JOURNAL OF AGRICULTURAL AND FOOD CHEMISTRY

Article

Simultaneous Analysis of 3-MCPD and 1,3-DCP in Asian Style Sauces using QuEChERS Extraction and Gas Chromatography – Triple Quadrupole Mass Spectrometry

Susan Genualdi, Patricia Jean Nyman, and Lowri S. deJager

J. Agric. Food Chem., **Just Accepted Manuscript** • DOI: 10.1021/acs.jafc.6b05051 • Publication Date (Web): 08 Jan 2017

ACS Publications

Journal of Agricultural and Food Chemistry is published by the American Chemical Society. 1155 Sixteenth Street N.W., Washington, DC 20036

Simultaneous Analysis of 3-MCPD and 1,3-DCP in Asian Style Sauces using QuEChERS Extraction and Gas Chromatography – Triple Quadrupole Mass Spectrometry

Susan Genualdi,[1] Patricia Nyman,[1,2] Lowri DeJager[1]

[1]**U.S. Food and Drug Administration**, 5001 Campus Drive; College Park, MD 20740, USA; susan.genualdi@fda.hhs.gov
[2] Retired

Corresponding Author:
Susan Genualdi
5001 Campus Drive
College Park, MD 20740
Ph: 240-402-2914
Email: susan.genualdi@fda.hhs.gov

Abstract

Acid hydrolyzed vegetable protein (aHVP) is used for flavoring a wide variety of foods and also in the production of non-fermented soy sauce. During the production of aHVP, chloropropanols including 3-monochloropropane-1,2-diol (3-MCPD) and 1,3 dichloropropane-2-ol (1,3-DCP) can be formed through the reaction of the hydrochloric acid catalyst and residual fat and the reaction of 3-MCPD with acetic acid, respectively. 3-MCPD is a carcinogen and 1,3-DCP has been classified as a genotoxic carcinogen. The European Union (EU) has set a maximum concentration of 0.02 mg/kg of 3-MCPD in aHVP, and the Food and Drug Administration (FDA) set a guidance limit of 1 mg/kg of 3-MCPD in aHVP. 1,3-DCP is not an approved food additive and the Joint FAO/WHO Expert Committee on Food Additives (JEFCA) has set a limit at 0.005 mg/kg which is close to the estimated method detection limit. Currently there are few analytical methods for the simultaneous determination of 3-MCPD and 1,3-DCP without derivatization due to differences in their physical chemical properties and reactivity. A new method was developed using QuEChERS (Quick, Easy, Cheap, Effective, Rugged, and Safe) with direct analysis of the extract without derivatization using gas chromatography-triple quadrupole mass spectrometry (GC-QQQ). Additionally, a market sampling of 60 soy sauce samples was performed in 2015 to determine if concentrations have changed since the FDA limit was set in 2008. The sampling results were compared between the new QuEChERS method and a method using phenylboronic acid (PBA) as a derivatizing agent for 3-MCPD analysis. The concentrations of 3-MCPD detected in soy sauce samples collected in 2015 (< MDL to 0.53 mg/kg) compared to 2003 (<MDL to 876 mg/kg) indicate that manufacturing controls currently in use during aHVP production have been effective at reducing 3-MCPD below current regulatory limits

Keywords: soy sauce, 3-MCPD, 1,3-DCP

Introduction

During the production of non-fermented soy sauce, 3-chloro-propane-1,2-diol (3-MCPD) can be formed from acid hydrolyzed vegetable protein (aHVP) through the reaction of hydrochloric acid and residual lipids.[1] Further reaction of (3-MCPD) with acetic acid can produce 1,3-dichloro-propan-2-ol (1,3-DCP).[1, 2] 3-MCPD is classified as a Group 2B carcinogen by the International Agency for Research on Cancer (IARC) and 1,3-DCP is classified as a genotoxic carcinogen by the Joint FAO/WHO Expert Committee on Food Additives (JEFCA).[3] In 1997, the Food Chemicals Codex (FCC) limited 3-MCPD and 1,3-DCP to 1 mg/kg and 0.050 mg/kg in aHVP.[4] The European Commission (EC) set a limit of 0.02 mg/kg for 3-MCPD and 1,3-DCP in aHVP and soy sauce in 2002 (EC No. 466/2001, EC No. 466/2002) and in 2008 the Food and Drug Administration (FDA) set a limit of 1 mg/kg of 3-MCPD in aHVP and soy sauce.[5] Because 1,3-DCP is considered a genotoxic carcinogen, JEFCA determined in 1993 that no concentration is safe and set a limit of 0.005 mg/kg which is estimated to be close to the method detection limit.[6] As a result of these limits being imposed, industry has made efforts to reduce the amount of 3-MCPD in aHVP with techniques that include control of the acid hydrolysis step, alkaline treatment after acid hydrolysis, and the use of sulfuric acid instead of hydrochloric acid.[7]

Although the structures of 1,3-DCP and 3-MCPD are quite similar, 3-MPCD has 2 hydroxyl groups that makes 3-MCPD more reactive and more polar than 1,3-DCP which only has one hydroxyl group. Because of their reactivity and polarity, 3-MCPD and 1,3-DCP are typically not analyzed directly but with derivatization that replaces the polar hydroxyl groups with non-polar functional groups. Without derivatization, these analytes may have poor peak shape. Additionally, the low molecular weights of 3-MCPD and 1,3-DCP result in low *m/z* fragmentation patterns following mass spectrometer (MS) ionization. Derivatizing agents previously used in the simultaneous analysis of 3-MCPD and 1,3-DCP include N-methyl-N-(trimethylsilyl) trifluoroacetamide (MSTFA),[8] bis(trimethylsilyl) trifluoroacetamide (BSTFA),[9, 10] heptafluorobutyric anhydride (HFBA),[11, 12] heptafluorobutyrylimidazole (HFBI),[1, 13-16]

hexamethyldisilazine-trimethylsilyl trifluoromethanesulfonate (HDMS-TMSOTf),[17] HFBA modified with trimethylamine (HFBA-Et_3N),[16] and 1-trimethylsilylimidazole (TSIM).[18] Phenylboronic acid (PBA)[19] can be used to derivatize 3-MCPD, but not 1,3-DCP since PBA needs two hydroxyl groups to form the cyclic phenyl boronate derivative.[20] Recently, the use of gas chromatography–triple quadrupole mass spectrometry (GC-QQQ) has been used for the simultaneous analysis of 3-MCPD and 1,3-DCP to avoid derivatization. [21, 22] Multiple reaction mode monitoring (MRM) by GC-QQQ reduces matrix interferences and allows for the monitoring of unique small ion fragments. Additionally, with a multi-mode inlet (MMI), the sample can be introduced at a low temperature and better sensitivity and peak shape is observed. To date, the methods using GC-QQQ without derivatization involve a combined clean-up and extraction step with glass chromatographic columns packed with Extrelut™ NT and primary secondary amine (PSA)[21] and another method using matrix solid-supported liquid liquid extraction on the Extrelut™ NT absorbent with the extract directly injected onto an online gel permeation chromatography (GPC) GC-QQQ system.[22]

A new method is presented here that uses QuEChERS (Quick, Easy, Cheap, Effective, Rugged, and Safe) extraction followed by direct injection of the acetonitrile extract with no nitrogen blowdown step and no derivatization. By using a GC-QQQ, these compounds can be analyzed directly and simultaneously at detection limits well below the limits set by the EU and JEFCA. QuEChERS has been previously used for the extraction of 3-MCPD in coffee. In coffee, the QuEChERS acetonitrile extract is blown to dryness under a stream of nitrogen.[23] The extract is brought up in a 20% salt solution, derivatized by PBA, and analyzed by single quadrupole GC-MS.[23]

Currently, at the Food and Drug Administration (FDA), the regulatory analysis of 3-MCPD is performed using the AOAC official method (2000.01).[13] This method is labor-intensive, time consuming and uses large amounts of solvents. The new QuEChERS method would provide rapid results for both 3-MCPD and 1,3-DCP with minimal solvent use and sample preparation. Since all regulatory labs might

not have access to a GC-QQQ, a previously published method was also validated for 3-MCPD that would provide rapid results using a single quadrupole GCMS. This method uses phenylboronic acid (PBA) as a derivatizing agent and is fast and simple. The derivatized 3-MCPD is directly extracted into a hexane layer and injected into the GCMS.[19] Although the PBA method cannot simultaneously analyze for 3-MCPD and 1,3-DCP, it provides a simple, rapid alternative to the AOAC method (2000.01).

Both of these methods were validated and used to perform a market sampling of 60 soy sauce samples in the Washington D.C. area to determine if concentrations in soy sauce have changed since the last sampling was performed in 2003, which was prior to the FDA limit of 3-MCPD being set to 1 mg/kg in 2008. In 2003, a market sampling of fifty-five samples in the Washington D.C. area found that 33% of the samples contained 3-MCPD above the FDA limit.[14] Thirty-nine of the samples were analyzed for 1,3-DCP and 56% of these samples had concentrations above the detection limit of 0.055 µg/kg. [14] The objectives of this research were to (1) develop and validate a new QuEChERS method for the simultaneous analysis of 3-MCPD and 1,3-DCP in soy sauce using GC-QQQ (2) validate the PBA method for 3-MCPD analysis in soy sauce (3) perform a market sampling of soy sauces in 2015 using both the new QuEChERS method and the PBA method to compare to results from 2003.

Materials and Methods

Samples

Sixty samples were collected in the Washington DC area in 2015. There were 51 soy sauces, 4 fish sauces, 4 oyster sauces, and 1 teriyaki sauce. Sauces were collected from chain grocery stores, Asian grocery stores, and from restaurants in the form of takeout containers and packets. These samples came from the USA, Mexico, Korea, Thailand, Japan, Indonesia, Taiwan, China, Germany, Hong Kong, Vietnam, and the Philippines. Twelve of these samples specifically had aHVP listed as an ingredient on the label.

Chemicals and Reagents

Phenylboronic acid (>97.0% purity), 3-MCPD (>98% purity), 1,3-DCP (>98% purity), and d_5 1,3-DCP (>98% purity) were purchased from Sigma Aldrich (St. Louis, MO) and d_5 3-MCPD (>98% purity) was purchased from Canadian isotopes (Point-Claire, Quebec, Canada). Sodium chloride crystals, acetonitrile, and LC-MS Optima™ water were purchased from Fisher Scientific (Fair Lawn, NJ, USA). For the QuEChERS method, salt extraction mylar pouches (AOAC 2007.1, 6 g MgSO4 with 1.5 g of sodium acetate) and dSPE pouches (400 mg of PSA, 400 mg of C18) were purchased from United Chemical (Bristol, PA). Additionally, a FAPAS® proficiency standard for 1,3-DCP and 3-MCPD in soy sauce was purchased from Sigma Aldrich (St. Louis, MO). Stock solutions of 3-MCPD and d_5 3-MCPD were initially prepared in 20% NaCl in water (PBA method) and 100% ethyl acetate (QuEChERS method) at concentrations of 2 mg/mL and further diluted for recovery and calibration solutions. Additionally, solutions of 1,3-DCP and d_5 1,3-DCP were also prepared in ethyl acetate for the QuEChERS method. For the PBA and QuEChERS methods, seven point calibration curves were prepared that corresponded to 3-MCPD and 1,3-DCP concentrations of 10 µg/kg – 2600 µg/kg in soy sauce.

Sample Preparation

PBA method

The derivatization of 3-MCPD in soy sauce was performed using the analytical method by Kusters et al. with slight modifications.[19] Briefly, 5 mL of soy sauce was added to a 20 mL glass vial along with the internal standard d_5 3-MCPD spiked to give a final concentration of 1 mg/kg. Then 5 mL of 5M NaCl was added to the soy sauce. The vial was sealed with a screw cap lid with PTFE septa and placed on a shaker/vortexer (Glas-Col Terre Haute, IN, USA) for 10 minutes. Then 200 uL of 25% phenylboronic acid (PBA) was added to the vial and the vial was placed in the oven for 90^{o}C for 10 minutes. After the sample cooled to room temperature, 2 mL of hexane was added to the vial. The sample was shaken/vortexed (Glas Col Terre Haute, IN, USA) for 3 minutes and centrifuged at 1800 rcf for 5 minutes. The hexane layer was removed with a 3 mL Norm-ject™ syringe and filtered using a 0.2 um Acrodisc®

PTFE filter. The extract was placed into an amber glass autosampler vial with a PTFE lined screw cap for further analysis

QuEChERS method

Soy sauce samples (10 mL) were placed in 50 mL polypropylene centrifuge tubes. The internal standards d_5 3-MCPD and d_5 1,3-DCP were added to the soy sauce to give a final concentration of 1 mg/kg. Then, 10 mL of acetonitrile was added followed by a salt extraction packet with 6000 mg $MgSO_4$ and 1500 mg NaCl (UCT, Bristol, PA). The samples were vortexed 1 minute and then centrifuged for 15 minutes at 10,000 rcf. The supernatant (6 mL) was transferred to a 15 mL polypropylene centrifuge tube containing a dSPE packet with 1200 mg $MgSO_4$, 400 mg PSA and 400 mg C18 (UCT, Bristol, PA). This tube was shaken for 2 minutes and centrifuged for 15 minutes at 10,000 rcf. The supernatant was directly transferred to an autosampler vial using a 1 mL Norm-ject™ syringe and filtered using a 0.2 um Acrodisc® PTFE filter.

Instrumentation

PBA method

Single quadrupole gas chromatography-mass spectrometry (GCMS) was used for separation and quantitation. A 7890A GC-5975C MS was used with a 60m DB-1 column (Agilent, Santa Clara, CA). The inlet temperature was set to 180°C, because at higher temperatures residues of the phenylboronic acid derivatizing agent can damage the column. [24] A 2:1 split injection was performed with an injection volume of 3 µL. The oven temperature ramp was optimized with an initial temperature of 75°C with a 2 min hold, followed by a 5°C/min ramp to 180°C and a final ramp of 25°C/min to 250°C with a 5 min hold. The longer column and slow temperature ramp were essential to separate out the derivatized 3-MCPD from a contaminant that elutes closely with similar ions. The auxillary temperature was set to 280°C and the source and quadrupoles at 230°C and 150°C respectively. The ions monitored in SIM mode were *m/z* 150 for the quantitative ion and *m/z* 93 and 201 as the qualifying ions for d_5 3-MCPD. For 3-MCPD,

144 *m/z* 147 was the quantitative ion and *m/z* 91 and 196 were monitored as qualifying ions. Details on the
145 corresponding structures to these ions has been previously described. [20]

146 **QuEChERS method**

147 A Restek column (Rtx-200, 30 m, 250 µm, 25 µm) with a trifluoropropylmethyl polysiloxane
148 stationary phase was used for separation and analysis was performed using gas chromatography-triple
149 quadrupole mass spectrometry (GC-QQQ) with an Agilent 7010. One microliter of sample was injected
150 into the multi-mode inlet (MMI) at a temperature of 100°C with a split ratio of 5:1. The inlet was then
151 ramped to 300°C at a speed of 700°C/min. The initial oven temperature was set to 60°C with a 2 minute
152 hold followed by a ramp of 15°C/min to 100°C with a 5 minute hold and a final ramp of 60°C/min to
153 300°C with a 2 minute hold. The auxillary and source were set to 300°C and the quadrupoles at 150°C.
154 The transitions monitored were *m/z* 84 → *m/z* 46 (Quant) and *m/z* 82 → *m/z* 46 (Qual) for d_5 1,3-DCP
155 and d_5 3-MCPD, and *m/z* 81 → 43 (Quant) and *m/z* 79 → 43 (Qual) for 1,3-DCP and 3-MCPD all with a
156 collision energy of 30. Chromatograms can be found in the supporting information (Figure S3).

157 **Results**

158 **Validation for PBA and QuEChERS methods**

159 Both methods were validated using internal FDA guidelines. [25] Recovery experiments were
160 performed by fortifying naturally fermented soy sauce with 3-MCPD and 1,3-DCP at the FDA action limit
161 for 3-MCPD (1 mg/kg), half of the FDA limit (0.5 mg/kg) and two times the FDA limit (2 mg/kg). A
162 minimum of 4 replicates were needed for validation samples. In this study 6 replicate extractions were
163 performed at each level for the PBA method and 4 replicate extractions were done at each level for the
164 QuEChERS method. For the PBA method, the recoveries ranged from 76 to 108 % (Figure 1) and the
165 method detection limit (MDL) was calculated to be 0.005 mg/kg for 3-MCPD. The MDL was calculated
166 based on the standard deviation of 7 replicate spikes at 0.010 mg/kg as described in 40 CFR part 136,
167 Appendix B. [26] For the QuEChERS method, the recoveries ranged from 84-117% for 3-MCPD and 101-

113% for 1,3-DCP (Figure 1). The MDLs were calculated from the standard deviation of 7 replicate spikes at 0.005 mg/kg and determined to be 0.002 mg/kg for 3-MCPD and 0.0004 mg/kg for 1,3-DCP. The percent RSDs for replicate extractions were 4-10 % for 3-MCPD using the PBA method, 2-13 % for 3-MCPD using the QuEChERS method and 2-4 % for 1,3-DCP with the QuEChERS method. A FAPAS soy sauce proficiency sample was obtained and both methods were used to analyze the sample. The testing included results from 31 participants. The assigned value was determined to be 44.1 µg/kg for 3-MCPD and 14.8 µg/kg for 1,3-DCP. Using the assigned value and the standard deviation for proficiency, individual z-scores were calculated. Under normal circumstances, the z scores should fall within the range of -2 to 2. For the PBA method, the calculated value for 3-MCPD was 56 µg/kg and the z-score was 1.22. For the QuEChERS method, the calculated value for 3-MCPD was 45 µg/kg with a z-score of 0.10 and for 1,3-DCP the calculated value was 16 µg/kg with a z-score of 0.38 (Figure 2).

Market sampling results & comparison

Eight out of sixty soy sauce samples contained 3-MCPD above the detection limit (Figure 3) with concentrations between 0.13 mg/kg and 0.53 mg/kg. All samples with positive detections were analyzed in triplicate and replicates ranged from 4-17 % RSD for 3-MCPD using the PBA method, 4-12 % for 3-MCPD and 2-10 % for 1,3-DCP using the QuEChERS method. All eight samples were below the FDA limit of 1 mg/kg, but were above the EU limit of 0.02 mg/kg. Comparatively 5 out of 8 samples showed no significant differences (p-value > 0.05) in the results obtained between the two methods, while 3 of the samples (#1, #2, #7) were significantly different (p-value < 0.05). The percent differences between the results obtained from the two methods for these 3 samples were 33%, 46% and 21% for samples 1, 2, and 7 respectively. Considering the FAPAS proficiency samples were both in the acceptable range, it is unclear why some of the samples had significantly different results between the two methods. In all three cases, the PBA method reported greater concentrations of 3-MCPD compared to the QuEChERS method. Soy sauce is a complex mixture, especially non-fermented soy sauces where additives and

192 flavors are added intentionally to artificially create aromas and flavors. The varying composition
193 between soy sauces may play a role in the yield of 3-MCPD in certain sauces by these two different
194 methods.

195 In the market sampling published in 2003, 33% of the samples had concentrations of 3-MCPD
196 greater than 1 mg/kg, while in 2015, none of the samples had concentrations above 1 mg/kg. It appears
197 that companies have made efforts to reduce the amount of 3-MCPD in the final soy sauce product since
198 the FDA limit was set in 2008. In 2003, 40% of the samples had 1,3-DCP concentrations greater than the
199 detection limit of 0.00005 mg/kg with samples as high as 9.8 mg/kg. In 2015 samples, 8% of samples
200 had detections of 1,3-DCP above the detection limit of 0.0004 mg/kg and all were below the JEFCA limit
201 of 0.005 mg/kg (Figure 4). Since 1,3-DCP is produced from the further reaction of 3-MCPD, it is not
202 surprising that the concentrations of 1,3-DCP are much lower in 2015 compared to 2003 since the 3-
203 MCPD concentrations in soy sauce have also decreased.

204 **Discussion**

205 A new QuEChERS method is presented here that allows for the simultaneous analysis of 3-
206 MCPD and 1,3-DCP without derivatization. This method has low detection limits (0.0004 mg/kg for 1,3-
207 DCP and 0.0023 mg/kg for 3-MCPD) which are both well below the JEFCA limit of 0.005 mg/kg for 1,3-
208 DCP and the EU limit of 0.020 mg/kg for 3-MCPD. QuEChERS is a rapid extraction and clean-up
209 technique and the acetonitrile extract can be directly injected into the GC-QQQ without the need for
210 solvent concentration, which avoids additional losses of 1,3-DCP due to evaporation. The Agilent 7010
211 GC-QQQ allows for the direct injection of 3-MCPD and 1,3-DCP with an MMI inlet which can be
212 temperature ramped and offers improved sensitivity and peak shape. Also, because the ions monitored
213 for these compounds are low molecular weight fragments, the use of the GC-QQQ allows for specific
214 transitions to be monitored and reduces the possibility of interferences. The PBA method is also a quick
215 technique that allows for the rapid analysis of 3-MCPD. Both of these methods were validated to be

used as options for monitoring 3-MCPD in a regulatory setting and to provide alternative methods for analysis other than the AOAC method (2000.01) which is solvent intensive and time consuming. Additionally these two methods were used to perform a market sampling in 2015 to compare to a sampling from 2003. The results indicate that the amount of 3-MCPD in soy sauces on the market in the Washington D.C. area in 2015 are all below the FDA limit and concentrations have decreased drastically since 2003. In 2003, all of the soy sauces produced in America had concentrations below 1 mg/kg, while all the soy sauce samples above 1 mg/kg were produced in Asia. In 2015, 93% of the samples were produced in Asia and the 8 samples with detections above the MDL were from Thailand (4) and Vietnam (4). Compared to 2003, where concentrations of 426 mg/kg and 120 mg/kg 3-MCPD were measured in soy sauces from Thailand and Vietnam, the concentrations measured in 2015 were all below 0.45 mg/kg or not detected (n.d) (Figure 5). Even with the limited sample size for sauces from 2003 (n=2 for Thailand and n=2 for Vietnam) compared to 2015 (n=14 for Thailand and n=5 for Vietnam), it is clear that samples are no longer being found with the high concentrations detected in 2003 from these countries (Figure 5). The other countries with high 3-MCPD concentrations in 2003 included Hong Kong (n.d – 876 mg/kg), China (n.d – 85 mg/kg), and the Philippines (n.d. – 36 mg/kg). Samples from these countries in 2015 found no detectable concentrations above the detection limit along with soy sauces tested from all the other countries (Japan, Taiwan, Korea, Indonesia, Mexico, USA, and Germany). Out of the 60 samples analyzed, 12 of them listed aHVP on the label. None of these samples contained detectable concentrations of 3-MCPD, indicating that manufacturing controls currently in use during aHVP production have been effective at reducing 3-MCPD below current regulatory limits.

Acknowledgements

We gratefully acknowledge Nicole Shyong and Nahyun Jeong for help with sample extraction and analysis.

Associated Content

240 **Supporting Information**

241 This material is available free of charge via the Internet at http://pubs.acs.org.

242 Structures of 3-MCPD and 1,3 DCP (Figure S1), GCMS chromatogram of derivatized 3-MCPD (Figure S2),

243 GC-QQQ chromatogram of analytes in standard (Figure S3) GC-QQQ chromatogram of analytes in

244 sample (Figure S4), soy sauce sample information (Table S1).

References

1. Vanbergen, C. A.; Collier, P. D.; Cromie, D. D. O.; Lucas, R. A.; Preston, H. D.; Sissons, D. J., Determination of chloropropanols in protein hydrolysates *J. Chromatogr. A* **1992**, *589*, 109-119.
2. Velisek, J.; Davidek, J.; Hajslova, J.; Kubelka, V.; Janicek, G.; Mankova, B., Chlorohydrins in protein hydrolysates. *Zeitschrift Fur Lebensmittel-Untersuchung Und-Forschung* **1978**, *167*, 241-244.
3. FAO/WHO, Joint FAO/WHO Expert Committee on Food Additives (JEFCA) fifty-seventh meeting, 2001, summary and conclusions. *Rome: Food Agriculture Organization of the United Nations/World Health Organization* **2001**.
4. Food Chemical Codex. *Acid Hydrolysates of Proteins* **1997**, *First Supplement to the 4th edition* 1-4.
5. FDA CPG Sec 500.500 guidance levels for 3-MCPD (3-chloro-1,2-propanediol) in acid-hydrolyzed protein and Asian-style sauces. . http://www.fda.gov/ICECI/ComplianceManuals/CompliancePolicyGuidanceManual/ucm074419.htm. (accessed 3/25/15).
6. Olsen, P., Toxicological evaluation of certain food additives and contaminants - chloropropanols. *WHO Food Additive Series* **1993**, *32*, 267-285.
7. Lee, B. Q.; Khor, S. M., 3-chloropropane-1,2-diol (3-MCPD) in soy sauce: a review on the formation, reduction, and detection of this potential carcinogen. *Compr. Rev. Food Sci. Food Saf.* **2015**, *14*, 48-66.
8. Lee, M. R.; Chiu, T. C.; Dou, J. P., Determination of 1,3-dichloro-2-propanol and 3-chloro-1,2-propandiol in soy sauce by headspace derivatization solid-phase microextraction combined with gas chromatography-mass spectrometry. *Anal. Chim. Acta* **2007**, *591*, 167-172.
9. Gonzalez, P.; Racamonde, I.; Carro, A. M.; Lorenzo, R. A., Combined solid-phase extraction and gas chromatography-mass spectrometry used for determination of chloropropanols in water. *J. Sep. Sci.* **2011**, *34*, 2697-2704.
10. Racamonde, I.; Gonzalez, P.; Lorenzo, R. A.; Carro, A. M., Determination of chloropropanols in foods by one-step extraction and derivatization using pressurized liquid extraction and gas chromatography-mass spectrometry. *J. Chromatogr. A* **2011**, *1218*, 6878-6883.
11. Abu-El-Haj, S.; Bogusz, M. J.; Ibrahim, Z.; Hassan, H.; Al Tufail, M., Rapid and simple determination of chloropropanols (3-MCPD and 1,3-DCP) in food products using isotope dilution GC-MS. *Food Control* **2007**, *18*, 81-90.
12. Chung, W. C.; Hui, K. Y.; Cheng, S. C., Sensitive method for the determination of 1,3-dichloropropan-2-ol and 3-chloropropane-1,2-diol in soy sauce by capillary gas chromatography with mass spectrometric detection. *J. Chromatogr. A* **2002**, *952*, 185-192.
13. Brereton, P.; Janet, K.; Crews, C.; Honour, S.; Wood, R.; Davies, A., Determination of 3-chloro-1,2-propanediol in foods and food ingredients by gas chromatography with mass spectrometric detection: collaborative study. *J. Chromatogr. A* **2001**, *84*, 455-465.
14. Nyman, P. J.; Diachenko, G. W.; Perfetti, G. A., Survey of chloropropanols in soy sauces and related products. *Food Addit. Contam., Part A* **2003**, *20*, 909-915.
15. Carro, A. M.; Gonzalez, P.; Lorenzo, R. A., Simultaneous derivatization and ultrasound-assisted dispersive liquid-liquid microextraction of chloropropanols in soy milk and other aqueous matrices combined with gas-chromatography-mass spectrometry. *J. Chromatogr. A* **2013**, *1319*, 35-45.
16. Xu, X. M.; Ren, Y. P.; Wu, P. G.; Han, J. L.; Shen, X. H., The simultaneous separation and determination of chloropropanols in soy sauce and other flavoring with gas chromatography-mass spectrometry in negative chemical and electron impact ionization modes. *Food Addit. Contam., Part A* **2006**, *23*, 110-119.

17. Lee, B. Q.; Radzi, C.; Khor, S. M., A simultaneous derivatization of 3-monochloropropanediol and 1,3-dichloropropane with hexamethyldisilazane-trimethylsilyl trifluoromethanesulfonate at room temperature for efficient analysis of food sample analysis. *J. Chromatogr. A* **2016**, *1432*, 101-110.
18. Cao, X. J.; Song, G. X.; Gao, Y. H.; Zhao, J. F.; Zhang, M. L.; Wu, W. P.; Hu, Y. M., A novel derivatization method coupled with GC-MS for the simultaneous determination of chloropropanols. *Chromatographia* **2009**, *70*, 661-664.
19. Kusters, M.; Bimber, U.; Ossenbruggen, A.; Reeser, S.; Gallitzendorfer, R.; Gerhartz, M., Rapid and simple micromethod for the simultaneous determination of 3-MCPD and 3-MCPD esters in different foodstuffs. *J. Agric. Food Chem.* **2010**, *58*, 6570-6577.
20. Rodman, L. E.; Ross, R. D., Gas-liquid-chromatography of 3-chloropropanediol. *J. Chromatogr. A* **1986**, *369*, 97-103.
21. Mo, W. M.; He, H. L.; Xu, X. M.; Huang, B. F.; Ren, Y. P., Simultaneous determination of ethyl carbamate, chloropropanols and acrylamide in fermented products, flavoring and related foods by gas chromatography triple quadrupole mass spectrometry. *Food Control* **2014**, *43*, 251-257.
22. Yi, Q.; Miao, H.; Wu, Y. N., Derivatization-free method for chloropropanols determination in food by online gel permeation chromatography-gas chromatography-triple quadrupole mass spectrometry. *Chin. J. Anal. Chem.* **2016**, *44*, 678-684.
23. Sadowska-Rociek, A.; Cieslik, E., Assessment of 3-MCPD levels in coffee and coffee substitutes by simplified QuEChERS method. *J. Verbraucherschutz Lebensmittelsicherh.* **2015**, *10*, 117-122.
24. Breitling-Utzmann, C. M.; Hrenn, H.; Haase, N. U.; Unbehend, G. M., Influence of dough ingredients on 3-chloropropane-1,2-diol (3-MCPD) formation in toast. *Food Addit. Contam., Part A* **2005**, *22*, 97-103.
25. FDA Guidelines for the validation of chemical methods for the FDA foods program. http://www.fda.gov/downloads/ScienceResearch/FieldScience/UCM298730.pdf (accessed 3/25/2015).
26. USEPA, Appendix B to Part 136 - definition and procedure for the determination of the method detection limit - revision 1.11. **1984**.

Figure captions

Figure 1. Method recoveries of soy sauce spiked at 0.5, 1, and 2 mg/kg using the PBA and QuEChERS methods. The median, 10^{th}, 25^{th}, 75^{th}, and 90^{th} percentiles are displayed as vertical boxes with error bars

Figure 2. Concentrations of 3-MCPD and 1,3-DCP measured in a FAPAS proficiency sample using the PBA and QuEChERS methods

Figure 3. Market sampling results of 3-MCPD in soy sauce analyzed using the PBA and QuEChERS methods

Figure 4. Market sampling results of 1,3-DCP in soy sauce analyzed using the QuEChERS method

Figure 5. Comparison between soy sauces sampled in 2003 and 2015 from Vietnam and Thailand

Figure graphics

Figure 1.

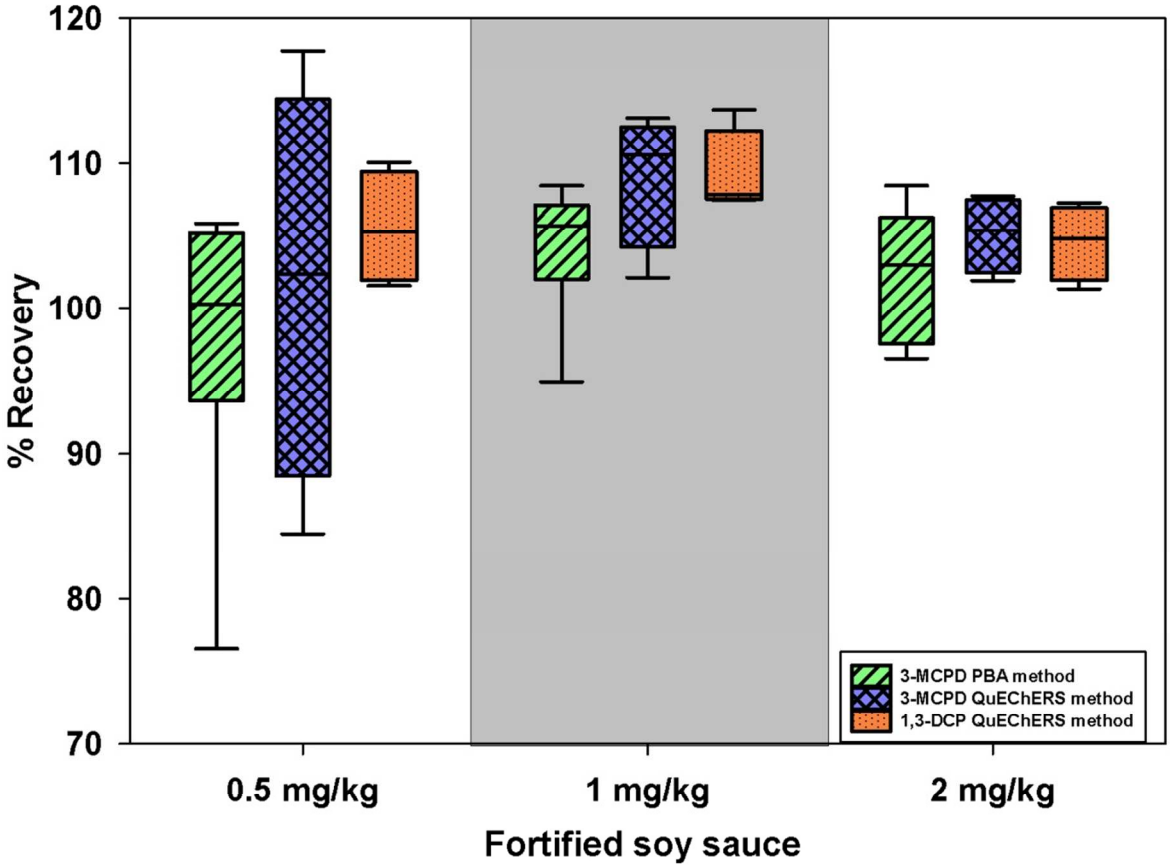


Figure 2

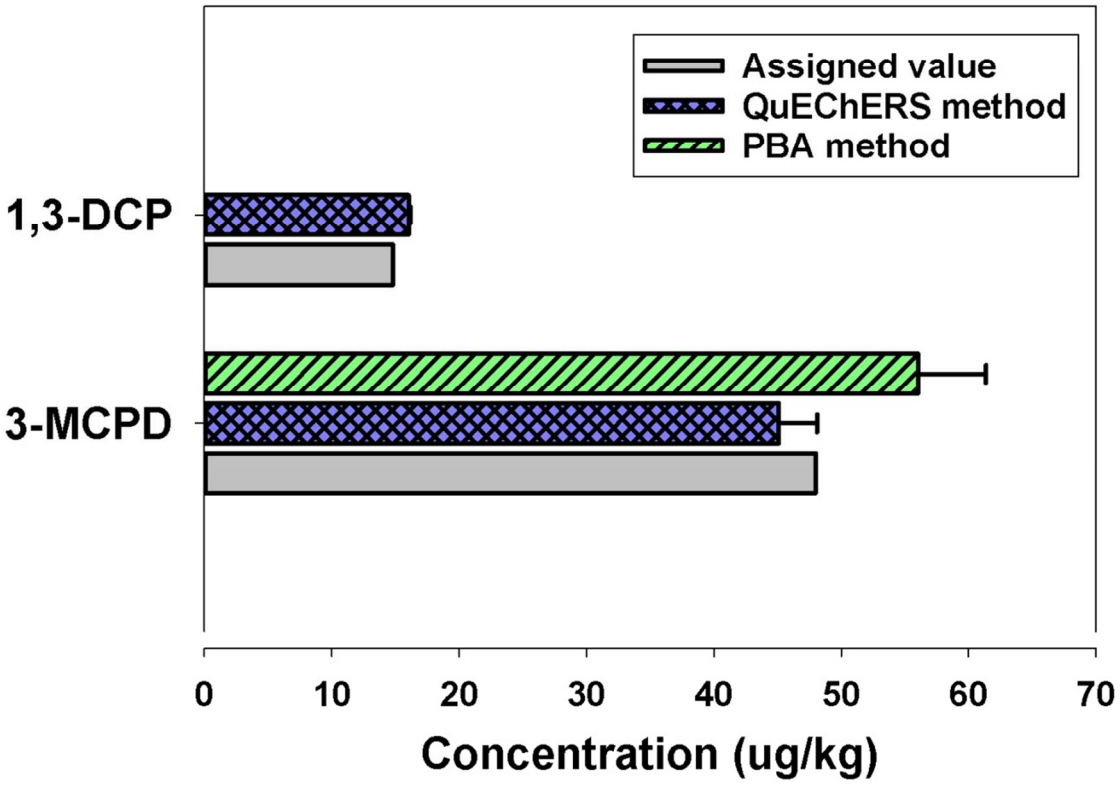

Assigned value
QuEChERS method
PBA method
1,3-DCP
3-MCPD
0
10
20
30
40
50
60
70
Concentration (ug/kg)

Figure 3

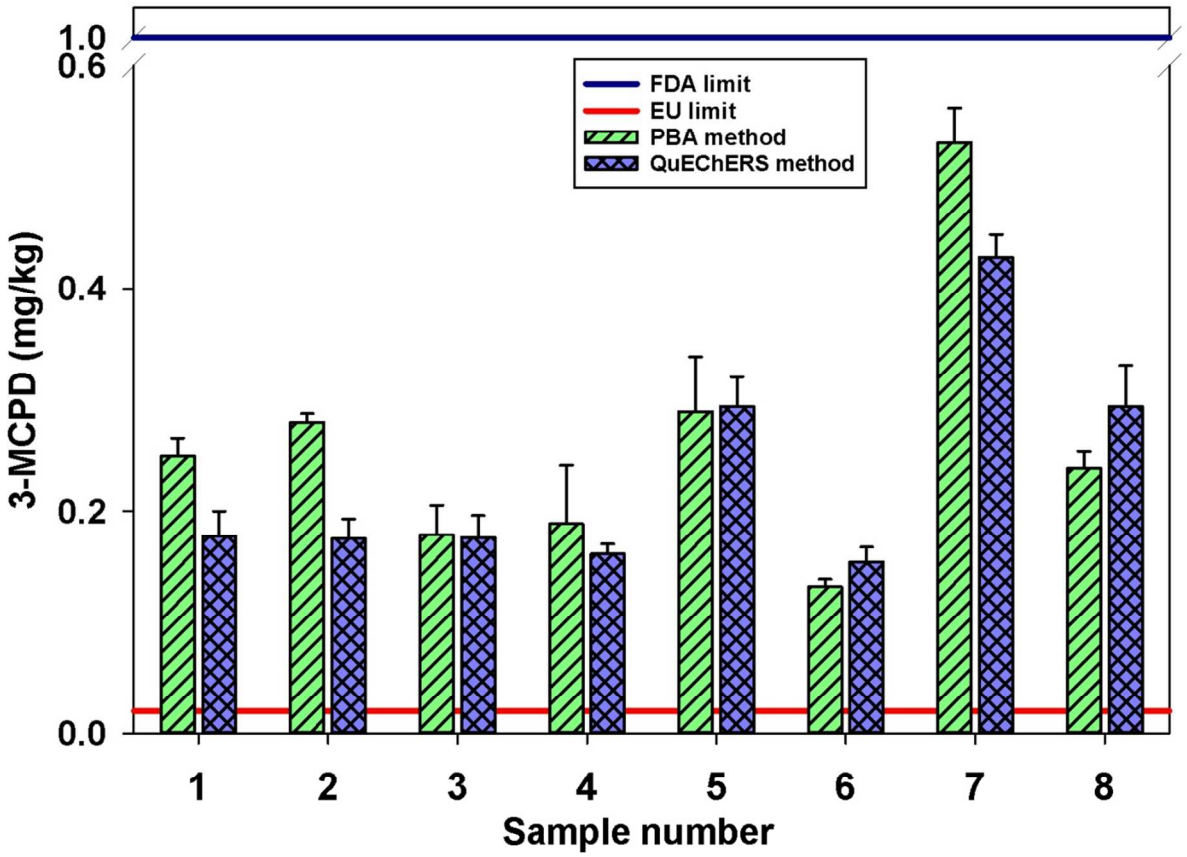

1.0
0.6
0.4
0.2
0.0
3-MCPD (mg/kg)
FDA limit
EU limit
PBA method
QuEChERS method
1
2
3
4
5
6
7
8
Sample number

Figure 4

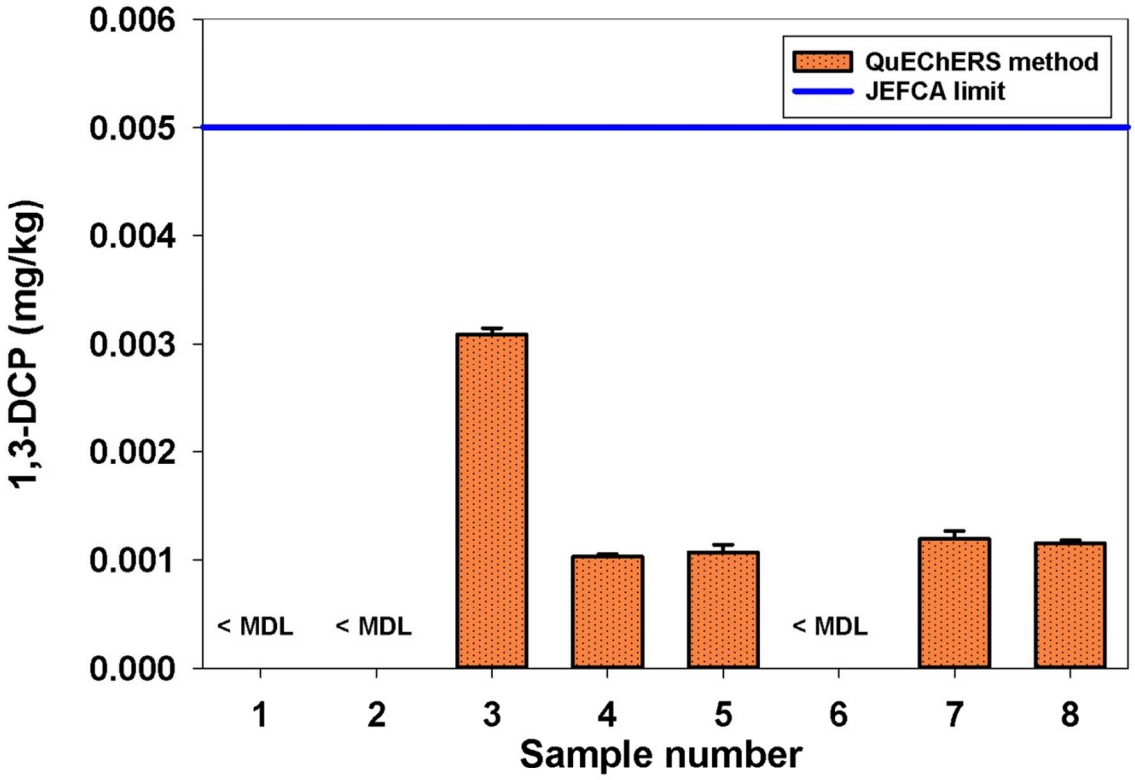
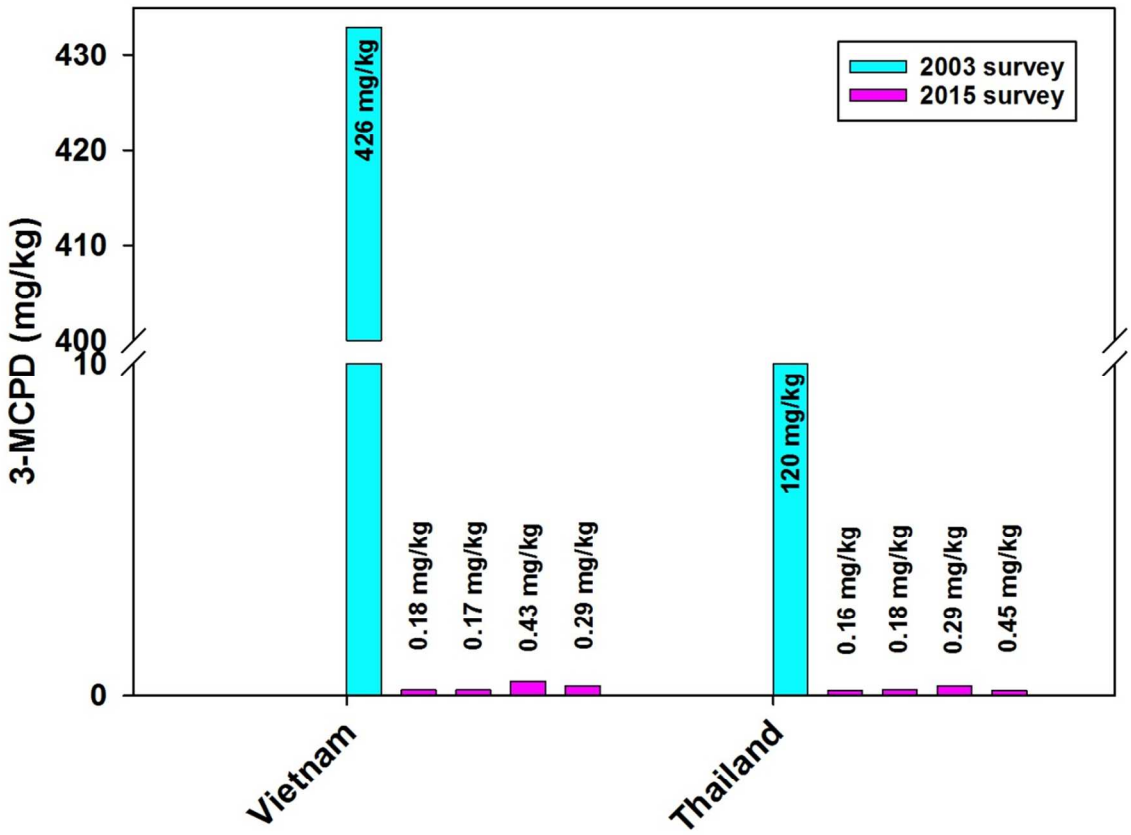

0.006
0.005
0.004
0.003
0.002
0.001
0.000
1,3-DCP (mg/kg)
QuEChERS method
JEFCA limit
< MDL
< MDL
< MDL
1
2
3
4
5
6
7
8
Sample number

Figure 5


3-MCPD (mg/kg)
430
420
410
400
10
0
2003 survey
2015 survey
426 mg/kg
0.18 mg/kg
0.17 mg/kg
0.43 mg/kg
0.29 mg/kg
120 mg/kg
0.16 mg/kg
0.18 mg/kg
0.29 mg/kg
0.45 mg/kg
Vietnam
Thailand

Graphic for table of contents