ORIGINAL ARTICLE

Double lateral flow immunosensing of undeclared pork and chicken components of meat products

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Abstract Adulteration of meat products is a serious problem in the modern society. Consumption of falsified meat products can be hazardous to health and/or lead to violating religious dietary principles. To identify such products, rapid and simple test systems for point-of-need detection are in demand along with complex laboratory methods. This study presents the first double lateral flow (immunochromatographic) test system, which allows simultaneous revealing two prevalent types of falsifications—undeclared addition of pork and chicken components to meat products. In the proposed test system, porcine myoglobin (MG) and chicken immunoglobulin Y (IgY) were used as specific biomarkers recognizable by antibodies. Within the optimization of the analysis, the concentrations of the immune reagents and regimes of their application on the working membrane were selected, which provided minimal limits of detection (LODs) for both analytes. The developed test system enables the detection of MG and IgY with the LODs of 10 and 12 ng/mL, respectively, which accords to addition of 0.1% of the undeclared meat compounds. The applicability of the test system to control the composition of raw meat mixtures and cooked food products was confirmed. The developed approach can be considered as a promising tool for monitoring composition of meat products.

Abbreviations

BSA	Bovine serum albumin
CZ	Control zone
DMSO	Dimethyl sulfoxide
ELISA	Enzyme-linked immunosorbent assay
GAMI	Goat anti-mouse immunoglobulins
GARI	Goat anti-rabbit immunoglobulins
GNPs	Gold nanoparticles
IgY	Chicken immunoglobulin of class Y
LOD	Limit of detection
Mab	Monoclonal antibodies
MG	Myoglobin
PBS	50 MM K-phosphate buffer with 0.1 M NaCl, pH 7.4
PBST	PBS containing 0.05% Triton X-100
RACI	Rabbit anti-chicken immunoglobulins
RACI–HRP	The conjugate of RACI with horseradish peroxidase
TEM	Transmission electron microscopy
TZ	Test zone

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Introduction

The adulteration of meat product seems an often case in modern food production because unscrupulous manufacturers are used to replacing declared components of foodstuffs with cheaper ones (Momtaz et al. 2023). Consequences of such falsifications are not limited to misinforming consumers. Consumption of counterfeit products may have serious negative effects for health and violate religious dietary requirements (Bansal et al. 2017; Hossain et al. 2020). In the

current analytical practice, the predominant methods for species identification in meat products are hybridization techniques with recognition and subsequent amplification of specific regions of nucleic acids (Azad et al. 2023). Although these methods are characterized by high selectivity and low limits of detection (LODs), their implementation involves use of complex and expensive equipment in special laboratories by highly qualified personnel. Recent developments of autonomous systems for polymerase chain reactions or isothermal amplifications are still far from wide application (Narasimhan et al. 2023). Therefore, test systems for rapid mass control of food composition, which can be used in out-of-laboratory conditions, are in great demand along the entire chain of food production—from the receipt of raw materials to meat products retailing.

Several lateral flow test strips have been reported as tools to detect proteins specific for different meat sources. Among the latter biomarkers, troponin I (Chen and Hsieh 2002; Zvereva et al. 2020a), porcine serum albumin (Masiri et al. 2016), and porcine immunoglobulins (Kuswandi et al. 2017; Hendrickson et al. 2021a; Banerjee et al. 2023) can be mentioned. However, a priority direction in the development of lateral flow immunoassay is the transformation of monoparametric testing to multiparametric one enabling simultaneous detection of several biomarkers using one test strip (Guan et al. 2022; Wu et al. 2020). Compared to monoparametric tests, the performance of multiple tests increases while the consumption of reagents and expendable materials for their manufacturing is significantly reduced. Their application in mass screening provides increased productivity and reduces cost of the testing per one analyte (Anfossi et al. 2019). Unlike immunochromatographic multiparametric tests created and used for various tasks, to date, there are no such tests developed to reveal undeclared additives in meat products. It should be noted that the development of multiparametric test strips is not an ordinary integration of several monoparametric ones. A combination of the immunoreagents having different specificities on one test strip modifies their interactions before reaching the binding zones. The integration of many immunoreagents on one test strip leads to other problems—choosing acceptable interaction conditions and ensuring similar binding levels for all of them. Simple combination of several reagents in one test strip without adapting the interaction conditions demonstrated the deterioration of analytical parameters (Bartosh et al. 2020). Besides, the best regimes cannot be complied with for all immune interactions simultaneously, which complicates the choice of new optimal conditions (Bartosh et al. 2020). Therefore, the development of multiparametric tests needs a special selection of interaction conditions, which are likely to differ from those for monoparametric testing.

Among possible ways of meat products counterfeiting, the most common ones include the use of chicken and pork raw meat as additives substituting the declared raw materials (Alikord et al. 2018). The additional importance of pork control is explained by the strong forbiddance of pork consumption in Islam and Judaism (Zia et al. 2020). As for chicken, relatively cheap production causes its frequent use for falsification of meat products composed from more expensive kinds of meat (Yang et al. 2022).

Therefore, the present study aims at the development and characterization of a double lateral flow test system for the detection of pork and chicken meat in food products using porcine myoglobin (MG) and chicken immunoglobulin Y (IgY) as specific biomarkers. The work includes obtaining reagents for immune detection of both analytes, selection of conditions for sensitive detection of both biomarkers, determination of analytical parameters for the developed double test, and validation of its applicability for the determination of pork and chicken meat in raw and cooked meat products.

Materials and methods

Reagents

MG and monoclonal antibodies (Mab) against MG of clone 7C3 (Mab7C3) were purchased from HyTest (Moscow, Russia). Mab against MG of clone A6 (MabA6) were obtained from the Russian Research Center of Molecular Diagnostics and Therapy (Moscow, Russia). Chicken IgY, rabbit anti-chicken immunoglobulins (RACI), goat anti-mouse immunoglobulins (GAMI), goat anti-rabbit immunoglobulins (GARI), and the conjugate of RACI with horseradish peroxidase (RACI–HRP) were obtained from Imtek (Moscow, Russia). GAMI–HRP and GARI–HRP were from Jackson ImmunoResearch (Cambridgeshire, UK).

Chloroauric acid, bovine serum albumin (BSA), streptavidin–HRP conjugate (STR-HRP), sodium citrate, sodium azide, sucrose, dimethyl sulfoxide (DMSO), and Triton X-100 were obtained from Sigma Chemicals (St. Louis, MO, USA). D-biotin-N-hydroxysuccinimide ester was from ICN Biomedicals (Irvine, CA, USA). One-component HRP substrate solution based on 3,3′,5,5′-tetramethylbenzidine was from Immunotech (Moscow, Russia). All other solvents and chemicals of analytical quality were obtained from Khimmed (Moscow, Russia).

Enzyme-linked immunosorbent assay (ELISA) was performed in Costar 9018 96-well transparent polystyrene microplates from Corning Costar (Corning, NY, USA). For the production of lateral flow test strips, CNPC-SS12 nitrocellulose working membrane (with a pore size of 15 μm) GFB-R4 separation pad, and AP045 adsorption pad (all from Advanced Mrodevices, Ambala Cantt, India) were applied.

Biotinylation of antibodies

Antibodies were preliminarily dialyzed against 50 mM K-phosphate buffer with 0.1 M NaCl, pH 7.4 (PBS) in Amicon Ultra centrifugal filters containing regenerated cellulose (Merck Millipore, Carrigtwohill, Ireland) by centrifugation at 10,000 g for 15 min. Covalent binding of biotin to antibodies was carried out at a molar ratio of 10:1 following Bayer and Wilchek (1990). A solution of D-biotin-N-hydroxysuccinimide ester in DMSO (3.1 mg/mL) was added to the antibody solution. The mixture was incubated at room temperature for 2 h and dialyzed against PBS as described above.

ELISA of biomarkers

The ELISA of MG was implemented in the sandwich format as described (Zvereva et al. 2020b). MabA6 or Mab7C3 were immobilized in microplate wells (2 μg/mL in PBS) and incubated overnight at 4 °C. Every step of the ELISA was followed by fourfold washing of the microplate with PBS containing 0.05% Triton X-100 (PBST). Solutions of MG (100 μL, 5 μg/mL–0.1 ng/mL in PBST) were added to the wells and incubated for 1 h at 37 °C. After that, 100 μL of biotinylated Mab (2 μg/mL, in PBST) and STR–HRP (1:5,000 dilution, in PBST) were successively added and incubated in the same regime.

Chicken IgY detection was carried out as described by Hendrickson et al. (2021b). For this, RACI (2 μg/mL in PBS) were absorbed in the microplate wells by overnight incubation at 4 °C. Solutions of IgY (100 μL, 30 μg/mL–0.5 ng/mL in PBST) and RACI–HRP (100 μL, diluted 1:5,000 in PBST) were successively added and incubated by the same way and with the same washing procedures as in the MG ELISA.

Finally, for both ELISAs, the same procedure of HRP activity detection was implemented. For this, HRP substrate solution (100 μL) was added to the wells and incubated for 15 min at room temperature. Then, the enzymatic reaction was stopped by the addition of 1 M H_2SO_4 (50 μL per well). The optical density (OD) was measured at 450 nm with a Zenyth 3100 microplate photometer (Anthos Labtec Instruments, Wals, Austria).

Preparation and characterization of gold nanoparticles

Gold nanoparticles (GNPs) with an average diameter of 25 nm were obtained by citrate reduction of $HAuCl_4$ to Au^0 as described by Frens (1973). The GNP preparation was characterized by transmission electron microscopy

(TEM) using a CX-100 microscope (Jeol, Japan) as reported by Zvereva et al. 2020a.

Preparation of antibody–GNP conjugates

The antibody–GNP conjugates were prepared as described by Zvereva et al (2020b), and Hendrickson et al. (2021b). Firstly, Mab7C3, MabA6, and RACI were dialyzed against 10 mM Tris–HCl buffer, pH 9.0. The pH of the GNP solution was adjusted to 8.5–9.0 by 0.1 M K_2CO_3. For the conjugation, the antibodies were added to the GNP solution with concentrations of 20 μg/mL for Mab7C3, 8 μg/mL—for MabA6, and 10 μg/mL—for RACI. The mixtures were incubated for 45 min with stirring at room temperature. After that, a solution of 10% BSA was added to its final content of 0.25%, and the mixtures were stirred for another 15 min. The obtained conjugates were precipitated by centrifugation at 12,800 g for 15 min at 4 °C and resuspended in 10 mM Tris–HCl buffer, pH 8.5, containing 1.0% BSA, 1.0% sucrose, and 0.05% sodium azide. The conjugates were stored at 4 °C.

Preparation of immunochromatographic test strips

The reagents listed below were applied on the working membranes using an Iso-Flow dispenser (Imagene Technology, Hanover, NH, USA) with a loading of 0.1 μL per 1 mm of the membrane. All solutions were prepared in PBS.

Individual test strips for chicken IgY detection. The test zone (TZ) was formed using RACI (0.75, 1.0, or 2.0 mg/mL). The control zone (CZ) was formed using GARI (0.5 mg/mL).

Individual test strips for MG detection. The TZ was formed using MAb7C3 or MabA6 (0.75, 1.0, or 2.0 mg/mL). The CZ was formed using GAMI (0.5 mg/mL).

Double test strip. The TZ for chicken IgY detection was formed using RACI (0.75 mg/mL), and the TZ zone for MG detection was formed using MAb7C3 (2.0 mg/mL). The CZ was formed using a mixture of GARI and GAMI solutions with concentrations of 0.35 mg/mL for every protein.

Working membranes with applied immunoreagents were dried for at least 20 h at room temperature, composed with separation and adsorption pads, and cut into test strips (with 3.0 mm width) using an automatic guillotine cutter (KinBio, Shanghai, China).

Sample pretreatment

Raw meat (chicken, turkey, pork, beef, goat, rabbit, and horse) as well as vegetarian and turkey meat sausages were purchased from local supermarkets. Beef and pork-based cooked sausages with a confirmed composition were produced and kindly provided by the V.M. Gorbatov Federal

Research Center for Food Systems of the Russian Academy of Sciences (Moscow, Russia). Meat mixtures were obtained in our laboratory from minced raw meat. To prepare heat-treated meat mixtures, they were incubated in vacuum bags for 45 min at 80 °C. After that, the mixtures were cooled to 35 °C by spraying the bags with water for 30 min. The prepared mixtures were stored at 4 °C before the analysis.

Meat samples preparation was carried out as proposed by Hendrickson et al. (2021a). Meat samples were minced by a household homogenizer. Then, to 250 mg of every homogenate, 5 mL of the extraction buffer (PBS containing 0.1% Triton X-100 and 0.5 M KCl) was added and incubated with stirring for 10 min at room temperature. Then, the samples were sonicated for 10 min and centrifuged for 10 min at 5,000 g. The resulting supernatants were analyzed by the test strips.

Lateral flow immunosensing

Individual assays. The tested sample (100 µL), RACI–GNP ($OD_{520} = 6$, 0.5 µL), or MabA6–GNP conjugate ($OD_{520} = 17.5$, 0.5 µL) were mixed and incubated with test strips for 15 min.

Double assays. The tested sample (100 µL), RACI–GNP ($OD_{520} = 6$, 0.5 µL), and MabA6–GNP ($OD_{520} = 17.5$, 0.5 µL) were mixed and incubated with test strips for 15 min.

After the analysis, test strips were scanned using the CanoScanLiDE 90 scanner (Canon, Tokyo, Japan). The obtained images were processed with TotalLab software (TotalLab, Newcastle upon Tyne, UK). The total level of coloration intensity in the binding zones was used to confirm the presence of target analytes in the samples and to estimate their content.

Processing of the assay results

Average and RSD values for the measured responses (OD_{450} for ELISAs or intensity of the TZ coloration for lateral flow tests) were determined based on triplicate measurements. The concentration dependences of the responses were approximated using the Origin software (Origin Lab, Northampton, MA, USA).

Results and discussion

Obtaining and characterization of reagents for lateral flow immunosensing

The analytical capabilities of the immunoreagents were characterized in the sandwich ELISA. To perform the ELISA of MG and find the best combination of capture and detection antibodies, both Mab7C3 and MabA6 were biotinylated.

It was shown that all variants of antibody combination as immobilized and biotinylated compounds provided specific detection of porcine MG. The minimal LOD of 9 ng/mL was achieved in the case of Mab7C3 immobilization (capture antibodies) and biotinylated MabA6 interacting with the STR–HRP conjugate (Fig. 1S a). The use of RACI immobilized in the microplate wells and the RACI–HRP conjugate in the sandwich ELISA allowed the revealing chicken IgY with the LOD of 2.5 ng/mL conjugate (Fig. 1S b). Therefore, the demonstrated high sensitivity of biomarker detection ensured using the considered antibodies for the development of lateral flow test systems.

As a colored marker and a carrier for antibodies, GNPs were synthesized and characterized by TEM. According to the obtained images, the average diameter of GNPs was 24.9 ± 4.6 nm with an ellipticity of 1.2 ± 0.1 (in total, 49 nanoparticles were processed) (Fig. 2S). Antibody concentrations for conjugation with GNPs were selected as described in our previous studies (Hendrickson et al. 2021b; Zvereva et al. 2020b): 20 µg/mL—for Mab7C3, 8 µg/mL—for MabA6, and 10 µg/mL—for RACI.

Obtaining and characterization of individual lateral flow test strips for chicken IgY

The works started with the production and characterization of individual lateral flow assays for both biomarkers to develop test systems for comparison with double lateral flow tests, where analytical parameters were expected to retain after the integration of reactants with different specificities. The development included the selection of the reactants' concentrations and conditions of their interactions to reach the minimal LODs and high coloration of the formed zones on the test strips. Two types of nitrocellulose working membranes were tested for the application of immunoreagents: the CNPC-SS12 (Advanced Microdevices, Ambala Cantt, India) and the UniSart CN95 (Sartorius, Goettingen, Germany) membranes. As a result, the CNPC-SS12 working membrane was chosen for further experiments because of the formation of more distinct and uniformly colored zones during immunochromatography. To form the TZ and the CZ, RACI and GARI were applied to the working membrane, respectively. To initiate the assay, the tested sample was mixed with the RACI–GNP conjugate, and the test strip was immersed in this mixture. In the presence of the analyte in the sample, specific complexes IgY—RACI–GNP were formed, which moved with the liquid along the test strip and bound to RACI forming a colored band in the TZ. The excess of labeled antibodies moved further, bound in the CZ forming the second colored band there or transferred to the adsorption pad of the test strip.

The best analytical characteristics of the individual test strip for IgY detection were achieved by immobilization

of RACI in the TZ at a concentration of 1 mg/mL and the addition of 0.5 µL of RACI–GNP conjugate ($OD_{520} = 6$) to the sample. The LOD of IgY in the individual test strips was 12.8 ng/mL; the assay duration was 15 min conjugate (Fig. 3S a).

Obtaining and characterization of individual lateral flow test strips for MG

To develop individual lateral flow test strips for MG, two options of reagent combination were first approbated. As capture/labeled antibodies, MabA6/Mab7C3–GNP and MabA6/MabA6–GNP were used. The coloration of the TZ was observed after the analysis in both variants. However, upon the MabA6/Mab7C3–GNP combination, non-specific coloration at the zero point (no MG in the sample) was demonstrated, whereas for the MabA6/MabA6–GNP combination, the coloration intensity of TZ was weak even for high (> 500 ng/mL) concentrations of MG. Upon immobilization of Mab7C3 in the TZ (2 mg/mL) and the use of the MabA6–GNP conjugate (0.5 µL, $OD_{520} = 17.5$), the coloration intensity was very high. As a result, the LOD of MG reached 6.8 ng/mL conjugate (Fig. 3S b). GAMI was applied to the CZ at a concentration of 0.5 mg/mL to reach comparable signals of both binding zones for high (> 500 ng/mL) concentrations of MG in the sample.

Development of double lateral flow test strips

The design of the double lateral flow test strips for the simultaneous determination of IgY and MG in a sandwich format of immunochromatography is shown in Fig. 1. The working membrane of the double test strip contained two TZ (TZ1 and TZ2 with the reagents of different specificities) and one CZ. Accordingly, the development of the double assay included the choice of the TZ arrangement and the concentrations of the capture and labeled antibodies. Because the CZ was formed by a mixture of secondary antibodies of different specificities, the amount of each antibody could be decreased in comparison with monoparametric tests. Thus,
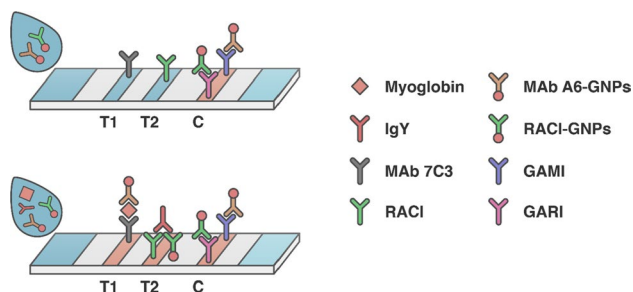
GAMI and GARI in the concentration of 0.35 mg/mL each were applied to form the CZ. It was demonstrated that the TZ coloration in the individual test of IgY was higher than that in the test system of MG at the same concentrations of both analytes. Therefore, we decided to arrange binding zones as follows: TZ1 (MG detection; Mab7C3) → TZ2 (IgY detection; RACI) → CZ. Upon such zone location, less dispersion of reagents during their movement along the membrane and more efficient binding MG were achieved. To converge the marker binding in the two TZ, the RACI concentration in TZ2 was slightly reduced compared to the individual IgY tests. Finally, Mab7C3 (2 mg/mL) and RACI (0.75 mg/mL) were applied to the test strip.

In the next step, the optimal concentrations of labeled Mab were chosen. The optimality criterion was high (and similar) coloration intensities for TZ1 and TZ2 at the same concentrations of both analytes. Figure 2 shows the dependence of the coloration intensity of TZ1 upon the change of MabA6–GNPs content in the reaction medium. TZ coloration was assessed in two variants of the analysis—upon zero and high (1,000 ng/mL) concentration of MG. To do this, analyte-free or IgY and MG-containing samples (50 µL) were mixed with the RACI–GNP conjugate (0.5 µL, $OD_{520} = 6$) and the MabA6–GNP conjugate (0.25–2.5 µL, $OD_{520} = 17.5$). It was shown that the optimal composition of the double test strip, which ensures the high intensity of TZ coloration and the absence of nonspecific coloration at the zero point, includes Mab7C3 (2 mg/mL) and RACI (0.75 mg/mL) immobilized in the TZ1 and TZ2, respectively, and RACI–GNP (0.5 µL, $OD_{520} = 6$) and MabA6–GNP (0.5 µL, $OD_{520} = 17.5$) added to tested

Fig. 1 Scheme of the developed double lateral flow test system for IgY and MG immunodetection in a sandwich format
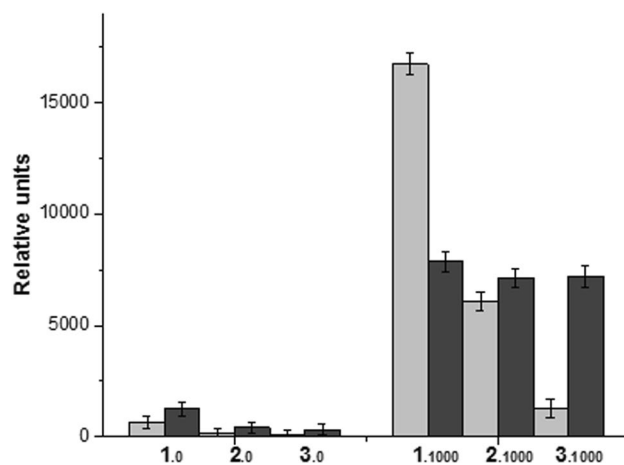

Fig. 2 The dependence of the coloration intensity of TZ1 (grey columns) and TZ2 (black columns) on the amount of the MabA6–GNP conjugate during the analysis of MG; 2.5 (1), 0.5 (2), and 0.25 µL of MabA6–GNP (3) are added to the tested sample. The TZ coloration is characterized in the absence of the analytes (columns 1.0, 2.0, and 3.0) and at their concentration of 1,000 ng/mL (columns 1.1000, 2.1000, and 3.1000)
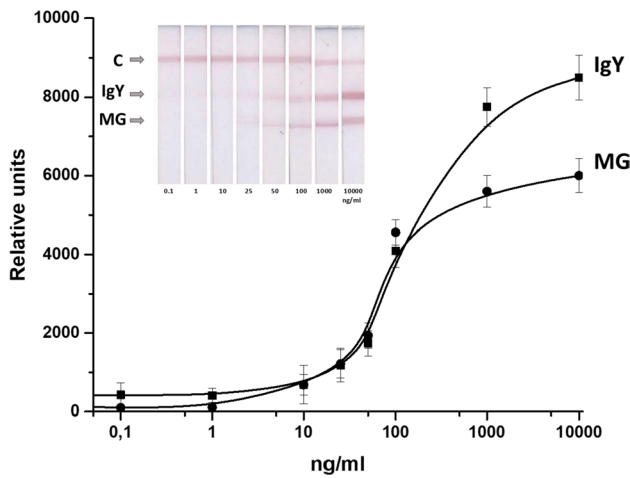
Fig. 3 Calibration curves of IgY and MG in the double test system and the appearance of test strips

sample (100 μL). The resulting calibration curves of IgY and MG are presented in Fig. 3. The LODs of IgY and MG for the double immunosensing were 12 ng/mL and 10 ng/mL, respectively. According to the literature data, both biomarkers are contained in relatively high concentrations in muscle tissues of the corresponding species. Thus, the average MG content is estimated as 1 mg per 1 g of porcine meat (Liu et al. 2021) while 1 g of chicken muscles contains 0.2 mg of IgY (Hendrickson et al. 2022). Therefore, the demonstrated detection sensitivity for both biomarkers gives reason for the application of the developed double test system to analyze real raw meat mixtures and cooked meat products.

Analysis of meat samples from different species using the developed double test system

To confirm the specificity of the developed test system, the meat of different animals and birds was tested. For this, extracts of pork, chicken, turkey, beef, lamb, horse, and rabbit meat were prepared and analyzed. The obtained data demonstrate that coloration of the TZ1 (MG detection) appeared only after the analysis of pork extract (column 1 in Fig. 4a), and the coloration in TZ2 (IgY detection) appeared for chicken and turkey meat extracts (columns 2, 3 in Fig. 4b). These results confirmed the high specificity of the developed test system and its suitability to control the undeclared additives.

Analysis of raw meat mixtures of different compositions

Mixtures of various compositions were prepared from minced meat of beef as the main component with the addition of pork and chicken (10, 5, and 1%). The results of the analysis of meat mixtures by the double test system are
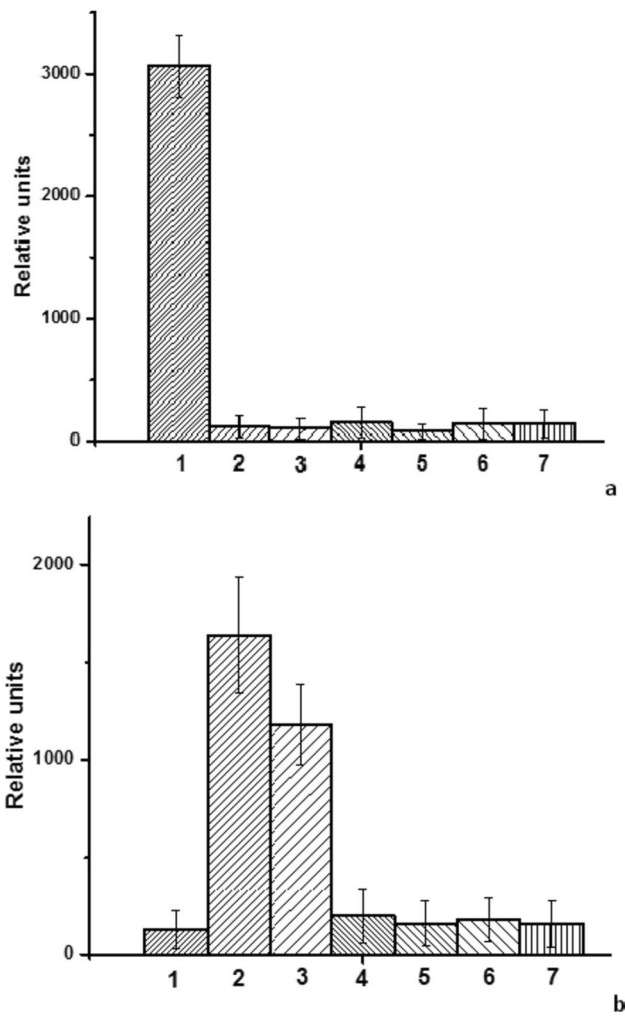

Fig. 4 Coloration intensities of TZ1 (**a**) and TZ2 (**b**) after the analysis of extracts of pork (1), chicken (2), turkey (3), beef (4), rabbit (5), horse (6), and lamb (7)

presented in Fig. 5a–c. The reliable coloration of TZ1 and TZ2 was detected for all mixtures with pork and chicken meat, respectively. The coloration of each TZ appeared independently of the signal in another TZ. Therefore, the developed test system confirmed its efficiency for qualitative («yes–no») control of counterfeit additives in raw meat mixtures. It should be noted that the regulations accepted for the meat industry consider only the presence of undeclared sources in the amount of at least 1% or more (and even 5% or more) as critical manifestations of falsifications committed by manufacturers (Feng et al. 2023). Such attitude is associated with a decrease in economic reasons for small-scale falsifying additives. However, evaluation of the analytical method's capability to detect undeclared additives with higher sensitivity is also required because small variations in the composition may also lead to health risks and violation of religious dietary requirements. It was demonstrated
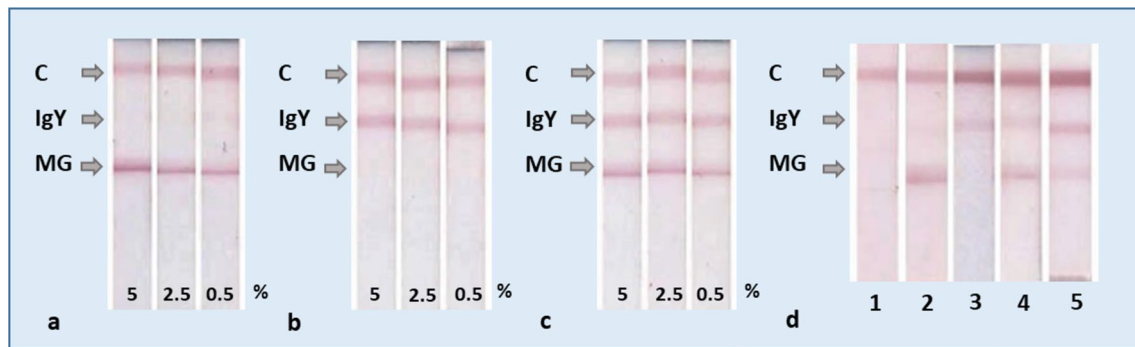
Fig. 5 Images of double test strips after testing meat mixtures (a-c) and cooked meat samples (d). **a** samples containing 5, 2.5, and 0.5% of porcine meat; **b** samples containing 5, 2.5, 0.5% of chicken meat; **c** samples containing 5, 2.5, and 0.5% of chicken meat and 5, 2.5, 0.5% of porcine meat; **d** vegetarian sausages (1), beef/pork cooked sausages (2), turkey sausages (3), heat-treated mixture of pork (90%) and chicken (10%) meat (4), heat-treated mixture of pork (60%) and chicken (40%) meat (5)

that the TZ coloration intensity is a concentration-dependent parameter and the results of its quantitative assessment satisfy linear approximations for both TZ1 and TZ2. The calculated LODs for the developed double test system indicate the possibility to reveal at least 0.1% of pork and chicken meat added to raw materials. The determined sensitivity is comparable (in terms of w/w content of undeclared meat) with that reported in the earlier proposed immune and other receptor-based techniques for individual control of porcine and chicken meat: 0.063% (Hendrickson et al. 2021b), 0.1% (Cheubong et al. 2021), 0.25% (Nhari et al. 2018), 0.5% (Chen and Hsieh 2020), and 1% (Ofori and Hsieh 2017).

Analysis of cooked meat products

The manufacturing of semi-finished and finished meat products usually includes thermal and enzymatic processing (Dao et al. 2022). Therefore, the use of morphological methods for identifying components in such foodstuffs is limited (Abdel Hafeez et al. 2016). On the other hand, food processing is associated with the risks of changing the structure or complete destruction of antigenic determinants recognized by antibodies. That is why testing the capabilities of the developed analytical system for the detection of the target biomarkers in processed meat products is of greatest interest. To perform this, extracts of sausages with different compositions were obtained, namely, vegetarian sausages, sausages containing beef and pork, and sausages containing turkey. Besides, extracts of thermally processed meat mixtures containing pork and chicken in the 90%/10% and 60%/40% ratios were detected. The obtained results are presented in Fig. 5d. It was shown that the developed double test system enabled the detection of proteins originated from the muscle tissue of pork, chicken, and turkey in finished meat products.

Conclusion

The developed test system allows for the detection of MG and IgY as biomarkers of porcine and chicken meat in meat-based products. The assay combines qualitative and quantitative modes. The achieved LODs were 10 ng/mL and 12 ng/mL for MG and IgY, respectively. They accord to the presence of the corresponding undeclared meat compounds in the concentration of at least 0.1%, whereas the concrete cut-off could depend on meat sources. The undoubted advantage of the developed test system is the possibility of simultaneous detection of two relevant analytes, which increases testing efficiency. The selectivity of the developed assay and its applicability for raw and cooked meat analysis was confirmed. Due to rapidity and methodological simplicity, the developed test system can be proposed as an effective tool for detecting added undeclared pork and chicken meat in meat products.

Author contributions Conceptualization, EAZ, AVZ and BBD; Methodology, EAZ; Validation, EAZ; Investigation, EAZ and ODH; Resources, BBD and AVZ; Writing—Original Draft Preparation, EAZ; Writing—Review & Editing, AVZ; Visualization, AVZ; Project Administration, BBD; Funding Acquisition, AVZ.

Declarations

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