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Development of ultrasensitive direct chemiluminescent enzyme immunoassay for determination of aflatoxin B1 in food products

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ABSTRACT

A direct competitive chemiluminescent enzyme-linked immunosorbent assay (CL-ELISA) for determination of aflatoxin B1 (AFB1) was developed. To improve the assay sensitivity, a mixture of 3-(10′-phenothiazinyl)-propane-1-sulfonate and 4-morpholinopyridine previously optimized by a factorial design was used as enhancer of horseradish peroxidase-induced chemiluminescence. Varying the concentrations of the coating anti-AFB1 antibody and conjugate of AFB1 and horseradish peroxidase the conditions of the chemiluminescent assay were optimized. The values of the detection limit value and dynamic working range of CL-ELISA of AFB1 were 0.0015 ng mL^{-1} and 0.003–0.03 ng mL^{-1}, respectively. It was shown that a dilution of rice and mung beans extracts in 5 and 10 times, respectively, prevented a matrix effect of the food products in CL-ELISA. The recovery values from the spiked samples of rice and mung beans were in the range of 90–104% and 102–117%, respectively. Studying 8 rice and 8 mung beans samples purchased in commercial stores the developed CL-ELISA allowed to find 3 samples (1 rice and 2 mung beans) containing AFB1, the content of AFB1 in one sample being higher than the maximum acceptable level established in the European Community.

1. Introduction

Mycotoxins are defined as fungal metabolites which, when ingested, inhaled or adsorbed through the skin cause lowered performance, sickness or death in man or animals [1,2]. The most important mycotoxins are the aflatoxins (AFs) and ochratoxin A that are produced as secondary metabolites by the fungi *Aspergillus* and *Penicillium* and are known to be carcinogenic, mutagenic, teratogenic and immunosuppressive [3,4]. Aflatoxin B1 (AFB1, Fig. 1) has the highest toxicity among all aflatoxins. In fact, the European Committee Regulations have established the maximum acceptable level for AFB1 in cereals, peanuts and dried fruits equal to 2 ng g^{-1} [5].

To minimize the risk of human exposure by AFB1 and to control the content of AFB1 in food and feed samples many techniques have been already developed [6–8]. The most widespread analytical method using in practice for quantitative determination of AFB1 is a liquid chromatography in a combination with ultraviolet spectroscopy, fluorescence, or mass spectrometry as detection methods [9–11]. However, this method is complex and time-consuming and also requires costly and bulky instrumentation.

Enzyme immunoassay is a good alternative to chromatography, because it allows an analysis of massive number of samples, may be easily automatized and does not require time-consuming procedures and sophisticated equipment. Presently enzyme-linked immunosorbent assay (ELISA) is widely accepted as the "gold standard" screening method [12].

In ELISA an isozyme *c* of horseradish peroxidase (HRP, EC 1.1.11.7) is commonly used as a label of immunoreagents [13,14]. Multiple detection methods for the enzyme activity of peroxidase-labeled immunoreagents are applied, including colorimetry, fluorimetry and chemiluminescence (CL). CL detection is markedly more sensitive than other methods [15,16]. This method is based on the enzymatic oxidation of luminol by hydrogen peroxide in the presence of peroxidases under mild alkaline conditions. Luminol oxidation leads to the formation of a 3-aminophthalate ion in an excited state which emits light when returning to the ground state [17].

Since HRP is a poor catalyst in luminol oxidation [18], certain compounds known as enhancers are added to the substrate mixture to increase CL intensity [19–21]. Previously the

Abbreviations: (AFB1), Aflatoxin B1; (HRP), Horseradish peroxidase; (ELISA), Enzyme-linked immunosorbent assay; (CL), Chemiluminescence; (SPTZ), 3-(10′-phenothiazinyl)-propane-1-sulfonate; (MORPH), 4-morpholinopyridine; (ECR), Enhanced chemuliminescence reaction; (RLU), Relative luminescence units; (CV), Coefficient of variation

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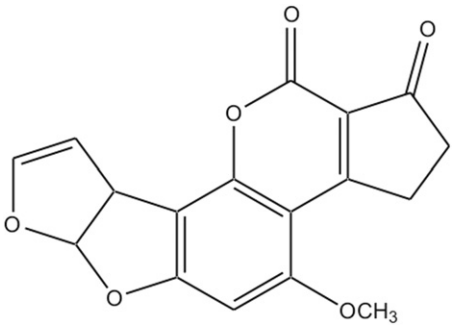

Fig. 1. Chemical structure of aflatoxin B1.

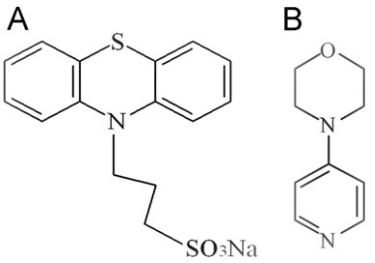

Fig. 2. Chemical structures of 3-(10′-phenothiazinyl)-propane-1-sulfonate (A) and 4-morpholinopyridine (B).

mechanism of enhanced chemiluminescence reaction (ECR), where luminol and an enhancer are oxidized simultaneously, was described in [22]. For a long time the most popular enhancer was 4-iodophenol. Using the substrate solution containing luminol-4-iodophenol-H_2O_2 the CL-ELISA for determination of AFB1 was developed previously. This method has been successfully applied to the evaluation of AFB1 in agricultural products with a good recovery [23].

Recently it was demonstrated that 3-(10′-phenothiazinyl)-propane-1-sulfonate (SPTZ) (Fig. 2a) in combination with 4-morpholinopyridine (MORPH) (Fig. 2b) are the most efficient enhancers for plant peroxidases [24–26]. The mechanism of the enhancement ability of SPTZ/MORPH combination was recently reported [27]. Under optimized conditions determined by a full 2^5 factorial design a ratio of CL intensity formed in HRP-induced ECR to background was higher than 140,000 [26].

In the present work we describe the ultrasensitive direct competitive enzyme-linked immunosorbent assay (ELISA) for determination of AFB1. To increase the sensitivity of the assay, we applied the chemiluminescent method for determination of HRP activity using SPTZ and MORPH as enhancers. The developed CL-ELISA was successfully applied for determination of AFB1 in samples of rice and mung beans.

2. Experimental

2.1. Reagents and materials

Aflatoxin B1 (AFB1) was purchased from Sigma Chemical Co. (St. Louis, MO, USA). AFB1 analytical standard solution (0.01 mg mL^{-1}), Certified Reference Material from Supelco; Bellefonte, PA, USA. Horseradish peroxidase (HRP, RZ 3.0) was purchased from Roche (Germany) and used without further purification. Sodium 3-(10′-phenothiazinyl)-propane-1-sulfonate (SPTZ) was prepared as described by Marzocchi et al. [24]. Luminol, Tween 20, Tris, *o*-(carboxymethyl)hydroxylamine hemihydrochloride (CMO), 4-morpholinopyridine (MORPH) were from Aldrich (USA). Black polystyrene plates (high protein binding) were obtained from Nunc (Denmark). 1-ethyl-3-(3′-dimethylaminopropyl)carbodiimide (EDC), *N*-hydroxysuccinimide (NHS), and bovine serum albumin (BSA) from Sigma Chemical Co. (St. Louis, MO, USA), *N*-dimethylformamide (DMF) and H_2O_2 (30%) were from J. T. Baker (Phillipsburg, NJ, USA). The concentration of H_2O_2 was estimated by measuring the absorbance using $\varepsilon_{240}=43.6$ [28].

2.2. Production of polyclonal anti-AFB1 antibody

The polyclonal antibodies specific to AFB1 (anti-AFB1-pAb) were produced by subcutaneous immunization of rabbits by a conjugate of AFB1-CMO and BSA. The purification of anti-AFB1-pAb was carried as follows: at first step saturated $(NH_4)_2SO_4$ was added to the rabbit antiserum to a 35% saturation. After incubation for 1 h and centrifugation (8000 rpm for 30 min) the precipitated proteins were discarded, and the additional quantity of $(NH_4)_2SO_4$ solution was added to a 50% saturation. The precipitated anti-AFB1-pAb was centrifuged and then redissolved in distilled water. The volume of the used water was equal to half of the original volume of antiserum. The anti-AFB1-pAb was dialyzed against 2 L of 0.01 M phosphate buffer with 0.15 M NaCl, pH 7.5 (PBS) for 72 h at 4 °C with two changes of the buffer. Finally, PBS was added to the obtained anti-AFB1-Ab up to the original antiserum volume. The antibody sample was stored at −20 °C or lyophilized for future use. The obtained antibody was used in the development of the CL-ELISA, because it showed a high affinity and selectivity [29].

2.3. Preparation of aflatoxin B1-CMO

To introduce carboxylic group in the chemical structure of AFB1 needed for the preparation of AFB1-HRP conjugate the synthesis of AFB1-CMO was carried out as described in [30]. For this, 10 mg AFB1 and 15 mg CMO were dissolved in a mixture containing 1.0 mL pyridine, 4.0 mL methanol and 1.0 mL water. After the reaction completion the mixture was gently refluxed for 2.5 h with continuous magnetic stirring, and then it was incubated at room temperature overnight. Using a rotary evaporator the reaction mixture was concentrated up to ~1 mL. To purify AFB1-CMO a thin-liquid chromatography (TLC) was carried out on silica gel plates using chloroform:methanol (9:1) in 1.5% acetic acid as an eluent. Localization of AFB1-CMO spot on the plate was detected under UV light (365 nm). Then, AFB1-CMO was removed from the TLC plate and dissolved in chloroform. Finally, AFB1-CMO was dried in the open air.

2.4. Synthesis of aflatoxin B1-HRP conjugate

AFB1 was conjugated with HRP by a carbodiimide method as follows: 1.0 mg of EDC freshly dissolved in 0.01 mL of DMF and 0.8 mg of NHS in 0.01 mL DMF were added to 0.1 mL of AFB1-CMO solution (0.25 mg mL^{-1} of DMF). The mixture was kept at room temperature for 2 h with continuous stirring. Then, 1.5 mg of HRP in 1.0 mL of 0.1 M $NaHCO_3$, pH 8.3 was added to the AFB1-CMO solution dropwise, and the reaction solution was kept at room temperature for next 2 h with stirring. The obtained conjugate was dialyzed against 2 L of PBS for 72 h with two changes of the buffer and stored at −20 °C or lyophilized for further use.

2.5. Determination of AFB1 by CL-ELISA

CL-ELISA was carried out using 96-wells black polystyrene plates (MaxiSorp, Nunc, Denmark). The plates were coated by adding into each well 100 μL of anti-AFB1-pAb (dilution 1:60,000) dissolved in PBS, and incubated at 4 °C overnight. The plate was

then washed using PBS with 0.05% Tween 20 (PBST) four times and blocked by adding 170 μL of PBS containing 0.1% BSA for 30 min at 37 °C. The plate was washed four times with PBST. Subsequently, 50 μL of AFB1-HRP (dilution 1:80,000) in 10 mM PBS, pH 7.4 and 50 μL of AFB1 (0.00002–2.0 ng mL^{-1}) were added to each well. The competitive step of the assay proceeded for 1 h at 37 °C. The plates were washed again as described above. Finally, 100 μL of freshly prepared substrate solution (80 mM Tris, pH 8.3, containing 0.17 mM luminol, 2.1 mM SPTZ, 8.75 mM MORP, and 1.75 mM H_2O_2 [26]) were added to each well and stirred. Chemiluminescence intensity was monitored at room temperature on a luminescence reader (FlexStation 3, Molecular Devices).

2.6. Preparation of spiked samples

Rice (*Oryza sativa*) and mung beans (*Vigna radiata*) samples were cultivated in Indonesia, Taiwan and Myanmar and purchased from Taiwanese stores. Each sample (10 g) was homogenized with 100 mL of extraction solvent (methanol/water 1:1, v/v) and incubated for 2 h with shaking (200 rpm) at 37 °C. After centrifugation at 14,000*g* for 10 min, the supernatant was passed through 0.45 μm and 0.22 μm syringe filters. Prior to ELISA the obtained extracts of rice and mung beans were diluted 5 and 10 times with PBS, respectively.

2.7. Data analysis

Standards and samples were run in triplicates, and the mean values were processed. Standard curves were obtained by plotting the light intensity against the logarithm of the analyte concentration and fitted to a four-parameter logistic equation using the Origin 6.0 Professional software (OriginLab Corp., United States):

$$Y = \{(A-D)/(1+(x/C)^B\}+D,$$

where A is the asymptotic maximum (intensity in the absence of an analyte, I_{max}), B is the curve slope at the inflection point, C is the x value at the inflection point, D is the asymptotic minimum (I_{min}, background signal), Y is CL intensity, and x is AFB1 concentration.

3. Results and discussion

3.1. Choice of the optimal concentrations of coating antigen and specific antibody

While performing a direct competitive ELISA (Fig. 3) the sensitivity of the assay depends on the concentration of capture antibody and enzyme-labeled antigen. By this, varying the concentrations of polyclonal anti-AFB1-pAb and AFB1-HRP conjugate, a set of calibration curves for AFB1 determination was obtained. All curves had a form of calibration curve typical of competitive ELISA (Fig. 4). The values of IC_{10}, IC_{50}, dynamic working range (IC_{20}–IC_{80}), the highest analytical signal (I_{max}) and background (I_{min}) were selected as the parameters used for estimation of the assay efficiency.

As seen in Table 1, the values of background were low and similar for all used combinations of concentrations of anti-AFB1-pAb and AFB1-HRP. Comparison of the analytical parameters for combinations 2, 3 and 4 obtained at fixed dilution of anti-AFB1-pAb (1:60,000) and varying the conjugate concentration showed that the lowest detection limit value (0.0015 ng mL^{-1}), good working range (0.003–0.03 ng mL^{-1}) and the highest value of I_{max} were obtained at the dilution of AFB1-HRP equal to 1:160,000. On the other hand, comparison of combinations 1, 3 and 5 obtained at fixed dilution of AFB1-HRP (1:160,000) and varying the specific antibody concentration showed that the analytical parameters for the combinations 3 and 5 were better than those for combination 1 and similar to each other. For further work we chose combination 3 (1:60,000/1:160,000) as optimal, because it had higher I_{max} value (Table 1) and, hence, higher sensitivity. The coefficient of variation (CV) for determination of AFB1 concentrations within the working range of the assay was lower than 4.7% ($n=6$).

Previously some immunochemical methods for AFB1 determinations were reported [23,31–33]. The most sensitive of these methods was CL-ELISA, where HRP activity was measured towards luminol/hydrogen peroxide/4-iodophenol solution. The linear range and the detection limit for this method was 0.05–10.0 ng mL^{-1} and 0.01 ng mL^{-1}, respectively [23]. The replacement of 4-iodophenol in the substrate solution composition with SPTZ/MORPH, which are more efficient enhancers [25,26], allowed us to significantly improve both the detection

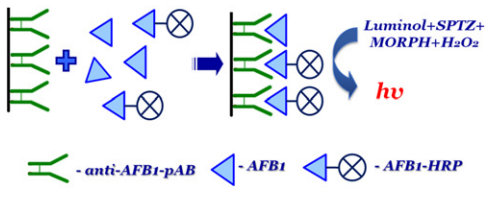

Fig. 3. Scheme of direct competitive CL-ELISA for determination of aflatoxin B1 used in this work.

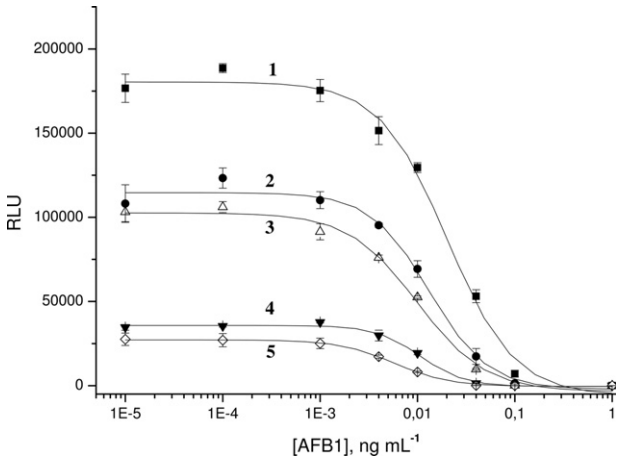

Fig. 4. Determination of aflatoxin B1 in buffered solutions by CL-ELISA. At the competitive step the reaction solution contained polyclonal anti-AFB1-antibody and the conjugate AFB1-HRP in the following dilutions: (1) 1:40,000/1:160,000, (2) 1:60,000/1:80,000, (3) 1:60,000/1:160,000, (4) 1:60,000/1:320,000, and (5) 1:80,000/1:160,000, respectively.

Table 1
Optimization of experimental conditions in competitive step of CL-ELISA for determination of AFB1 in buffered solutions.

No.	Anti-AFB1-pAb/ AFB1-HRP	I_{max}	I_{min}	IC_{10} (ng mL^{-1})	IC_{50} (ng mL^{-1})	IC_{20}–IC_{80} (ng mL^{-1})
1	1:40,000/1:160,000	180,400	14	0.003	0.02	0.006–0.04
2	1:60,000/1:80,000	114,600	14	0.0025	0.01	0.005–0.03
3	1:60,000/1:160,000	102,600	15	0.0015	0.009	0.003–0.03
4	1:60,000/1:320,000	35,780	20	0.003	0.009	0.005–0.02
5	1:80,000/1:160,000	27,180	12	0.001	0.006	0.002–0.01

limit value of AFB1 (0.0015 ng mL^{-1}) and the dynamic working range (0.003–0.03 ng mL^{-1}).

3.2. Analysis of the spiked samples

To demonstrate a practical usefulness of the developed CL-ELISA we measured AFB1 concentration in spiked samples of food products. For this, the assay produced for determination of AFB1 in buffered solutions was adapted first to rice samples. It is well known that at a transition from buffered solutions to extracts of real samples the compounds present in the extracts usually change a behavior of calibration curve. This effect was named a matrix effect.

As the developed CL-ELISA is highly sensitive, to prevent the matrix effect we tried to use a dilution of rice samples. For this, different quantity of AFB1 were added in a AFB1-free extract of rice, diluted in different times using PBS, and then AFB1 concentration was measured in the obtained solutions by the CL-ELISA. We showed that when the sample dilution was 1:5, the recovery values were in range 90–104% (Table 2). Therefore, the matrix effect of rice may be completely prevented by 5-fold dilution of rice extracts. Also, we showed that the additional dilution of rice samples did not improve the assay.

Similar results were obtained in the analysis of mung beans extracts. When these extracts containing different concentrations of AFB1 (0.008–0.025 ng mL^{-1}) were diluted in 10 times, values of recovery and coefficient of variation (CV) were in range of 102% to 117% and 2% to 7%, respectively, whereas values of recovery and CV from the spiked samples diluted in 5 times were in the range from 135% to 182% and to 22%, respectively (Table 3), i.e., the obtained values were significantly higher at 5-fold dilution than at 10-fold dilution of mung bean extracts. It allowed us to conclude that at the determination of AFB1 in mung beans extracts by the CL-ELISA prevention of the matrix effect was observed at higher dilution of samples (1:10) than in the case of rice extracts.

Table 2
Recovery of aflatoxin B1 from spiked rice samples (dilution 1:5) using CL-ELISA ($n=4$).

Rice samples	Spiked AFB1 (ng mL^{-1})	Found AFB1 (ng mL^{-1})	Recovery (%)	CV (%)
Rice 1	0.008	0.0081 ± 0.0004	101.3 ± 5.0	4.9
	0.012	0.0118 ± 0.0008	98.3 ± 6.7	6.8
	0.016	0.0158 ± 0.0019	98.5 ± 11.9	12.0
	0.025	0.0259 ± 0.0021	103.6 ± 8.4	8.1
Rice 2	0.008	0.0078 ± 0.0003	97.5 ± 3.8	3.9
	0.012	0.0117 ± 0.0003	97.5 ± 2.5	2.6
	0.016	0.0149 ± 0.0007	93.1 ± 4.4	4.7
	0.025	0.0226 ± 0.0010	90.4 ± 4.0	4.4
Rice 3	0.008	0.0081 ± 0.0004	101.3 ± 5.0	4.9
	0.012	0.0124 ± 0.0010	103.3 ± 8.3	8.1
	0.016	0.0152 ± 0.0019	95.0 ± 11.9	12.5
	0.025	0.0242 ± 0.0044	96.8 ± 17.6	18.2
Rice 4	0.008	0.0080 ± 0.0004	100 ± 5.0	5.0
	0.012	0.0123 ± 0.0007	102.5 ± 5.8	5.7
	0.016	0.0149 ± 0.0004	93.1 ± 2.5	2.7
	0.025	0.0251 ± 0.0021	100.4 ± 8.4	8.4

3.3. Analysis of the real samples

Using the developed method we analysed 8 samples of rice cultivated and purchased in Taiwanese stores. Our results demonstrated that except one, all samples did not contain AFB1. The content of AFB1 in this rice sample was 2.3 ng g^{-1}. The obtained value was a bit higher than that the maximum acceptable level established by the European Committee Regulations for AFB1.

Also, we estimated AFB1 content in 8 samples of commercially available samples of mung beans. It was demonstrated that 6 samples did not contain AFB1; 2 of the samples contained AFB1 at concentrations equal 1.7 ng g^{-1} and 1.6 ng g^{-1} that lower than the maximum acceptable level.

4. Conclusion

In this work we have developed the ultra-sensitive CL-ELISA for determination of AFB1 in buffer solution. The high sensitivity of the assay was achieved by using chemiluminescent method of measurement of peroxidase activity under the optimized conditions in the presence of SPTZ and MORPH (enhancers). The values of the detection limit and dynamic working range of CL-ELISA of AFB1 were 0.0015 ng mL^{-1} and 0.003–0.03 ng mL^{-1}, respectively. The matrix effect of rice and mung beans was completely prevented by 5-fold and 10-fold dilution of the extracts. At analysis of 8 rice and 8 mung beans samples purchased in commercial stores the developed CL-ELISA allowed finding 3 samples (1 rice and 2 mung beans) containing AFB1, the content of AFB1 in one sample being higher than the maximum acceptable level established in the European Community. Therefore, the developed CL-ELISA could provide a valuable tool for sensitive determination of AFB1 in food products.

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Table 3
Dependence of dilution of mung beans samples on the matrix effect at determination of AFB1 by CL-ELISA.

No. of sample	Spiked AFB1 (ng mL^{-1})	Dilution of mung beans samples					
		1/5 ($n=2$)			1/10 ($n=4$)		
		Found AFB1 (ng mL^{-1})	Recovery (%)	CV (%)	Found AFB1 (ng mL^{-1})	Recovery (%)	CV (%)
1	0.008	0.0133 ± 0.0019	166.3 ± 23.8	14.3	0.0088 ± 0.0004	110.0 ± 5.0	4.5
	0.012	0.0203 ± 0.0	169.2 ± 0.0	0.0	0.0132 ± 0.0009	110.0 ± 7.5	6.8
	0.016	0.0281 ± 0.0015	175.6 ± 9.4	5.3	0.0179 ± 0.0003	111.9 ± 1.9	1.7
	0.025	0.0454 ± 0.0101	181.6 ± 40.4	22.2	0.0254 ± 0.0013	101.6 ± 5.2	5.1
2	0.008	0.0125 ± 0.0005	156.3 ± 6.3	4.0	0.0086 ± 0.0006	107.5 ± 7.5	7.0
	0.012	0.0172 ± 0.0006	143.3 ± 5.0	3.5	0.0138 ± 0.0010	115.0 ± 8.3	7.2
	0.016	0.0234 ± 0.0013	146.3 ± 8.1	5.6	0.0187 ± 0.0008	116.9 ± 5.0	4.3
	0.025	0.0338 ± 0.0007	135.2 ± 2.8	2.1	0.0280 ± 0.0007	112.0 ± 2.8	2.5

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References

[1] F. Ricci, G. Volpe, L. Micheli, G. Palleschi, Anal. Chim. Acta 605 (2007) 111.
[2] G.S. Shephard, F. Berthiller, P.A. Burdaspal, C. Crews, M.A. Jonker, R. Krska, S. MacDonald, R.J. Malone, C. Maragos, M. Sabino, M. Solfrizzo, H.P. van Egmond, T.B. Whitaker, World Mycotoxin J. 5 (2012) 3.
[3] Y. Liu, F. Wu, Environ. Health Perspect. 118 (2010) 818.
[4] L.A. Corcuera, A. Vettorazzi, L. Arbillaga, E. Gonzalez-Penas, A. Lopez de Cerain, Food Chem. Toxicol. 50 (2012) 3440.
[5] Commission Regulations no. 472/2002 of 12 March 2002 and no. 123/2005 of 26 January 2005.
[6] D. Zhang, P. Li, Y. Yang, Q. Zhang, W. Zhang, Z. Xiao, X. Ding, Talanta 85 (2011) 736.
[7] Z. Li, Z. Wang, X. Sun, Y. Fang, P. Chen, Talanta 80 (2010) 1632.
[8] J. Hashemi, G.A. Kram, N. Alizadeh, Talanta (2008) 1075.
[9] Y. Wang, X. Liu, C. Xiao, Z. Wang, J. Wang, H. Xiao, L. Cui, Q. Xiang, T. Yue, Food Control 28 (2012) 131.
[10] W.S. Khayoon, B. Saad, T.P. Lee, B. Salleh, Food Chem. 133 (2012) 489.
[11] L. Afsah-Hejri, S. Jinap, S. Arzandeh, H. Mirhosseini, Food Control 22 (2011) 381.
[12] E.N. Ediage, J.D. Di Mavungu, I.Yu. Goryacheva, C. Van Peteghem, S. De Saeger, Anal. Bioanal. Chem. 403 (2012) 265.
[13] C. Regalado, B.E. García-Almendárez, M.A. Duarte-Vázquez, Phytochem. Rev. 3 (2004) 243.
[14] M. Farre', L. Kantiani, D. Barcelo, Trends Anal. Chem. 26 (2007) 1100.
[15] A. Roda, M. Guardigli, Anal. Bioanal. Chem. 402 (2012) 69.
[16] Ch.A. Marquette, L.J. Blum, Bioanalysis 1 (2009) 1259.
[17] F. McCapra, Methods Enzymol. 305 (2000) 3.
[18] I.S. Alpeeva, I.Yu. Sakharov, J. Agric. Food Chem. 53 (2005) 5784.
[19] Y. Dotsikas, Y. Loukas, Talanta 71 (2007) 906.
[20] Z.W. Zhao, Spectrosc. Spect. Anal. 22 (2002) 895.
[21] Y.L. Liang, F.A. Yu, S.C. Yu, Y.J. Wu, H.Q. Zhang, L.B. Qu, J. Lumin. 132 (2012) 1021.
[22] P.M. Easton, A.C. Simmonds, A. Rakishev, A.M. Egorov, L.P. Candeias, J. Am. Chem. Soc. 118 (1996) 6619.
[23] L. Fang, H. Chen, X. Ying, J.M. Lin, Talanta 84 (2011) 216.
[24] E. Marzocchi, S. Grilli, L. Della Ciana, L. Prodi, M. Mirasoli, A. Roda, Anal. Biochem. 377 (2008) 189.
[25] M.M. Vdovenko, L. Della Ciana, I.Yu. Sakharov, Anal. Biochem. 392 (2009) 54.
[26] M.M. Vdovenko, A.S. Demiyanova, T.A. Chemleva, I.Yu. Sakharov, Talanta 94 (2012) 223.
[27] I.Yu. Sakharov, M.M. Vdovenko, Anal. Biochem. 434 (2013) 12.
[28] R.J. Kulmacz, Arch. Biochem. Biophys. 249 (1986) 273.
[29] B.H. Liu, Y.T. Hsu, C.C. Lu, F.Y. Yu, Food Control 30 (2013) 184.
[30] F.S. Chu, M.T.S. Hsia, P. Sun, J. AOAC 60 (1977) 791.
[31] N. Arroyo-Manzanares, L. Gámiz-Gracia, A.M. García-Campana, J.J. Soto-Chinchilla, L.E. García-Ayuso, Electrophoresis 31 (2010) 2180.
[32] A.Yu. Kolosova, W.B. Shim, Z.Y. Yang, S.A. Eremin, D.H. Chung, Anal. Bioanal. Chem. 384 (2006) 286.
[33] D. Tang, Y. Yu, R. Niessner, M. Mir, D. Knopp, Analyst 135 (2010) 2661.