. This, in turn, requires suitable analytical methods for a reliable quantification of residual allergenic agents ensuring a proper wine labeling. In absence of prescribed official methods, the only available reference is the European regulation 1234 of 2007 (article 120g) concerning the performances of the analytical methods for wine analysis [4]. In the latter, a direct reference to the International Organization of Vine and Wine (OIV) resolution was reported, setting up the analytical requirements to be fulfilled for the quantification of potentially allergenic residues of fining agent in wine (resolution 427-2010 modified by the OIV/COMEX 502-2012) [5, 6].

The most widespread and easy-to-use analytical method for routine monitoring of allergenic ingredients in food commodities is based on enzyme-linked immunosorbent assays (ELISAs) [7–9]. For specific application to wines, several ELISA kits are already commercially available, and recently some collaborative tests have been performed to

✉ Linda Monaci
linda.monaci@ispa.cnr.it

¹ Institute of Sciences of Food Production, National Research Council (ISPA-CNR), Via G. Amendola 122/O, 70126 Bari, Italy

² Thermo Fisher Scientific, Tempe, AZ 85284, USA

³ Dipartimento di Chimica, Università degli Studi di Bari Aldo Moro, Via Orabona, 4, 70126 Bari, Italy

validate the performances of the assay according to the OIV resolution [10, 11].

Mass spectrometry (MS) has been also successfully applied to the detection of allergens in food, thanks to several advantages including multitarget detection, unambiguous allergen identification, and accurate quantitative data [12, 13]. Focusing on allergenic fining agents used for wine clarification, several investigations on MS-based methods to trace milk [14–16] and egg allergens [17], even simultaneously [18–22], have been reported, most of them differing in terms of sample pre-treatment and instrumental setup.

Recent technological advances in instrumentation and sample preparation have led to the development of MS-based targeted protein assays as an attractive alternatives to classical ELISAs. Several applications of such approaches can be found in the literature, appearing under different format and acronyms: mass spectrometry immunoassay (MSIA), probe affinity mass spectrometry (PAMS), immuno-matrix-assisted laser desorption ionization (iMALDI) MS, and surface-enhanced laser desorption ionization (SELDI) MS to cite just a few [23]. All of these approaches couple an immunoaffinity capture system (allowing enrichment of the low abundance target molecule) with mass spectrometric detection, enabling unambiguous detection and multiplexed analyses. In this way, the advantages derived from the combination of two complementary approaches are guaranteed with an additional benefit that the process can be automated for high-throughput sample pre-treatment procedure. The MSIA approach, as originally proposed in 1995, is based on the microscale immunoaffinity capture of a target antigen followed by identification and quantitation by MALDI MS [24]. Since its introduction, MSIA counts a widespread application in the biomedical field, in particular in biomarker screening for potential application to cardiovascular disease [25–28], diabetes [29, 30], cancer [31, 32], and growth disorders [33, 34], among the most recent.

The MSIA immunoaffinity step can greatly benefit from the recently introduced disposable automation research tips (D.A.R.T.'STM) and electronic pipetting technology [29, 30, 35]; a capture antibody was immobilized onto a micro-column and the target-containing sample interacts with the tailored stationary phase through repetitive pipetting cycles. After several washing and elution steps, the target antigen can be either directly analyzed as such by mass spectrometry or enzymatically digested and the resulting peptide mixture analyzed by, e.g., MALDI MS (peptide mass fingerprinting) and/or electrospray ionization (ESI) MS. Protein digestion provides a more reliable detection since antigen identification relies on a number of simultaneously detected peptide markers each giving specific MS/MS transitions observable by, e.g., selected reaction monitoring (SRM) in an ion trap mass analyzer. The further addition of a third dimension represented by a

liquid chromatography (LC) separation step should finally provide a practically unambiguous identification of a given allergen.

Here, a MSIA workflow based on a semi-automated immunoaffinity enrichment step (performed by antibody customized D.A.R.T.'S fitted on a multichannel electronic pipette) and a LC-ESI-SRM platform for the detection/quantification of ovalbumin in wine is described for the first time.

Materials and methods

Reagents

Albumin from chicken egg white (ovalbumin, OVA), bovine serum albumin (BSA), sodium hydroxide, phosphate buffer saline, sodium chloride, acetonitrile, formic acid, hydrochloric acid, iodoacetamide (IAA), and dithiothreitol (DTT) were purchased from Sigma Aldrich. *N*-Methyl-2-pyrrolidone, 1,1'-carbonyldiimidazole, and acetone were purchased from VWR international PBI. Rabbit anti-ovalbumin polyclonal antibody (poly-anti-OVA) raised against the native protein was purchased from Millipore. Trypsin (proteomic grade) was purchased from Promega (Milan, Italy). Custom MSIA Disposable Automation Research Tips (D.A.R.T.'STM) were provided by Thermo Fisher Scientific. A local retailer provided allergen-free white wine.

D.A.R.T.'S customization and MSIA application

The customized MSIA D.A.R.T.'STM were tailored to the specific capture of purified OVA in its native form, by covalent immobilization of an OVA-specific antibody; to this aim, a polyclonal antibody (Ab), previously evaluated by our research group, was utilized [36]. The preparation of the customized D.A.R.T.'STM was performed according to the manufacturer protocol following three main steps: (1) surface activation by 1,1'-carbonyldiimidazole, (2) rinsing with acetone followed by vacuum assisted drying, and (3) antibody covalent linking. After Ab immobilization, the MSIA D.A.R.T.'STM were stored at 4 °C in PBS buffer solution and used for immunoassays within 1 week from preparation.

As for the assay development, preliminary investigations were carried out on OVA standard solutions in PBS buffer, with the inclusion of BSA as blocking agent to reduce non-specific adsorption. Artificially contaminated wine samples were prepared by spiking variable amounts of OVA standard solution (1 mg/mL) according to the final concentration desired. Afterwards, the wine samples were diluted 1:4 with 25 mM sodium hydroxide solution for pH adjustment and further added with BSA at the final concentration of 1 µg/mL. Up to 12 samples could be simultaneously processed with

a semi-automated procedure, thanks to the aid of a 12-channel electronic pipette (Finnpipette™ Novus I—Thermo Fisher Scientific). The optimized protocol for MSIA based purification is illustrated at point 2 of Scheme 1. Briefly, after a rapid washing step with phosphate buffer saline (10 mM PBS at pH 7.4, 30 aspirate/dispense cycles of 150 μ L), the customized tips were first saturated with a 1 mg/mL aqueous solution of BSA (100 aspirate/dispense cycles of 150 μ L) to block any non-reacted site and reduce non-specific binding. The ovalbumin immune enrichment, from a 5-mL aliquot of the wine samples, was performed by 999 aspirate/dispense cycles of 250 μ L. Finally, after a rapid washing step with PBS (5 aspirate/dispense cycles of 150 μ L) analyte elution was performed by two consecutive steps of 200 aspirate/dispense cycles of 100 μ L for each step [elution mixture 0.5 M NaCl + 25 mM NaOH in H₂O/ACN (90:10 v/v)].

Enzymatic digestion and LC-ESI-MS/MS detection

Protein enzymatic digestion was carried out in the final eluate (200 μ L) after pH adjustment; 12 μ L of 1% formic acid was added to lower the pH value down to 8. Before digestion, protein reduction was performed by adding 10 μ L of 50 mM

DTT solution (to 200 μ L of sample volume), followed by 30-min incubation in a thermoshaker at 60 °C. After cooling the sample at room temperature, 20 μ L of 100 mM IAA solution was added and the mixture was incubated in the dark for 30 min at room temperature. Finally, 5 μ L of a trypsin solution (0.1 μ g/ μ L in 50 mM acetic acid) was added to the sample. The reaction was stopped after about 14 h by acidifying the sample with 1 M HCl, and the final extract was filtered through 0.2- μ m PTFE filters before chromatographic injection.

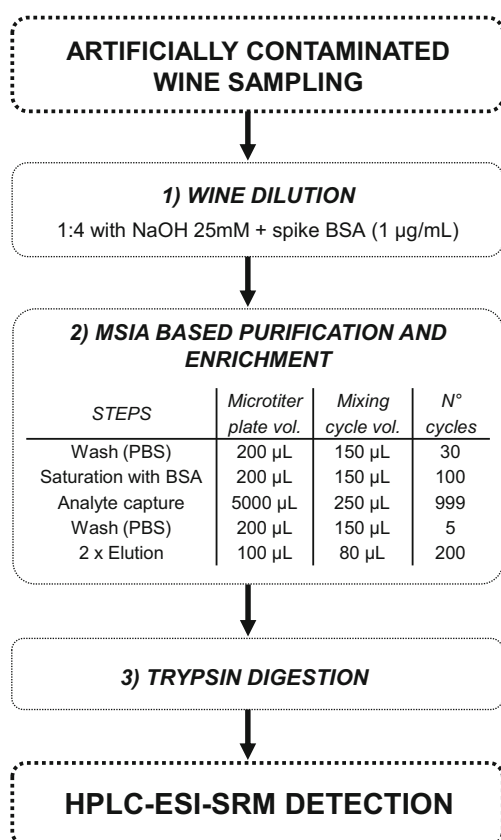
For LC-ESI-MS analyses, a platform consisting of a UHPLC pumping system, an autosampler, an ESI interface, and a Linear Ion Trap Mass Spectrometer (Velos Pro—Thermo Fisher Scientific, San Jose, USA) was used. Peptide separation was accomplished on an Aeris Peptide XB-C18 column (150 $\times$ 2.1 mm, 3.6 μ m, 100 Å; Phenomenex) at a flow rate of 200 μ L/min, using a binary gradient composed of H₂O + 0.1% formic acid (solvent A) and CH₃CN + 0.1% formic acid (solvent B). The gradient elution program was: 0–20 min linear from 12 to 50 % B; step change to 90% B then isocratic for 10 min; step change to 12% B then isocratic for 15 min for column conditioning. The ESI source settings were the following: sheath gas flow (arbitrary units) 25, auxiliary gas flow (arbitrary units) 5, spray voltage 4 kV, capillary temperature 320 °C, and heater temperature 30 °C. According to a previous study [21], the peptide markers and relevant transitions included in the MS/MS (linear ion trap-SRM) acquisition method were

- Peptide LTEWTSSNVMEER (in the following abbreviated as LTE): precursor ion 791.4 m/z (charge state +2); selected transitions: y_4^+ (564.2 m/z), y_8^+ (951.4 m/z), y_9^+ (1052.5 m/z);
- Peptide GGLEPINFQTAADQAR (in the following abbreviated as GGL): precursor ion 844.4 m/z (charge state +2); selected transitions: y_{12}^{+2} (666.3 m/z), y_7^+ (732.4 m/z), y_{10}^+ (1121.5 m/z);
- Peptide YPILPEYLQCVK (in the following abbreviated as YPI): precursor ion 761.9 m/z (charge state +2); selected transitions: b_4^+ (487.3 m/z), y_8^+ (1036.5 m/z), y_9^+ (1149.6 m/z).

Two chromatographic segments were set up in which each peptide was isolated and activated by collisional induced dissociation (CID) with a normalized collision energy of 35%, and the ion current related to the three most intense transitions were recorded within a 3 m/z wide window.

Results and discussion

The method development started with preliminary experiments on OVA standard solution aimed to evaluate some



Scheme 1 General workflow of the analytical approach devised by combining MSIA and SRM detection

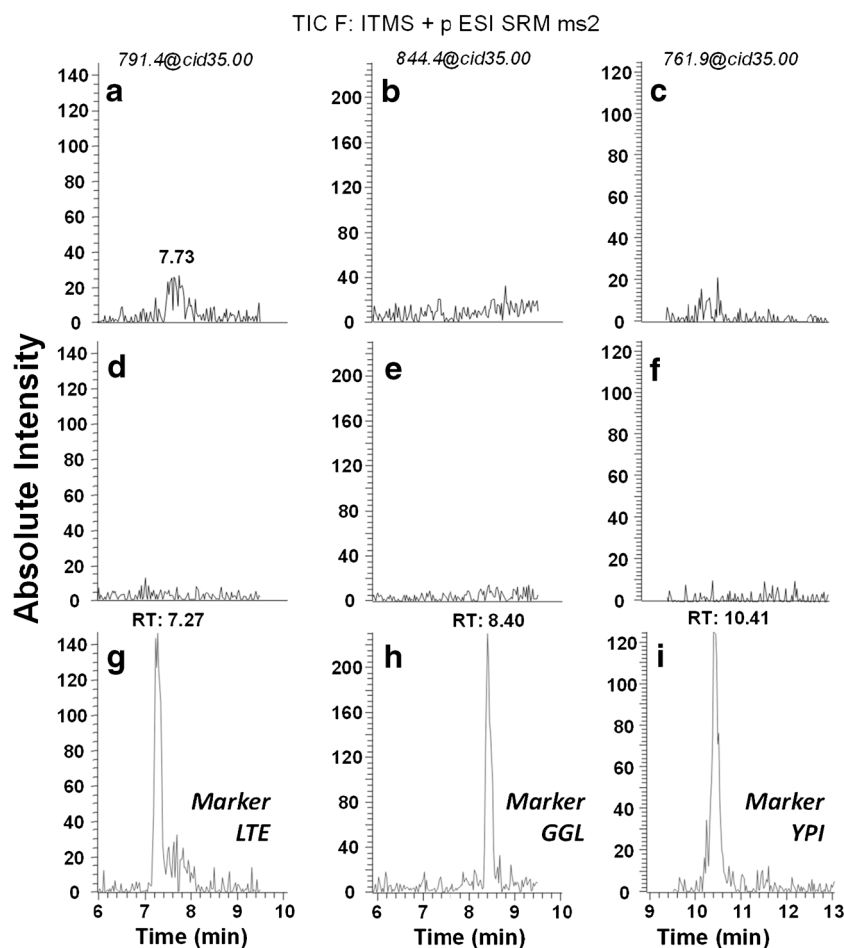
critical aspects of the immuno-enrichment step such as the maximum analyte loading on the antibody modified tips, the elution conditions, and the role of non-specific adsorption (targeted experiments were planned accordingly). Given the poor multicharge ionization efficiency of OVA, as an intact protein, and the low resolution/mass range of the mass analyzer used, the inclusion of a digestion step and the analysis of specific tryptic peptides (as markers for egg residual traces in wine) was mandatory. Quantitation was performed by LC-ESI-ion trap MS in Selected Reaction Monitoring mode [21]; in particular, three most intense transitions (see “Materials and methods” section) of the selected marker peptides, namely, LTEWTSSNVMEER (LTE, m/z 791.4), GGLEPINFQTAADQAR (GGL, m/z 844.4), and YPILPEYLQCVK (YPI, m/z 761.9), were acquired.

First of all, OVA standard solutions at two concentrations (0.5 and 2 $\mu\text{g/mL}$) in PBS were prepared. Aliquots of the fresh-made OVA standard solutions were digested and saved to be used as reference for further evaluation (see later). To assess roughly the efficiency of the analyte capture, OVA standard solutions were “loaded” on antibody modified MSIA D.A.R.T.’STM and protein depletion in the loading sample evaluated. A significant depletion was observed (data not shown),

proving that the in-house customization of MSIA D.A.R.T.’STM by immobilization of the anti-OVA polyclonal antibody and the proposed loading procedure by repeated aspiration/dispense cycles (see Experimental) were, indeed, both effective.

The second step was to evaluate the elution efficiency. To this purpose, a strongly basic saline solution with a low percentage of organic solvent (0.5 M NaCl + 25 mM NaOH in $\text{H}_2\text{O}/\text{ACN}$ 90:10) was selected based on previous findings in our laboratory [36, 37]. Sequential elution steps with fresh eluent were carried out to increase analyte recovery (the eluted fractions were combined and digested after pH neutralization by formic acid addition); unfortunately, a recovery considerably lower than expected was found. At first glance, it could be argued that eluent composition could be responsible for such a finding. Indeed, trypsin activity could be affected by the presence of an organic solvent impairing the digestion yield, whereas the relatively high electrolyte concentration (0.5 M NaCl) could cause ion suppression effects in the electrospray ionization. In order to prove/reject this hypothesis, OVA solutions prepared at the same concentrations (0.5 and 2 $\mu\text{g/mL}$) in the same eluting mixture were subjected to direct trypsin digestion omitting the loading step on MSIA D.A.R.T.’STM. The MS signals of the marker peptides were

Fig. 1 Overlay of the SRM chromatograms referred to peptide markers LTE, GGL, and YPI acquired for different samples purified with differently customized MSIA D.A.R.T.’S: **A–C** chromatograms related to allergen-free wine sample, purified with poly-anti-OVA customized MSIA D.A.R.T.’S; **D–F** chromatograms of spiked wine sample (0.5 $\mu\text{g/mL}$) purified with blank MSIA D.A.R.T.’S (no antibody); **G–I** chromatograms of spiked wine sample (0.5 $\mu\text{g/mL}$) purified with poly-anti-OVA custom MSIA D.A.R.T.’S



now from 3- to 10-fold higher than those previously observed, suggesting that the low recovery has to be mainly ascribed to the immunoaffinity step on MSIA D.A.R.T.'STM. The effect most likely involved could be a significant non-specific (and irreversible) OVA interaction with the MSIA D.A.R.T.'STM stationary phase competing with the antigen-antibody recognition.

Thus, the MSIA D.A.R.T.'STM preparation procedure was adjusted including a saturation step with BSA (1 mg/mL) before the immunoaffinity capture step; in addition, all processed samples were supplemented with BSA (1 µg/mL) to ensure the presence of the blocking agent also during the capture cycles. This approach proved to reduce significantly the analyte loss due to non-specific adsorption on the MSIA D.A.R.T.'STM stationary phase.

Assay optimization on artificially contaminated wines

A white wine from Pinot Grigio grapes, provided by a local retailer and deemed allergen-free was taken as model matrix. A 5-mL wine aliquot was artificially contaminated with OVA at 0.5 µg/mL concentration for consistency with the previously selected experimental conditions. Afterwards, both spiked and allergen-free wine samples were diluted 1:1 (v/v) with a basic aqueous solution to raise the pH around neutrality and facilitate antigen-antibody recognition occurring at physiological pH; 1 µg/mL BSA was also added to prevent non-specific interactions (vide supra).

In order to prove that the observed signal could be ascribed only to specific enrichment based on immunological recognition, poly-anti-OVA customized MSIA D.A.R.T.'STM and “blank” MSIA D.A.R.T.'STM were prepared in parallel; the latter was subjected to the whole functionalization procedure except for the inclusion of poly-anti-OVA into buffer solution after activation. A comparison of the SRM chromatograms referred to the LTE, GGL, and YPI marker peptides was reported in Fig. 1. Panels A, B, and C refer to allergen-free wine processed with poly-anti-OVA customized MSIA D.A.R.T.'STM; panels D, E, and F refer to spiked wine sample processed with antibody-free MSIA D.A.R.T.'S; panels G, H, and I show chromatograms of spiked wine sample processed with poly-anti-OVA customized MSIA D.A.R.T.'S. As it can be seen, peptide markers signals could be detected only in the last case, demonstrating that the described workflow was, indeed, effective and that non-specific absorption could be considered negligible either with OVA standard (panels A, B, C) or allergen-free wine (panels D, E, F).

After this preliminary feasibility test, experimental conditions were further tuned in order to improve the final performance in terms of sensitivity and reproducibility. In particular, two factors were considered: (1) total elution volume and (2) matrix dilution factor required to maximize recovery and minimize matrix effects, respectively, without compromising the

assay sensitivity. As for point (1), two aliquots (0.5 µg/mL) of a spiked wine sample were processed in parallel for the immuno-enrichment on two customized MSIA D.A.R.T.'S that were separately eluted using two or three elution steps (100 µL each). Panel A of Fig. 2 clearly indicates that two consecutive elution steps provided the best compromise (note that at least two steps must be performed since 200 µL is the volume required by the autosampler for three replicate injections). As for point (2), aliquots of a spiked (0.5 µg/mL) wine sample were diluted 1:2, 1:4, and 1:10 with NaOH solutions of different concentrations in order to ensure, in all cases, a final pH around neutrality. Samples were then processed for the immuno-enrichment step followed by (2 × 100 µL) elution steps. The results reported in panel B of Fig. 2 clearly show that a 1:4 dilution factor provided the best compromise between matrix effects reduction and sensitivity.

Assay evaluation and comparison with alternative methods

The following step was the evaluation of the main figures of merit of the method whose final workflow is reported in Scheme 1. Therefore, several wine aliquots were prepared and fortified with variable amounts of OVA in the concentration range 0.05–1.0 µg/mL. After a simple 1:4 dilution with 25 mM NaOH solution, the

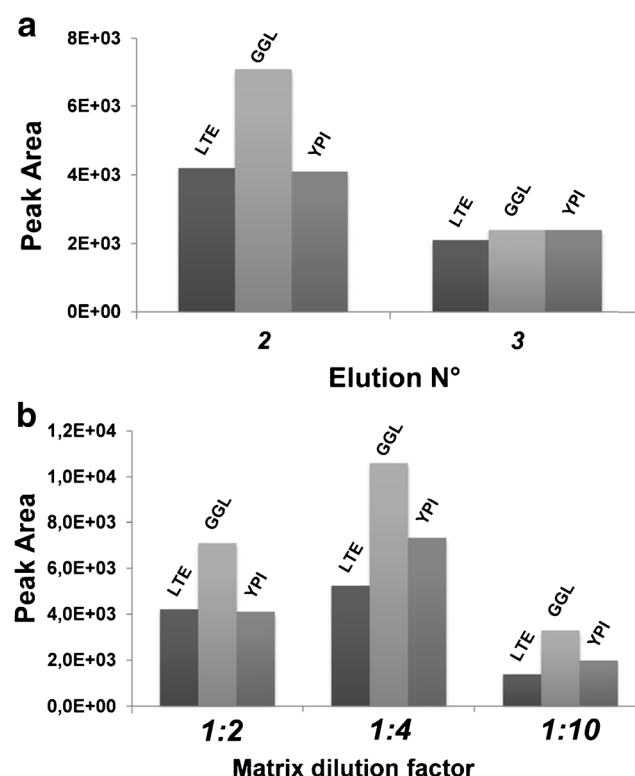


Fig. 2 Comparison of chromatographic peak areas obtained for the peptide markers under different assay conditions in order to optimize the elution volume (A) and matrix dilution factor (B). A spiked wine sample (0.5 µg/mL) was used

sample was subjected to immuno-enrichment, and peptides obtained from the tryptic digestion were separated by LC and analyzed in SRM acquisition mode (three transitions detected for each peptide). Figure 3 showed typical chromatograms of the three peptide markers recorded for blank wine samples fortified with OVA at 0.05 µg/mL concentration. Raw data in Fig. 3 give an immediate direct experimental proof of the detection performance of the proposed method, considering that this is the lowest concentration ever investigated for tracing egg residues in wines by LC-MS. The GGL peptide presents, indeed, among the selected peptide markers, the most favorable signal-to-noise (S/N) ratio whereas YPI presents the lowest S/N ratio (around 6 in the specific case). Since the unambiguous identification of OVA relies on the simultaneous detection of the three peptide markers at a minimum S/N ratio of three, the above finding indicates that even at the lowest investigated concentration of 0.05 µg/mL, OVA contamination can be reliably identified. For quantitative purposes, the most sensitive marker, GGL, can be used. Note that at a concentration as low as 0.05 µg/mL, the observed S/N ratio was around 10 for LTE and 20 for GGL, which means that quantitation can be readily performed, GGL being the preferred marker. A calibration curve “peak area (arbitrary units) versus [OVA] (µg/mL)” was then generated in the concentration range from 0.05 to 1.0 µg/mL (at least three replicates for each concentration). Unfortunately, at

higher concentration values, the responses tend to level off indicating that the dynamic range cannot be widened. Data were fitted by a second-order polynomial model $Y = \beta_0 + \beta_1 x + \beta_2 x^2$ obtaining the following parameter estimates ($\pm 95\%$ confidence interval): $\beta_0 = 400 (\pm 500)$, $\beta_1 = 18,000 (\pm 3000)$, and $\beta_2 = -10,000 (\pm 3000)$ ($R^2 = 0.98$). Limit of detection (LOD) and quantitation (LOQ) were extrapolated from the above calibration line using the standard deviation of the intercept as an estimate of the noise. The LOD and LOQ values calculated at S/N = 3 and S/N = 10, respectively, were 0.01 and 0.03 µg/mL, respectively. These extrapolated figures agreed well with the experimentally observed minimum concentration of 0.05 µg/mL, which showed a S/N ratio higher than 20 for the GGL peptide.

Finally, the within-day precision, evaluated analyzing eight independent samples (fortified with OVA at 0.1 µg/mL), was found equal to 18% for GGL peptide marker.

In order to appreciate the performance of the devised method, an overview of the main analytical methods proposed in the last 4 years for the detection of egg allergen in wine is provided in Table 1. Compared to other MS-based methods, MSIA proved to be quite promising in terms of sensitivity gain. Additional advantages are the limited sample pretreatment required (a simple dilution step) and the possibility to fully automate the whole

Fig. 3 Typical chromatographic peaks acquired for the three peptide markers LTE (791.4), GGL (844.4), and YPI (761.9) at the lowest concentration investigated of 0.05 µg/mL. Raw (unsmoothed) data are shown. The final workflow sketched in Scheme 1 was followed

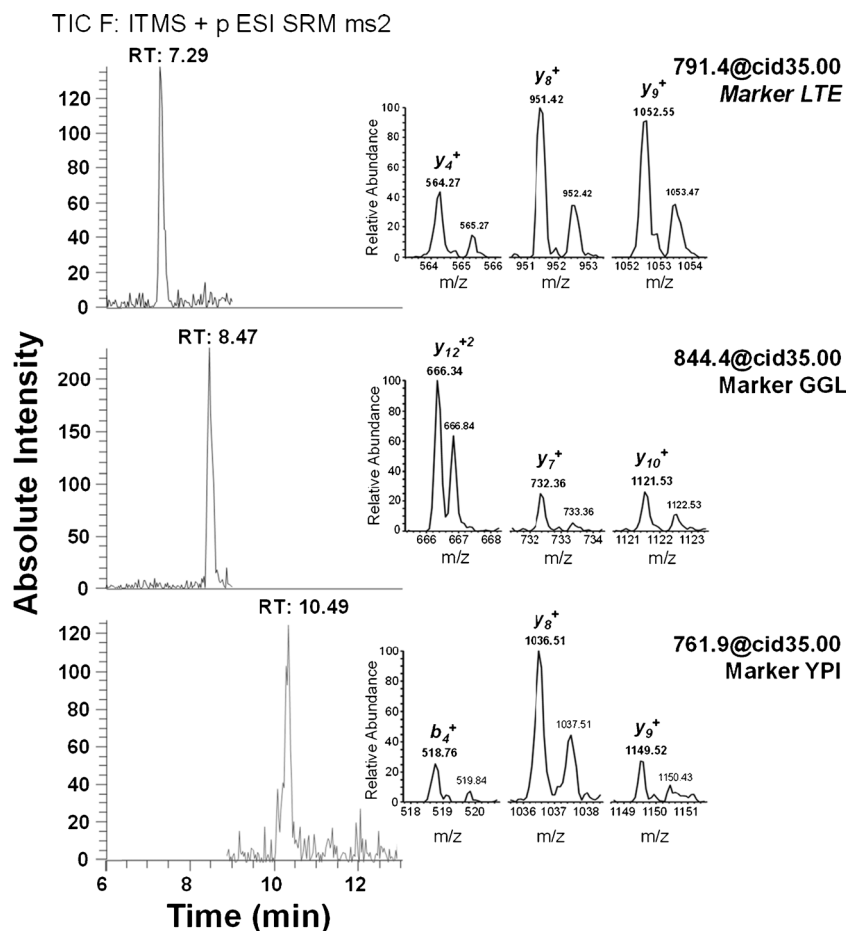


Table 1 Overview of the main analytical methods proposed in the last 4 years for the detection of egg allergens in wine

Method	Allergen	Matrix	Dynamic/linear range (DR/LR)	LOD ($\mu\text{g/mL}$)	LOQ ($\mu\text{g/mL}$)	Reference
HPLC/LTQ linear ion trap-SRM	Ovalbumin	Red wine	LR: 2–100 $\mu\text{g/mL}$	0.8	2	[17]
HPLC/quadrupole-time-of-flight-SIM	Albuclar®	Red wine	ND	0.5	ND	[18]
HPLC/Orbitrap™ HR-MS	Ovalbumin	White wine	LR: 0.2–2 $\mu\text{g/mL}$	0.3	ND	[21]
MicroHPLC/linear ion trap-SRM			LR: 0.2–2 $\mu\text{g/mL}$	0.19	ND	
HPLC/Orbitrap™ HR-MS	Egg white	White wine	LR: 0.25–10 $\mu\text{g/mL}$	0.4–0.7 ^a	ND	[20]
On-line SPE-HPLC/linear ion trap SRM	Egg white	White wine	LR: 0.1–2 $\mu\text{g/mL}$	0.036	0.121	[22]
Commercial ELISA kit	Egg white	Red wine	DR: 0.061–2 $\mu\text{g/mL}$	0.056	0.16	[11, 38]
Commercial ELISA kit	Whole egg	Red wine	DR: 0.5–13.5 mg/kg	0.32	ND	[10]
		White wine	DR: 0.5–13.5 mg/kg	0.20–0.35 ^b	ND	
ELISA	Ovalbumin	White/red wines	LR: 0.005–3 $\mu\text{g/mL}$	0.005	0.005	[39]
	AlbuVin	White wines	LR: 0.021–10 $\mu\text{g/mL}$	0.010	0.021	
		Red wines	LR: 0.010–3 $\mu\text{g/mL}$	0.010	0.010	
Western blotting	Blancoll/Ovocoll	Red wines	LR: 0.05–0.5 $\mu\text{g/mL}$	ND	0.05	[40]
Label-free SPR	Egg white	White wine	DR: 0.09–10 $\mu\text{g/mL}$ ^a	0.03–0.16 ^a	0.09–0.3 ^a	[36]
		Rosé wine	DR: 0.08–40 $\mu\text{g/mL}$ ^a	0.04–0.2 ^a	0.08–0.5 ^a	
Label-free SPR	Egg white	Red wine	DR: 0.3–20 $\mu\text{g/mL}$	0.2	0.3	[37]
MSIA HPLC-linear ion trap-SRM	Ovalbumin	White wine	DR: 0.05–0.5 $\mu\text{g/mL}$	0.01 ^c	0.03 ^c	This work

^a LOD/LOQ values and dynamic ranges calculated on wine matrices obtained by different grape varieties

^b LOD values calculated with different standard deviations on blank samples (repeatability versus reproducibility standard deviations)

^c Extrapolated from the GGL peptide calibration function (see also text)

procedure. Furthermore, MSIA approach shortens the distance between MS-based methods and ELISAs in terms of sensitivity, offering at the same time an unambiguous detection of the allergenic protein, through the simultaneous detection of three unique peptide markers.

In order to prove the relevance of the devised MS-based immunoassay for egg residues in wine, preliminary experiments aimed to widen the method application to different wine matrices were performed. In particular, six more matrices were tested

differing both for grape selection (Vermentino di Sardegna, Greco di Tufo, Fiano di Avellino, Müller Thurgau, Bombino nero, and San Severo) and vinification procedure (white and rosé). All the matrices were artificially fortified with ovalbumin at the same concentration and treated with the same approach developed for the previous reference matrix (Pinot Grigio). Typical resulting chromatograms were reported in Fig. 4 providing clear evidence that all three peptide markers were unambiguously detected in all the investigated matrices.

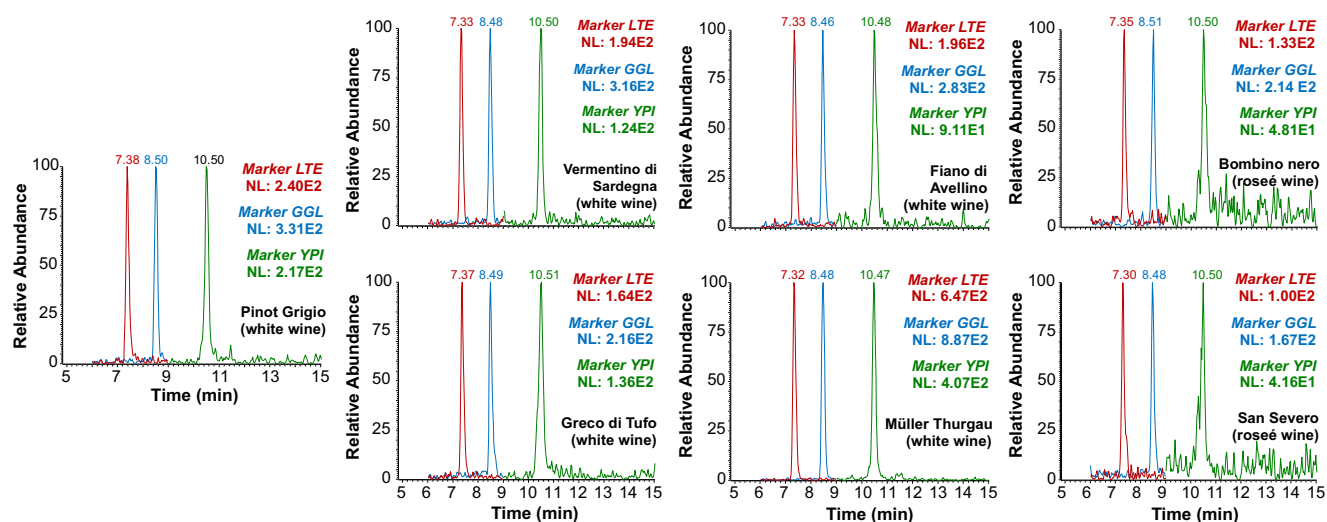


Fig. 4 Comparison of typical chromatograms recorded for the three peptide markers LTE (791.4), GGL (844.4), and YPI (761.9) (the three traces were overlapped in the same chromatogram) in different wine matrices, at the same OVA concentration of 0.25 $\mu\text{g/mL}$

Conclusions

The feasibility of MSIA approach tailored to the detection of residual egg allergens in wines was proved. Figures of merit (specificity, dynamic range, precision, sensitivity) were provided. The proposed MSIA approach outperforms other MS-based methods and gets closer to ELISAs in terms of sensitivity, boasting the unquestionable advantage of an unambiguous detection of the allergenic protein by the simultaneous detection of three unique peptide markers. The proposed method was developed on a multichannel electronic pipette system capable of processing, in a semi-automated way, up to 12 samples simultaneously. In prospective, the same approach could be scaled up onto a fully automated system, which allows more samples to be processed simultaneously throughout the whole workflow from the antibody immobilization to the elution of the purified target, providing a fully automated, high-throughput, time-effective sample purification procedure.

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Compliance with ethical standards

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Conflict of interest R.P. declares that she has no conflict of interest. L.M. declares that she has no conflict of interest. F.P. declares that he has no conflict of interest. R.C. is an EMEA Specialist for MSIA platform at Thermo Fisher Scientific.

Ethical approval This article does not contain any studies with human participants or animals performed by any of the authors.

Informed consent Not applicable.

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