Article

pubs.acs.org/JAFC

Development of a Competitive Indirect Enzyme-Linked Immunosorbent Assay Based on Monoclonal Antibodies for the Detection of 2-Dodecylcyclobutanone in Irradiated Beef

Yueliang Zhao, Feng Wang, Weiming Li, and Yiming Ha*

Institute of Agro-food Science and Technology, Chinese Academy of Agricultural Sciences/Key Opening Laboratory of Agricultural Products Processing and Quality Control, Ministry of Agriculture, Beijing 100193, China

Ⓢ *Supporting Information*

ABSTRACT: A highly sensitive and specific competitive indirect enzyme-linked immunosorbent assay (ciELISA) based on monoclonal antibodies was developed for the detection of 2-dodecylcyclobutanone (2-DCB), a chemical marker for irradiated lipid-containing foods. 2-Oxocyclobutane undecanoic acid was used as an alternative to 2-DCB and conjugated to BSA and OVA via a conventional carbodiimide condensation reaction to prepare the immunogen and the coating antigen for 2-DCB. The monoclonal antibody against 2-DCB was obtained using the hybridoma technique, with a high specificity and low cross-reactivity for 2-tetradecylcyclobutanone (2-TCB; <8%) and other structurally related compounds (<0.1%). The ciELISA method was applicable at optimal experimental conditions of 0.001−100 μg/mL 2-DCB in a buffer solution, with an IC_{50} value of 0.25 μg/mL and a limit of detection (defined as the IC_{20}) of approximately 0.004 μg/mL. The recovery efficiency of 2-DCB from ground beef patties ranged from 84.4 to 109.8%. The intra-assay and interassay coefficients of variation were <10.0 and <12.0%, respectively. The proposed method was validated by gas chromatography−mass spectrometry with high correlation. The same method was used to detect 2-DCB in ground beef patties irradiated at 0.5, 1.0, 3.0, 5.0, and 7.0 kGy; the 2-DCB concentration linearly increased with the radiation dose.

KEYWORDS: *2-dodecylcyclobutanone, irradiated beef, monoclonal antibody, ELISA*

■ INTRODUCTION

Irradiation is used to improve food safety; this process maintains food quality by decreasing the microbial load and extending the shelf life.[1,2] Although this food preservation process is considered safe, a reliable, convenient, and rapid method to distinguish irradiated from nonirradiated products should still be developed to ensure that food products are correctly labeled; such labeling would allow for informed consumer choice in compliance with both national and international legislation.[3,4]

2-Alkylcyclobutanones are radiolytic products that include 2-dodecylcyclobutanone (2-DCB) from palmitic acid and 2-tetradecylcyclobutanone (2-TCB) from stearic acid; these compounds have been used to detect irradiated lipid-containing foods.[5−11] 2-Alkylcyclobutanones are considered unique radiolytic products because they are not formed during cooking or any other heat-processing methods. To date, the detection of 2-DCB heavily relies on gas chromatography−mass spectrometry (GC-MS), which is accurate but expensive and time-consuming and often requires complicated sample preparation before analysis. By contrast, immunoassays are generally rapid, sensitive, selective, and cost-effective.[12−14] To the best of our knowledge, only one previous paper has described a direct competitive enzyme-linked immunosorbent assay based on polyclonal antibodies for the detection of 2-DCB.[6] These polyclonal antibodies against 2-DCB were produced in a rabbit by inoculating a synthesized 2-(tetradec-5′-enyl)-cyclobutanone−bovine thyroglobulin conjugate. Chicken meat samples irradiated at doses ranging from 2.5 to 10 kGy were assayed and correctly identified.[6]

This investigation aimed to prepare highly sensitive and specific monoclonal antibodies against 2-DCB and to develop a competitive indirect enzyme-linked immunosorbent assay (ciELISA) method for efficiently screening and quantifying 2-DCB in irradiated ground beef patties. The cELISA method developed in this paper is a potential alternative to chromatography or spectrometry for the regulatory analysis of 2-DCB in irradiated lipid-containing foods. This method may help to improve the sensitivity and specificity of commercially available ELISA kits for 2-DCB.

■ MATERIALS AND METHODS

Chemicals and Instruments. Standard 2-DCB, 2-TCB, bovine serum albumin (BSA), ovalbumin (OVA), casein, polyethylene glycol (PEG 4000), hypoxanthine aminopterin thymidine (HAT), Freund's complete adjuvant, Freund's incomplete adjuvant, *N*,*N*-dimethylformamide (DMF), and the horseradish peroxidase-labeled goat anti-mouse IgG conjugate (HRP−GaM IgG) were purchased from Sigma-Aldrich (Buchs, Switzerland). 1-Ethyl-3-(3-dimethylaminopropyl)-carbodiimide hydrochloride (EDC) was purchased from Thermo, USA. 2-Oxocyclobutane undecanoic acid was purchased from Santa Cruz Biotechnology, Inc. (Santa Cruz, CA, USA). Hexane was purchased from Fisher Scientific (Pittsburgh, PA, USA). Carbamide

Received: May 6, 2013
Revised: July 10, 2013
Accepted: July 22, 2013
Published: July 22, 2013

© 2013 American Chemical Society

peroxide, tetramethylbenzidine (TMB), acetonitrile, 2-ethylcyclobutanone, 2-oxocyclobutane, carboxylic acid, tridecanoic acid, *n*-tridecane, RPMI-1640 cell medium, fetal calf serum, and other reagent grade chemicals were obtained from Guangda (Beijing, China). Mouse SP2/0 myeloma cells were procured from the Cell Bank of the Chinese Science Academy (Shanghai, China). The BALB/c mice were purchased from the Experimental Animal Center of the Chinese Academy of Sciences (Shanghai, China). The 96-well polystyrene microtiter plates were obtained from Greiner Bio-One, GmbH (Frickenhausen, Germany). The MK3 microplate reader was purchased from Thermo, USA. The Mega Bond Elut-SI column (1 g/6 mL) was obtained from Varian Inc. (Palo Alto, CA, USA).

Buffers and Solutions. The routinely used buffers in the experiments were (1) coating buffer, 0.05 mol/L carbonate buffer, pH 9.6; (2) coating antigen stock solution, 1 mg/mL coating antigen prepared with coating buffer; (3) assay buffer, 0.01 mol/L phosphate-buffered saline (PBS), pH 7.4, containing 140 mmol/L NaCl; (4) washing buffer, PBS buffer with 0.05% (v/v) Tween-20 (PBST); (5) blocking solution, 1% casein in assay buffer; (6) acetate buffer, 100 mmol/L sodium acetate acid buffer, pH 5.7; (7) substrate solution (TMB + H_2O_2), 200 μL 10 mg/mL TMB dissolved in DMF (20 μL of 5% H_2O_2 and 1 mL of acetate buffer were added to 20 mL of pure water); and (8) stopping solution, 5% sulfuric acid.

The 2-DCB standard solutions at concentrations of 0.001, 0.005, 0.01, 0.1, 1, 10, 50, and 100 μg/mL were prepared by dissolving 2-DCB powder in a methanol/PBS solution (10:90, v/v).

Preparation of the 2-Oxocyclobutane Undecanoic Acid-Conjugated Immunogen and Coating Antigen. 2-Oxocyclobutane undecanoic acid (Figure 1) is a cyclobutanone derivative with a 10-carbon side chain and a terminal acid group. This compound was conjugated with BSA and OVA to prepare the immunogen and coating antigen, respectively, against 2-DCB. The conjugates were synthesized using EDC, according to the manufacturer's instructions but with slight modifications. Briefly, 2 mg of lyophilized BSA and 2 mg of 2-oxocyclobutane undecanoic acid were dissolved in 700 μL of PBS (pH 7.4) to obtain solution I. Meanwhile, 10 mg of EDC was dissolved in 1 mL of PBS (pH 7.4) to obtain solution II. Subsequently, 100 μL of solution II was immediately added to solution I (700 μL) and stirred at room temperature for 5 h. This mixture was then dialyzed against PBS for 3 days, with the dialysis medium regularly changed twice daily to remove the free haptens in the solution. Finally, the mixture was centrifuged at 7000*g* for 30 min. The supernatant was collected to recover the 2-oxocyclobutane undecanoic acid−BSA immunogen. The recovered immunogen was stored at −20 °C for future use. The 2-oxocyclobutane undecanoic acid−OVA conjugate was synthesized in a similar manner for use as the coating antigen.

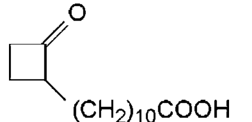

Figure 1. Structure of 2-oxocyclobutane undecanoic acid.

Immunization. BALB/c mice (6−8 weeks old) were subcutaneously immunized with 100 μg of 2-oxocyclobutane undecanoic acid−BSA conjugate (100 μg in 0.1 mL of PBS, pH 7.4) using an equal volume of Freund's complete adjuvant. The subsequent injections were intraperitoneally administered using the same immunogen concentration in the same volume of Freund's incomplete adjuvant solution at the third, fifth, seventh, and ninth weeks after the first injection. Starting from the third immunization, a small volume of blood was collected from the tail of the immunized mice on the 10th day after each injection for the antibody titer measurement. Immunized mice with serum antibody titers of 10^4 or higher were selected as the spleen donors for hybridoma production. These animals received intravenous injection boosts containing 100 μg of the conjugated immunogen in PBS (pH 7.4).

Cell Fusion. The mice were sacrificed on the third day after the final booster injection. Their spleen cells were collected and fused with mouse SP2/0 myeloma cells at a ratio of 8:1 using 50% PEG 4000 as the fusion agent. The fused cells or hybridomas were distributed in 96-well culture plates supplemented with HAT medium containing 20% fetal calf serum. Peritoneal macrophages from young BALB/c mice were used as feeder cells. The hybridomas were incubated at 37 °C with a 5% CO_2 atmosphere in a standard cell culture incubator (Thermo Scientific, Waltham, MA, USA).

Hybridoma Selection and Cloning. After 2 weeks of incubation, the culture supernatants were screened by ciELISA, as described below. The hybridomas that produced antibodies targeting 2-DCB were subcloned three times using the limiting dilution method.[15] The stable antibody-producing clones were expanded and cryopreserved in liquid nitrogen.

Purification of Monoclonal Antibodies. Aliquots of the selected stable subclones were expanded and intraperitoneally injected into female BALB/c mice. These mice were intraperitoneally preinjected with 0.5 mL of liquid paraffin during a week before the injection of antibodies. On the 10th day after antibody injection, the ascites were collected and purified using the ammonium sulfate precipitation method. The purified monoclonal antibodies were stored at −20 °C.

ciELISA Analysis. A ciELISA was proposed for 2-DCB analysis. Each well of the 96-well polystyrene microtiter plates was coated with 100 μL of the 1 μg/mL 2-oxocyclobutane undecanoic acid−OVA conjugate in coating buffer by incubating the plates overnight at 4 °C. The plates were then washed three times with the washing buffer using an automated plate washer. Each well was blocked with 200 μL of the blocking solution, and the plates were incubated at 37 °C for 2 h. The plates were washed, as previously described, followed by the addition of a series of diluted standard solutions or sample extracts (100 μL/well). The 2-DCB monoclonal antibody solution (100 μL/well) was subsequently added, and the plates were incubated for 1 h at 37 °C. After washing, HRP−GaM IgG was added (100 μL/well), and the plates were incubated for 1 h at 37 °C. The plate was then washed again, and the substrate solution (100 μL/well) was added. The plates were incubated with shaking for approximately 15 min at 37 °C before the stopping solution (50 μL/well) was added. The absorbance of each well was determined at 450 nm using a microplate reader. Serum was extracted before immunization and used as a negative control. The inhibition percentage is defined as follows: % inhibition = $B/B_0 \times 100$, where B and B_0 are the absorbance values of the well with and without the analyte, respectively. The IC_{50} is the analyte concentration that causes a 50% inhibition of antibody binding.

Antibody Specificity Test. The specificity of the produced monoclonal antibody was investigated using the proposed ciELISA method by detecting the cross-reactivity of compounds structurally related to 2-DCB. These compounds included 2-TCB, 2-ethylcyclobutanone, 2-oxocyclobutanecarboxylic acid, tridecanoic acid, and *n*-tridecane (Table 1). The IC_{50} value for each compound was determined according to their inhibition curves. The percent cross-reactivity (CR%) values were calculated using the following equation: % CR = $IC_{50,\ 2\text{-DCB}}/IC_{50,\ \text{compounds}} \times 100$.

Meat Samples. Fresh chilled ground beef patties (30% fat) were purchased from local markets in Beijing. The patties were irradiated at 0.5, 1.0, 3.0, 5.0, and 7.0 kGy using a ^{60}Co radiation source at room temperature in the Radiation Center of the Chemistry Institute of Peking University. After irradiation, the samples were immediately stored at −20 °C.

Sample Pretreatment for Analysis. Sample pretreatment was performed according to a previous study.[11] A 5 g sample of each set of ground beef patties was homogenized with 10 g of anhydrous sodium sulfate. The homogenate was blended with 100 mL of acetonitrile using a JJ-2 (2003-61) tissue-mashing machine (Beijing Guang-da-heng-yi Technology Co., Ltd., China) working at 6000 rpm for 1 min. The mixture was left to stand for 1 min before it was blended again for another 1 min. The extraction solvent was carefully passed through filter paper as it was transferred to a 500 mL round-bottom flask. The reaction mixture was retained in the tissue-mashing instrument. Another 100 mL of acetonitrile was then added, and the entire

Table 1. Cross-Reactivity of the Monoclonal Antibodies toward Selected Compounds

Compound	Chemical structure	IC_{50}	CR (%)
2-DCB	$(CH_2)_{11}CH_3$	0.25[a]	100
2-TCB	$(CH_2)_{13}CH_3$	3.4	7.35
2-Ethylcyclobutanone	CH_2CH_3	>1000	<0.1
2-Oxocyclobutanecarboxylic acid	COOH	>1000	<0.1
Tridecanoic acid	$CH_3(CH_2)_{11}COOH$	>1000	<0.1
n-Tridecane	$CH_3(CH_2)_{11}COOH$	>1000	<0.1

[a]IC_{50} = 0.25 μg/mL, based on CR testing.

extraction procedure was repeated, as described above. Finally, the extract solutions were combined and evaporated until dry using a rotary evaporator. The round-bottomed flask was washed with hexane, and the obtained solution was collected into a 50 mL volumetric flask. Ten milliliters of hexane (10 mL) was evaporated to 1 mL via a nitrogen stream, added to a silica SPE cartridge column, and immediately rinsed with 10 mL of *n*-hexane for conditioning before use. A 10 mL *n*-hexane aliquot was eluted and discarded. Subsequently, a 10 mL aliquot of a 2% diethyl ether/*n*-hexane solution (2:98, v/v) was eluted and collected from the 2-alkylcyclobutanone fraction. The eluted solution was concentrated until dry and resuspended in 50 μL of methanol and 450 μL of PBS (pH 7.4). A 100 μL aliquot of the resulting solution was then assayed by ciELISA. For the GC-MS analysis, after concentration to dryness, the remaining eluate was resuspended in 500 μL of *n*-hexane. A 1 μL aliquot of the resulting solution was subsequently used in the GC-MS analysis.

Validation of the ciELISA Method by GC-MS. To validate the proposed immunoassay, the 2-DCB-spiked ground beef patties were analyzed using a QP2010 Plus GC-MS system (Shimadzu, Kyoto, Japan). The GC conditions were as follows: column, Rtx-5MS (30.0 m × 0.25 μm × 0.25 mm); column temperature program, 55 °C (1 min), raised from 55 to 180 °C at 10 °C/min, raised from 180 to 250 °C at 2 °C/min, held at 250 °C (2 min); carrier gas, helium at 1.00 mL/min; injection temperature, 250 °C; injection mode, splitless; and injection volume, 1 μL. The MS conditions were as follows: ionization mode, electron ionization; ion detection, selected ion monitoring (SIM); detector voltage, 0.8 kV; ion source temperature, 200 °C; and transfer line temperature, 250 °C. The monitored ions were m/z 98 and 112; m/z 98 was likewise selected for determination.

RESULTS AND DISCUSSION

Synthesis of Immunogen and Coating Antigen. Similarly to other small molecular compounds, 2-DCB is a nonimmunogenic hapten that must be conjugated to a protein carrier to confer immunogenicity. 2-DCB does not have spacers that can be coupled covalently to the carrier protein. The molecular structure of a hapten generally remains nearly unchanged.[16,17] 2-Oxocyclobutane undecanoic acid has only one fewer C atom in its side chain, as compared with 2-DCB. This compound was used as an alternative to 2-DCB because it could conjugate with BSA and OVA via a conventional carbodiimide condensation reaction to prepare the immunogen and coating antigen of 2-DCB. The matrix-assisted laser desorption/ionization−time-of-flight mass spectrometry (MALDI-TOF-MS) spectra of BSA and the 2-oxocyclobutane undecanoic acid−BSA conjugate and the MALDI-TOF-MS spectra of OVA and the 2-oxocyclobutane undecanoic acid−OVA conjugate show that the molecular weights of BSA and OVA were clearly increased after conjugation. Therefore, the coupling of 2-oxocyclobutane undecanoic acid to BSA and OVA was successful. The coupling ratios of 2-oxocyclobutane undecanoic acid conjugated to BSA and OVA were 9.9:1 and 1.4:1, respectively, as calculated using the equation coupling ratio = $(MW_{conjugate} - MW_{carrier\ protein})/MW_{hapten}$

Optimization of Assay Conditions. To develop a highly sensitive and specific ciELISA method, the optimum assay conditions should be determined. These properties would include the coating antigen concentration and antiserum dilution. The assay conditions were optimized according to the checkerboard titration method.[18] The optimal concentration of the coating antigen was found to be 1 μg/mL, whereas the best dilution for the monoclonal antibody was 1:8000.

Characterization of the Assay. Under optimal assay conditions, the sensitivity and specificity of the proposed ciELISA were investigated. The competitive inhibition curve of 2-DCB is presented in Figure 2. The standard calibration curve

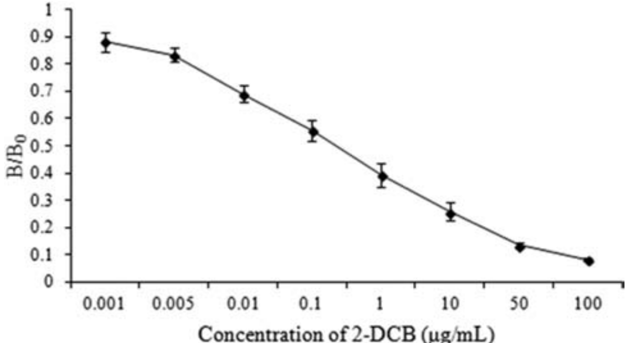

Figure 2. Competitive inhibition curve of 2-DCB.

of the ciELISA for 2-DCB determination was constructed at concentrations from 0.001 to 100 μg/mL in the buffer solution. This curve could be described using the linear equation of $B/B_0 = -0.168 \log C_{2\text{-DCB}} + 0.3993$ (R^2 = 0.9957). The sensitivity was expressed in terms of the IC_{50} values, wherein lower IC_{50} values indicated higher assay sensitivity. The IC_{50} value in this study was 0.25 μg/mL. The LOD of approximately 0.004 μg/mL was defined as the IC_{20} value from the inhibition curve, thereby indicating the high sensitivity of the assay.

The assay specificity was evaluated by determining the cross-reactivity (CR%) of antibodies against 2-DCB for five structurally related compounds. The molecular structures of the test compounds, as well as the IC_{50} and CR values for each compound, are given in Table 1. As shown in the table, the monoclonal antibody against 2-DCB had high specificity against 2-DCB, as well as low cross-reactivity with 2-TCB (<8%) and other structurally related compounds (<0.1%). These results suggested that the monoclonal antibodies were

specific against the cyclobutanone ring and the chain length of the aliphatic region of the molecule. Both regions appeared to function as a single epitope.

Accuracy and Precision of the Assay. To test the accuracy and precision of the modified ciELISA method, ground beef patties were spiked with 2-DCB at concentrations of 0.01, 0.1, and 0.5 mg/kg sample to determine recovery, intra-assay variation, and interassay variation of each treatment. Unspiked samples were used as blanks. The samples were extracted with acetonitrile via direct solvent extraction with a tissue-mashing instrument before they were purified using a 1 g silica cartridge. The elution was concentrated to dryness, resuspended in 50 μL of methanol, and diluted with 450 μL of PBS (pH 7.4) to completely eliminate the matrix effect. The diluted extracts were then analyzed by ciELISA. 2-DCB was not detected in the unspiked samples. The recovery efficiency was calculated using the standard calibration curve; the results are shown in Table 2. ciELISA gave acceptable recovery efficiency in the range from 84.4 to 109.8%. The coefficient of variation of the intra-assay was <10.0%, whereas that of the interassay was <12.0%, thereby demonstrating the high accuracy and precision of the assay.

Table 2. Results of the Recovery, Interassay Variation, and Intra-assay Variation of Ground Beef Patties Spiked with 2-DCB (n = 3)

concn fortified (mg/kg beef)	av recovery (%)	intra-assay variation[a] (%)	interassay variation[b] (%)
0.01	95.2	9.0	10.6
0.1	84.4	8.4	11.2
0.5	109.8	3.4	8.4

[a]Intra-assay variation was determined by analyzing one extraction sample three times on a single day. [b]Interassay variation was determined by analyzing one extraction sample on three different days.

Validation of the ciELISA Method by GC-MS. To evaluate the quality of the proposed ciELISA method, ground beef patties spiked with 2-DCB at concentrations of 0.01, 0.1, and 0.5 mg/kg were simultaneously analyzed by GC-MS and the ciELISA. The GC-MS calibration curve for 2-DCB was constructed in the range of 0.01, 0.02, 0.05, 0.10, 0.50, and 1.0 μg/mL; its linear equation was $y = 263175.6x - 2513.994$ (R^2 = 0.9995). As shown in Figure 3, the results from the two methods were highly correlated. The linear regression equation of $y = 0.8142x + 0.01$ (R^2 = 0.995) indicated the reliability of the proposed ciELISA test. These results suggest that 2-DCB in food samples could be simply, rapidly, and accurately detected by ciELISA.

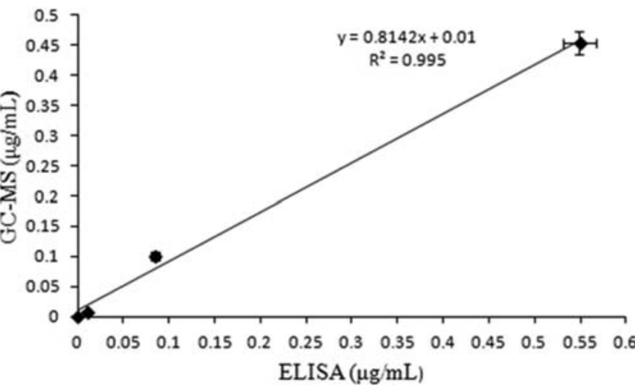

Figure 3. Correlation between the proposed ciELISA and GC-MS for ground beef patties spiked with 2-DCB at concentrations of 0.01, 0.1, and 0.5 mg/kg beef.

2-DCB Content of Irradiated Ground Beef Patties. The developed ciELISA was used to evaluate the amount of DCB in the ground beef patties irradiated at 0.5, 1.0, 3.0, 5.0, and 7 kGy. 2-DCB was not detected in the nonirradiated beef samples. The linear relationship ($y = 0.0626x + 0.0022$; R^2 = 0.9838) between the irradiation dose and 2-DCB content is presented in Figure 4. The equation can be used to immediately estimate the original absorbed doses in the irradiated ground beef patties. This result agrees with similar studies on the effect of the irradiation dose on the 2-DCB concentration.[3,7,8,19,20]

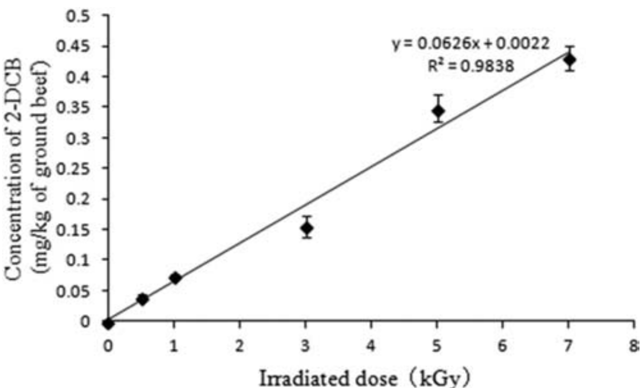

Figure 4. Effect of irradiation dose on the concentration of 2-DCB in irradiated ground beef patties.

In summary, the immunogen and the coating antigen for 2-DCB were successfully prepared and the monoclonal antibody against 2-DCB was likewise prepared using a hybridoma technique. A highly sensitive and specific ciELISA method for the detection of 2-DCB in irradiated ground beef patties was developed. The IC_{50} value of the optimized ciELISA was 0.25 μg/mL, with a detection limit of 0.004 μg/mL. Interferences caused by sample matrix were easily overcome by a simple dilution step before immunochemical analysis. The recovery efficiency was from 84.4 to 109.8%, whereas the intra-assay and interassay coefficients of variation were <10.0 and <12.0%, respectively, thereby demonstrating the high accuracy and precision of the assay. The proposed method was validated by its good correlation with GC-MS results. The proposed method was likewise used to detect 2-DCB in ground beef patties irradiated at 0.5, 1.0, 3.0, 5.0, and 7.0 kGy. The concentration of 2-DCB linearly increased with the irradiation dose. The proposed ciELISA method has high sensitivity, great simplicity, rapid reaction times, low cost, and high sample throughput. Thus, the ciELISA could be used as a feasible quantitative or screening method for the 2-DCB analysis of irradiated ground beef and other lipid-containing foods.

■ ASSOCIATED CONTENT

Ⓢ Supporting Information

MALDI-TOF-MS spectra of BSA and the 2-oxocyclobutane undecanoic acid−BSA conjugate and of OVA and the 2-oxocyclobutane undecanoic acid−OVA conjugate. This material is available free of charge via the Internet at http://pubs.acs.org.

AUTHOR INFORMATION

Corresponding Author

*(Y.H.) E-mail: hayimingketizu@hotmail.com. Phone: +86 10 62815972. Fax: +86 10 62829727.

Funding

This work was supported by the Special Fund for Agro-scientific Research in the Public Interest (Grant 201103007) and the The National Science and Technology Supported Program for the Control of Food Biological Toxin Pollution and Early Warning Technology of the Radioactive Pollution (Grant 2012BAK17B13).

Notes

The authors declare no competing financial interest.

ACKNOWLEDGMENTS

We thank the Key Laboratory of Agricultural Product Processing and Quality Control, Ministry of Agriculture, for the supply of the GC-MS instrument.

REFERENCES

(1) Gadgil, P.; Smith, J. S.; Hachmeister, K. A.; Kropf, D. H. Evaluation of 2-dodecylcyclobutanone as an irradiation dose indicator in fresh irradiated ground beef. *J. Agric. Food Chem.* **2005**, *53*, 1890–1893.

(2) Ha, Y. M.; Li, W. M.; Wang, F. Application of ESR spectroscopy to identify and estimate original dose in irradiated cumin and white pepper. *Eur. Food Res. Technol.* **2011**, *233*, 625–630.

(3) Gadgil, P.; Hachmeister, K. A.; Smith, J. S.; Kropf, D. H. 2-Alkylcyclobutanones as irradiation dose indicators in irradiated ground beef patties. *J. Agric. Food Chem.* **2002**, *50*, 5746–5750.

(4) Crews, C.; Driffield, M.; Thomas, C. Analysis of 2-alkylcyclobutanones for detection of food irradiation: current status, needs and prospects. *J. Food Compos. Anal.* **2012**, *26*, 1–11.

(5) Boyd, D. R.; Crone, A. V. J.; Hamilton, J. T. G.; Hand, M. V.; Stevenson, M. H.; Stevenson, P. J. Synthesis, characterization, and potential use of 2-dodecylcyclobutanone as a marker for irradiated chicken. *J. Agric. Food Chem.* **1991**, *39*, 789–792.

(6) Elliott, C. T.; Hamilton, L.; Stevenson, M. H.; McCaughey, W. J.; Boyd, D. R. Detection of irradiated chicken meat by analysis of lipid extracts for 2-substituted cyclobutanones using enzyme-linked immunosorbent assay. *Analyst* **1995**, *120*, 2337–2341.

(7) Obana, H.; Furuta, M.; Tanaka, Y. Analysis of 2-alkylcyclobutanones with accelerated solvent extraction to detect irradiated meat and fish. *J. Agric. Food Chem.* **2005**, *53*, 6603–6608.

(8) Soncin, S.; Panseri, S.; Rusconi, M.; Mariani, M.; Chiesa, L. M.; Biondi, P. A. Improved determination of 2-dodecylcyclobutanone in irradiated ground beef patties by gas-chromatography–mass-spectrometry (GC/MS) coupled with solid-phase microextraction (SPME) technique. *Food Chem.* **2012**, *134*, 440–444.

(9) Obana, H.; Furuta, M.; Tanaka, Y. Detection of irradiated meat, fish and their products by measuring 2-alkylcyclobutanones levels after frozen storage. *J. Food Hyg. Soci. Jpn.* **2007**, *48*, 203–206.

(10) Horvatovich, P.; Miesch, M.; Hasselmann, C.; Delincée, H.; Marchioni, E. Determination of monounsaturated alkyl side chain 2-alkylcyclobutanones in irradiated foods. *J. Agric. Food Chem.* **2005**, *53*, 5836–5841.

(11) Zhao, Y.; Wang, F.; Li, W.; Li, A.; Ha, Y. 2-Dodecylcyclobutanone as an irradiation dose indicator to identify and estimate original dose in irradiated ground beef. *Eur. Food Res. Technol.* **2012**, *235*, 901–906.

(12) Burks, A. W.; Brooks, J. R.; Sampson, H. A. Allergenicity of major component proteins of soybean determined by enzyme-linked immunosorbent assay (ELISA) and immunoblotting in children with atopic dermatitis and positive soy challenges. *J. Allergy Clin. Immunol.* **1988**, *81*, 1135–1142.

(13) You, J.; Li, D.; Qiao, S.; Wang, Z.; He, P.; Ou, D. Development of a monoclonal antibody-based competitive ELISA for detection of β-conglycinin, an allergen from soybean. *Food Chem.* **2008**, *106*, 352–360.

(14) Garber, E. A. Detection of melamine using commercial enzymelinked immunosorbent assay technology. *J. Food Prot.* **2008**, *71*, 590–594.

(15) Lu, Z.; Morinaga, O.; Tanaka, H.; Shoyama, Y. A quantitative ELISA using monoclonal antibody to survey paeoniflorin and albiflorin in crude drugs and traditional chinese herbal medicines. *Biol. Pharm. Bull.* **2003**, *26*, 862–866.

(16) Sherry, J. P. Environmental chemistry: the immunoassay option. *Crit. Rev. Anal. Chem.* **1992**, *23*, 217–300.

(17) Marco, M. P.; Gee, S.; Hammock, B. D. Immunochemical techniques for environmental analysis II. Antibody production and immunoassay development. *Trends Anal. Chem.* **1995**, *14*, 415–425.

(18) Kim, I. S.; Setford, S. J.; Saini, S. Determination of polychlorinated biphenyl compounds in electrical insulating oils by enzyme immunoassay. *Anal. Chim. Acta* **2000**, *422*, 167–177.

(19) D'oca, M. C.; Bartolotta, A.; Cammilleri, M. C.; Giuffrida, S. A.; Parlato, A.; Di Noto, A. M.; Caracappa, S. The gas chromatogarphy/mass spectrometry can be used for dose estimation in irradiated pork. *J. Radiat. Phys. Chem.* **2009**, *78*, 687–689.

(20) Tewfik, I. Extraction and identification of cyclobutanones from irradiated cheese employing a rapid direct solvent extraction method. *Int. J. Food Sci. Nutr.* **2008**, *59*, 590–598.