Hindawi Publishing Corporation
Journal of Chemistry
Volume 2016, Article ID 8592962, 7 pages
http://dx.doi.org/10.1155/2016/8592962

Research Article

Colloidal Gold Probe-Based Immunochromatographic Strip Assay for the Rapid Detection of Microbial Transglutaminase in Frozen Surimi

Daming Fan,[1] Yi Li,[1] Zhennan Gu,[1] Jianlian Huang,[2] Wenguo Zhou,[2] Wenhai Zhang,[2] Jianxin Zhao,[1] and Hao Zhang[1]

[1]*State Key Laboratory of Food Science and Technology, School of Food Science and Technology, Jiangnan University, Wuxi 214122, China*
[2]*Fujian Anjoy Food Share Co. Ltd., Xiamen 361022, China*

Correspondence should be addressed to Hao Zhang; zhanghao@jiangnan.edu.cn

Received 1 May 2016; Revised 4 July 2016; Accepted 17 July 2016

Academic Editor: Tatyana N. Ermolaeva

Adding microbial transglutaminase (MTGase) to frozen surimi to enable the surimi to be sold as a higher-grade product at a higher price defrauds surimi product manufacturers and undercuts legitimate industry prices. Therefore, it is important to develop an accurate method of detecting the presence of MTGase in surimi. In this study, an immunochromatographic strip assay with a colloidal gold antibody probe was successfully developed and used to rapidly and qualitatively detect MTGase in surimi samples. The results were obtained in less than 10 min. The limit for the qualitative detection of MTGase using the immunochromatographic strip assay was identified as 1.0 μg/mL. The results of the immunochromatographic strip analysis of frozen surimi samples were verified by comparison with the results of a sandwich enzyme-linked immunosorbent assay. The colloidal gold probe-based immunochromatographic strip assay was thus found to be a rapid, economical, and user friendly method of detecting MTGase in surimi.

1. Introduction

Transglutaminase (glutaminyl-peptide:amine γ-glutamyl-transferase, EC 2.3.2.13; TGase) catalyses the intra- and/or intermolecular crosslinking of proteins by ε-(γ-glutamyl) lysine side-chain bridge [1]. The enzyme has unique effects on protein properties, gelation capability, thermal stability, water holding capacity, and so forth [2–4]. It plays an essential role in a wide range of organisms and has been found in various animal tissues, fish, plants, and microorganisms [1, 5–7]. TGase can be isolated from the culture supernatant of a variant of *Streptoverticillium mobaraense*, facilitating the mass production and use of TGase as a food additive.

Surimi is composed of stabilized myofibrillar proteins obtained from mechanically deboned fish flesh that is washed with water and blended with cryoprotectants. Surimi is an intermediate product used in a variety of products ranging from traditional Japanese kamaboko to various surimi-based seafood products. The quality of surimi is determined by a number of factors, such as gel strength, colour, moisture content, impurities, and microbiological count. However, its gelation properties are of primary interest in surimi production and trade [8]. Some manufacturers use MTGase as a texture modifier to improve the gelation properties of surimi, enabling it to be sold at a higher price. Although MTGase rarely presents a health hazard, its addition to surimi defrauds surimi product manufacturers and undercuts legitimate industry prices. Therefore, it is important to develop an accurate method of detecting MTGase in surimi.

Several methods of detecting MTGase have been proposed over the last few decades, such as enzymatic activity determination, liquid chromatography-mass spectrometry (LC-MS), and the enzyme-linked immunosorbent assay (ELISA) method. Enzymatic activity determination is the conventional method of detecting MTGase [9]. However, this method is unsuitable for use with surimi, as it fails to

differentiate MTGase from the endogenous TGase found in fish. LC-MS offers a sensitive and precise means of detecting MTGase as shown by Kaufmann et al. [10]. However, the use of this analytical technique is limited, as it requires a well-equipped laboratory and trained personnel. Ohtsuka et al. [9], Kaufmann et al., and Schloegl et al. [11] showed that ELISA is an effective means of quantitatively detecting MTGase. However, surimi product manufacturers require a more convenient and quicker method of detecting MTGase, as it takes a full day to obtain the results of an ELISA analysis.

Research has shown that the use of a colloidal gold probe-based rapid immunochromatographic strip assay has advantages over other detection systems. As this method does not require trained personnel or costly equipment, its results can be obtained easily and rapidly. Immunochromatographic strip assays have a wide range of commercial applications in diagnostics, agriculture, environmental testing, and product quality evaluation [12–15]. Colloidal gold nanoparticles are the most commonly used detector reagents in immunochromatographic strip assays, as they are stable and capable of generating signals without time-consuming procedures such as incubation, washing, and enzymatic reactions. As a result, the colloidal gold probe-based immunochromatographic strip is ideally suited for on-site testing even by untrained personnel and reduces the time required for analysis.

To the best of our knowledge, this study is the first to investigate the use of a colloidal gold probe-based immunochromatographic strip assay to detect MTGase. The assay was developed, optimised, validated, and used to analyse MTGase in frozen surimi samples, and the results were verified by comparison with those of sandwich ELISA. The colloidal gold probe-based immunochromatographic strip assay was found to offer a promising means of reducing the cost and time involved in detecting MTGase in frozen surimi. This method also has potential for commercial development.

2. Materials and Methods

2.1. Materials. The transglutaminase was provided by Ajinomoto (Shanghai, China). The rabbit anti-bacterial-transglutaminase antibody (polyclonal antibody) was purchased from Zedira (Darmstadt, Germany). The mouse anti-bacterial-transglutaminase antibody (monoclonal antibody) was purchased from Covalab (Villeurbanne, France). The rat anti-mouse-IgG1 antibody (secondary antibody) was purchased from SouthernBiotech (Birmingham, USA). The bovine serum albumin (BSA) was purchased from Sigma (St. Louis, USA). The nitrocellulose membrane was purchased from Millipore (USA). The microtiter plate type: 468667, surface: MaxiSorp, was purchased from NUNC. The sample pad, conjugate pad, absorbent pad, and adhesive plastic backing plate were purchased from Kinbio (Shanghai, China). The other reagents were purchased from Sinopharm (Shanghai, China).

2.2. Methods

2.2.1. Preparation of Colloidal Gold Nanoparticles. The colloidal gold nanoparticles were prepared according to the method described by Frens [16] with a few modifications. 50 mL of 0.01% gold chloride solution was heated to boiling point, and 1 mL of 1% trisodium citrate solution was added rapidly under continuous stirring. The solution was kept at boiling point for a few minutes until its colour changed to a brilliant wine-red. Finally, the solution was cooled at room temperature and the prepared colloidal gold was stored at 4°C in the dark for further study.

2.2.2. Optimisation of Conditions for Labelling Monoclonal Antibody (MAb) with Colloidal Gold Nanoparticles

(1) Optimisation of Colloidal Gold pH for Conjugation. Identical amounts of colloidal gold were placed in the wells of a 96-well microplate, and different amounts of 0.2 M K_2CO_3 solution were added to adjust the pH of the colloidal gold from 6 to 10 with an increment of 0.5. Next, identical amounts of anti-MTGase MAb were added to give a concentration of 10 μg/mL in each well. The mixtures were gently stirred and allowed to react for 20 min at room temperature. Finally, 10% NaCl solution was added to each of the wells, and the absorbance of each mixture was taken at 520 nm once its colour change was complete.

(2) Optimisation of MAb Concentration for Conjugation. The pH value of the colloidal gold was optimised by the addition of 0.2 M K_2CO_3 solution, and identical amounts of colloidal gold were placed in the wells of a 96-well microplate. Next, different amounts of anti-MTGase MAb were added to give concentrations of 0, 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 12, and 14 μg/mL. The mixtures were gently stirred and allowed to react for 20 min at room temperature. Finally, 10% NaCl solution was added to each of the wells, and the absorbance of each mixture was taken at 520 nm once its colour change was complete.

2.2.3. Preparation of MAb-Gold Conjugate. At least 10% more than the minimum amount of MAb was added to the colloidal gold at the optimum pH. The mixture was incubated at room temperature with gentle stirring for 30 min. To stabilize the MAb-gold conjugate further, 10% BSA solution (with a final concentration of 0.5%) was added to the solution and incubated at room temperature with gentle stirring for 15 min; subsequently, 5% PEG20000 solution (with a final concentration of 0.1%) was added to the solution and incubated at room temperature with gentle stirring for 15 min. After incubation, the solution was centrifuged at 8000 rpm at 4°C for 1 h. The pellet was resuspended in 0.01 M pH 7.4 phosphate-buffered saline (PBS) (containing 15% sucrose, 3% BSA, 0.5% NaCl, 1% Tween 20, and 0.03% NaN_3). The final MAb-gold conjugate was stored at 4°C for further study.

2.2.4. Characterisation of Colloidal Gold Nanoparticles and the MAb-Gold Conjugate. The average size of the colloidal gold nanoparticles prepared was measured by dynamic light scattering using a particle size/zeta potential analyser (Zetasizer Nano ZS, Malvern Instruments Ltd., England). Each sample was measured in triplicate and the mean values were reported. The visible spectra of the colloidal gold nanoparticles and the gold-MAb conjugate were recorded using

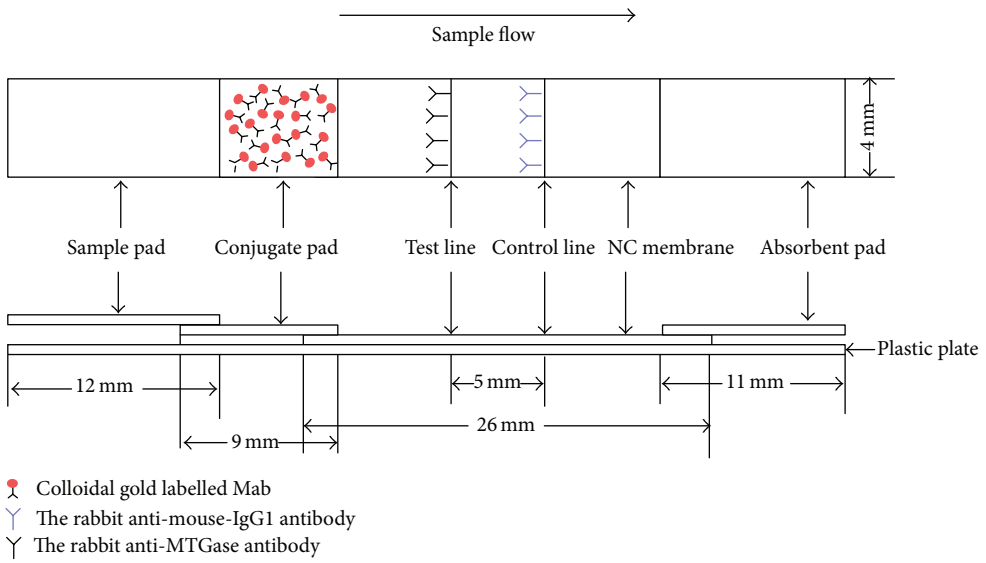

Figure 1: Description of the immunochromatographic strip.

an ultraviolet-visible (UV-Vis) spectrophotometer (UV-2450, Shimadzu, Japan). The spectra were obtained at wavelengths ranging from 400 to 700 nm (spectral resolution 1 nm). The reference beam sample was water. The fluorescence spectra of the colloidal gold nanoparticles and the MAb conjugate were recorded using fluorescence spectroscopy (F-7000, Hitachi, Japan) at an excitation wavelength of 280 nm. The widths of the two slits were 1.0 nm in excitation and 3 nm in emission. The emission band was monitored at wavelengths from 200 to 800 nm (spectral resolution 0.1 nm).

2.2.5. Preparation of the Immunochromatographic Strip. The immunochromatographic strip was composed of a sample pad, a conjugate pad, a nitrocellulose membrane, an absorbent pad, and an adhesive plastic backing plate. The sample pad and the conjugate pad, both made of polyester fibre, were soaked in 0.01 M PBS (containing 1% BSA, 0.5% NaCl, and 1% Tween 20), dried at 37°C for 1 h, and stored in a dry environment at room temperature for further study.

The rabbit anti-MTGase antibody (20 μg/mL) was spotted on the nitrocellulose membrane at a volume of 0.5 μL per dot to become the test line of the strip, as shown in Figure 1. The rabbit anti-mouse-IgG1 antibody (50 times diluted) was spotted on the nitrocellulose membrane at a volume of 0.5 μL per dot to become the control line. The test line and the control line were positioned about 5 mm apart. The membrane was dried at 37°C for 2 h. The gold-labelled antibody was spotted on the conjugation pad at a volume of 2 μL, and the pad was dried at 37°C for 1 h. The nitrocellulose membrane was pasted at the centre of the adhesive plastic backing plate. The conjugate pad was pasted in a position overlapping the nitrocellulose membrane by 2 mm. The sample pad overlapped the conjugate pad by 2 mm. The absorbent pad overlapped the nitrocellulose membrane by 3 mm. All of the other dimensional details are provided in Figure 1. Once fully assembled, the immunochromatographic strip was stored in a dry environment at room temperature for further study.

2.2.6. Sample Preparation. Frozen surimi was tempered to −2°C and mixed with MTGase (1 mg per gram surimi). Subsequently, 10 g surimi was homogenised with 100 mL Tris-HCl solution (0.05 M, pH 8.0), followed by two rounds of centrifugation at 4°C for 15 min (6000 rpm). The supernatant was used as the sample solution.

To prepare the negative control solution, 10 g prethawing surimi was homogenised with 100 mL Tris-HCl solution (0.05 M, pH 8.0) after being tempered to −2°C, followed by two rounds of centrifugation at 4°C for 15 min (6000 rpm). The supernatant was used as negative control.

2.2.7. Immunochromatographic Assay Procedure. 100 μL of the sample solution was added to the sample pad of the immunochromatographic strip. After 5 to 10 min, the result could be judged by the naked eye. If both the test line and the control line were coloured, the sample solution was taken to contain MTGase (i.e., a positive test result). If only the control line was coloured, the sample solution was assumed not to contain MTGase (i.e., a negative test result). A coloured control line indicated that the strip was effective, and a colourless control line indicated that the strip was ineffective.

2.2.8. Sensitivity of the Immunochromatographic Strip. MTGase standard curve for the immunochromatographic assay was prepared by diluting the MTGase solution obtained from Section 2.2.6 to different concentrations. The results were judged as positive or negative by visual inspection and the detection limit was defined as the minimum MTGase concentration at which the test line was unambiguously red in colour.

2.2.9. Detection of MTGase by Sandwich ELISA. The wells of a 96-well microtiter plate were coated with anti-MTGase rabbit

antibody solution and kept at 4°C overnight and then washed three times in 0.01 M pH 7.2 PBS containing 0.05% Tween 20 (PBST). The microplate wells were blocked with 0.5% BSA in PBST at 37°C for 1 h and washed three times with PBST. The sample solution and the standard solution obtained from Section 2.2.6 were added to the wells, incubated at 37°C for 1 h and washed three times with PBST. The anti-MTGase mouse antibody solution was added to the wells and incubated at 37°C for 1 h and then washed three times with PBST. The anti-mouse-IgG1 antibody-horseradish peroxidase solution was added to the wells and incubated at 37°C for 1 h and then washed four times with PBST. A tetramethylbenzidine substrate solution was added to the wells and incubated at 37°C for 20 min. This reaction was terminated by the addition of 0.2 M H_2SO_4 solution. The absorbance was measured at 450 nm using a microplate reader (Multiskan Go, Thermo, USA).

2.2.10. Testing of Commercial Frozen Surimi Samples. Nine samples of commercial frozen surimi were obtained from Fujian Anjoy Food Share Co., Ltd., China. Experimental samples were prepared according to Section 2.2.6. The immunochromatographic strip assay was used to analyse the concentration of MTGase in the frozen surimi sample solutions. The results were compared with those obtained by sandwich ELISA. The preparation of the samples for the ELISA analysis was the same as that for the immunochromatographic strip test.

3. Results and Discussion

3.1. Optimisation of Conditions for Labelling MAb with Colloidal Gold Nanoparticles. Electrostatic charge adsorption was the primary method used to label the antibodies with the colloidal gold nanoparticles. A partially protonated protein at a suitable pH can form ionic bonds with colloidal gold nanoparticles, as these nanoparticles are negatively charged over a wide range of pH [17]. Therefore, the pH of the colloidal gold nanoparticles plays a significant role in the labelling process. If the colloidal gold is not saturated with antibodies, the solution will turn from red to blue when 10% NaCl solution is added, which indicates that aggregation has taken place and that the antibody gold conjugate solution is not stable. Therefore, antibody concentration also plays a significant role in the labelling process.

Salt-induced precipitation was used to determine the optimal pH and the minimum amount of MAb, and the absorbance at 520 nm was recorded by a microplate reader. In Figure 2, the absorbance of the colloidal gold in response to the addition of 10% NaCl solution is plotted against the change in pH. The absorbance of the solution clearly increases as the pH increases. When the pH reaches 9.0, the absorbance curve is in saturation. Therefore, the optimal pH of the colloidal gold is 9.0. In Figure 3, the absorbance of the colloidal gold in response to the addition of 10% NaCl solution is plotted against the change in antibody concentration. The absorbance of the solution is shown to increase as MAb concentration increases. At a MAb concentration of 10 μg/mL, the curve is in saturation. To label the MAb

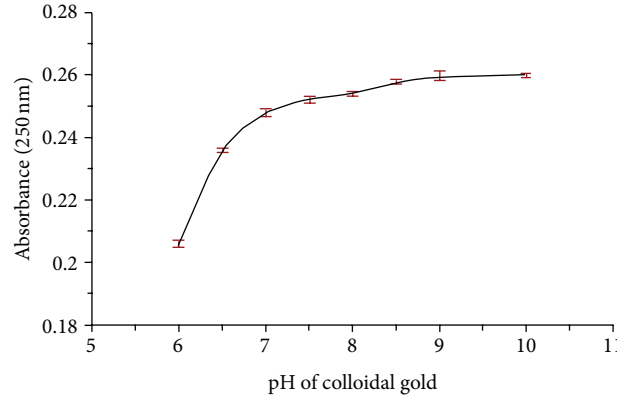

Figure 2: Optimum pH to produce a stable gold conjugate.

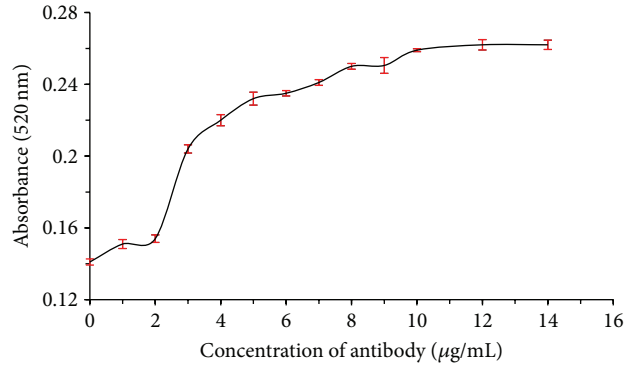

Figure 3: Determining the minimum protective amount of MAb.

with the colloidal gold nanoparticles, MAb concentration 10% higher than the minimum was used. Therefore, the optimal MAb concentration for the labelling reaction with the colloidal gold nanoparticle solution is 11 μg/mL.

3.2. Characterisation of Colloidal Gold Nanoparticles and the MAb-Gold Conjugate

3.2.1. Particle Size. The results of measuring the colloidal gold nanoparticles by dynamic light scattering are shown in Figure 4. The average diameter of the particles is 20.87 nm, the polydispersity index of the Z-average is 0.244, and the diameter of the particles ranges from approximately 20 nm to 40 nm. As colloidal gold nanoparticles with diameters of 20 nm to 40 nm are most commonly used by researchers in colloidal gold probe-based immunochromatographic assays [18], the colloidal gold nanoparticles prepared are assumed to be suitable for use in the immunochromatographic assay.

3.2.2. Optical Characterisation. The adsorption spectra of the MAb-gold conjugate and the colloidal gold were recorded using a UV-VIS spectrophotometer in the range of 400 to 700 nm. The results are shown in Figure 5. Curve (a) represents the spectrum of the MAb-gold conjugate, and curve (b) represents the spectrum of the colloidal gold solution. Curve (b) peaks at 520 nm due to surface plasmon resonance caused

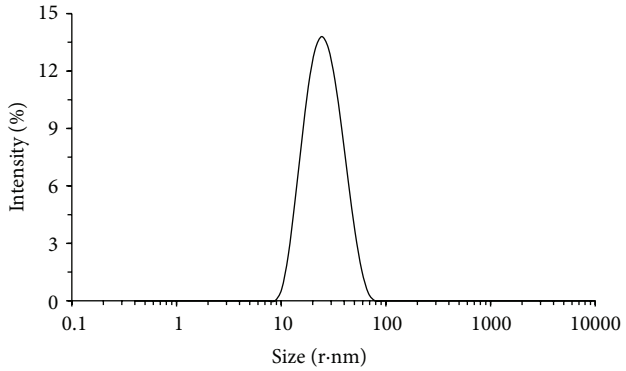

Figure 4: Dynamic light scattering spectrum of colloidal gold nanoparticles.

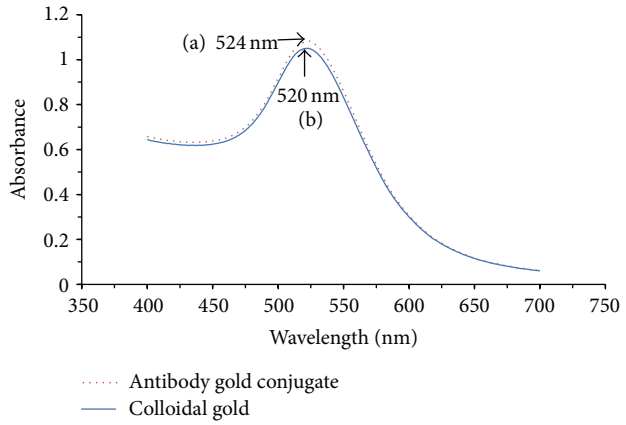

Figure 5: Visible spectra of the antibody gold conjugate and colloidal gold.

by the collective oscillation of the conduction electrons of the gold nanoparticles in response to irradiation by visible light. The surface plasmon resonance band at 520 nm corresponds to the standard optical response of colloidal gold nanoparticles with a diameter of 20.87 nm. Curve (a) shows that, after the addition of the MAb, absorbance increases noticeably and a slight red-shift of 4 nm occurs in the maximum absorbance of the plasmonic band, due to the interaction between the antibodies and the colloidal gold nanoparticles. These results clearly indicate the formation of bioconjugates.

Proteins contain three intrinsic fluorophores that can be quenched, tryptophan, tyrosine, and phenylalanine. However, as phenylalanine has a very low quantum yield, tryptophan and tyrosine residue are chiefly responsible for the intrinsic fluorescence of antibodies. As tryptophan is highly sensitive to changes in the local environment, its fluorescence emission spectra can be used to investigate antibody conformational changes and nanoparticle labelling [19]. In this study, fluorescence spectroscopy was used to record the fluorescence spectra of the MAb-gold conjugate and the colloidal gold between 200 nm and 800 nm, as shown in Figure 6. The excitation wavelength is 280 nm, which is distant from the plasmon resonance band of the colloidal gold nanoparticles. Curve (a) represents the spectrum of the MAb-gold conjugate

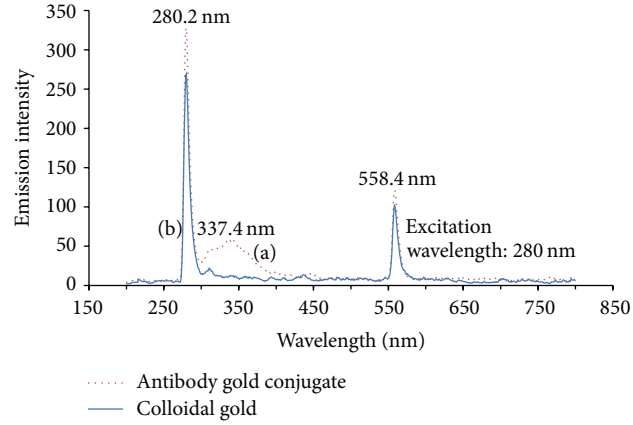

Figure 6: Fluorescence spectra of the MAb-gold conjugate and colloidal gold.

and curve (b) illustrates the spectrum of the colloidal gold. The curve representing the MAb-gold conjugate solution shows an intrinsic fluorescence band centred at 337.4 nm, which originates from the emission of tryptophan residue. In contrast, the colloidal gold does not exhibit fluorescence. The fluorescence emission caused by the tryptophan residue in the MAb can be identified in the fluorescence spectrum of the MAb-gold conjugate solution, indicating that the MAb is successfully labelled with the colloidal gold nanoparticles.

3.3. Sensitivity of the Immunochromatographic Strip Test. The sensitivity of the strip was determined by visual inspection. Standard solutions of MTGase at each final concentration (1000, 100, 10, 1, 0.1, and 0 μg/mL) were tested. The lowest titer of MTGase to give an unambiguously positive test strip result is 1 μg/mL, as shown in Figure 7. At this concentration, the test line is clearly light red in colour. A concentration of 0.1 μg/mL gives a pale-red colour, but this observation is considered to be ambiguous. Therefore, 1 μg/mL of MTGase is taken as the minimum visual detection limit of the immunochromatographic strip. Thus the LOD of MTGase in surimi was 10 mg/kg in the present study. Kaufmann reported that the LOD of MTGase in meat and meat products was 25 mg/kg *via* LC-MS/MS and ELISA methods. Moreover, it was also reported by Schloegl et al. that the LOD of MTGase in collagen was 5 mg/kg by ELISA. However, either LC-MS/MS or ELISA was time-consuming, and it takes several hours or more than ten hours. In contrast, MTGase concentration could be determined within 10 min by the immunochromatographic strip assay in the present study. Thus, it is more convenient and faster at the same level of MTGase content.

3.4. Testing of Commercial Frozen Surimi Samples. Nine commercial frozen surimi samples were analysed using the immunochromatographic strip assay and sandwich ELISA. The results of the immunochromatographic strip analysis are displayed in Figure 8. Both the control line and the test line are red, indicating, respectively, that the strip is effective and that the sample contains MTGase. The higher

Table 1: The results of the sandwich ELISA.

Sample name	MTGase detected by ELISA (g MTGase/100 g surimi)	P/N
Chujianghongsurimi	0.0469 ± 0.0029	Positive
Rizhao natural surimi	0.2255 ± 0.0205	Positive
Feihuasurimi	0.1493 ± 0.0236	Positive
Hongyesurimi	0.0009 ± 0.0002	Negative
Jinhai red sea bream surimi	0.0002 ± 0.0002	Negative
Jinhaihairtailsurimi	0.0017 ± 0.0002	Negative
Wenlingsurimi	0.0034 ± 0.0002	Negative
KISIMEX surimi	0.0009 ± 0.0002	Negative
Haizhiweisurimi	0.0010 ± 0.0002	Negative

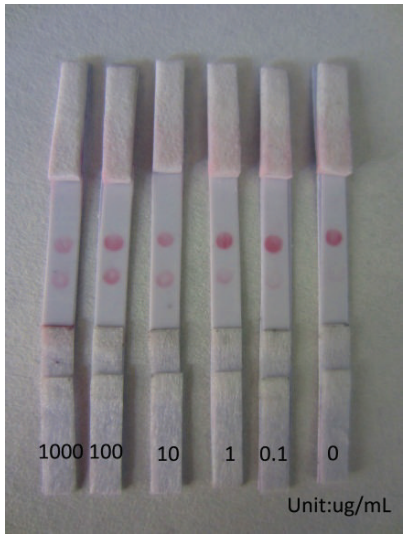

Figure 7: Detection of the sensitivity of the immunochromatographic strip.

the concentration of MTGase in the sample, the darker the sample's test line. Three of the samples of commercial frozen surimi test positive for MTGase, and six test negative for MTGase, which correlates well with the results of the sandwich ELISA, as shown in Table 1. The table shows that, of all the samples, the natural surimi from Rizhao had the highest concentration of MTGase. It also had the darkest test line. Therefore, the immunochromatographic strip test is shown to detect MTGase effectively and quickly.

4. Conclusion

In this study, a simple and rapid method of detecting MTGase in surimi samples using an immunochromatographic strip assay with colloidal gold nanoparticles as labels was developed. The average diameter of the colloidal gold nanoparticles synthesized for use in the strip was 20.87 nm. The labelling reaction was carried out at a pH of 9.0 and a MAb concentration of 11 μg/mL. The whole process of analysis using the strip assay was completed in less than 10 min. The lower detection limit of the immunochromatographic strip was judged by visual observation to be 1 μg/mL. The results obtained using the immunochromatographic strip correlated well with the results of the sandwich ELISA, indicating that immunochromatographic strips offer a rapid, effective, and convenient tool for detecting MTGase in surimi samples. Compared with sandwich ELISA, however, the immunochromatographic strip assay is a quicker and simpler method of MTGase detection, as it does not require skilled laboratory technicians or expensive equipment. In this study, the immunochromatographic strip method was tested with several frozen surimi samples. However, a larger number of surimi samples should be tested to validate the method fully.

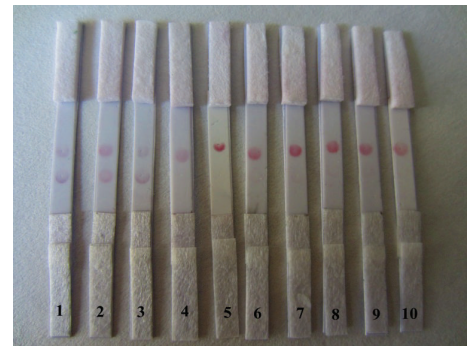

Figure 8: The results of the immunochromatographic strip assay. (1) Chujianghongsurimi. (2) Rizhao natural surimi. (3) Feihuasurimi. (4) Hongyesurimi. (5) Jinhai red sea bream surimi. (6) Jinhaihairtailsurimi. (7) Wenlingsurimi. (8) KISIMEX surimi. (9) Haizhiweisurimi. (10) Negative sample.

Competing Interests

The authors declare that they have no competing interests.

Authors' Contributions

Daming Fan and Yi Li are the co-first authors of this manuscript.

Acknowledgments

This research was supported by the planned project of China for the "Twelfth Five-Year Plan" in the field of country (no. 2012BAD28B05-04), the program for Amoy technological breakthrough of significant industry (no. 3502Z20121034), and the Teaching and Researching Joint Innovation Funds of Jiangsu Province (no. BY2013015-01).

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