Postmarket Laboratory Surveillance for Forbidden Substances in Halal-Certified Foods in Thailand

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ABSTRACT

Limited information exists regarding adulteration of Halal-certified food by substances forbidden under Islamic law (Haram substances). This study was conducted using forensic laboratory testing to investigate the prevalence of this type of adulteration. In this large-scale survey of Halal-certified food products randomly collected from markets in Thailand, 4,829 food samples from 10 food groups were tested in the laboratory for four potentially Haram substances: porcine DNA, porcine fatty acids, ethanol, and hydroxyproline (gelatin). No samples were adulterated with porcine DNA or fatty acids. However, 62 samples (approximately 1.3%) were positive for ethanol (>0.5% for non-naturally fermented products and >1% for naturally fermented products). The hydroxyproline concentration in the samples was compared with that of a negative control. Gelatin, as indicated by the presence of hydroxyproline, was the major suspicious substance found in these products. Further investigations are required to determine whether the gelatin is of Halal origin. These results from this first large-scale postmarket surveillance of Halal-certified food products for forbidden substances reveals the important role of forensic laboratory testing for supporting Halal supervision and certification. These findings provide useful information for government agencies seeking to encourage Halal compliance by food enterprises and for Muslim consumers and Halal food importers and exporters.

HIGHLIGHTS

• A total of 4,829 Halal food samples were tested for four substances forbidden by Islamic law.
• Of the total analyzed samples, 1.3% were questionable and needed further investigation.
• Gelatin was the major suspicious substance with concerns for its Halal origin.
• Laboratory testing may play an important role in preserving the Halal integrity of foods.

Key words: Adulteration; Food protection; Food safety; Halal food product; Haram

The Muslim population worldwide has been steadily increasing, now estimated as 25% of the world population (17). As this population rapidly grows, the demand for Halal food and nonfood supplies is also rising. Muslims spent US $1.3 trillion in 2017 on food and beverages, and this spending is predicted to reach US$1.9 trillion by 2023, creating significant opportunities for investment and the creation of global Halal food brands (18). The global market potential for Halal food is not limited to Muslims; other consumers may also be interested in Halal food products. Hence, consumer awareness of the quality of Halal food products is encouraging many industries to propose Halal as an interesting target market in global commerce. According to Islamic principles, Muslims must consume only products defined as Halal, which means permissible and lawful. Products that are Haram, which means unlawful, must be avoided (13, 34). Although Halal-certified products are increasingly available, some of these food products may be adulterated with Haram substances.

Investment in Halal food production in developing countries and non-Muslim countries requires adopting complex and advanced food technology for competition in the global Halal market. However, new Halal entrepreneurs sometimes lack Halal awareness. Haram substances, including raw food materials and food additives, have been used in Halal food production. Generally, the major Haram substances that commonly adulterate Halal food products have pork- and/or ethanol-based ingredients, including lard oil or fat, ethanol, and porcine gelatin (3, 13). Many scientific methods have been developed to detect these adulterations using various advanced technologies such as PCR (10, 21, 25, 35), chromatography, mass spectrometry, and infrared spectroscopy (37). However, some analytical
techniques were unable to detect some Haram substances due to the lack of specific markers, e.g., for lard or pork fat. In most studies, the ability to detect Haram adulteration has been investigated with simulated or spiked food samples but not with food samples collected from the food production system (24). Large-scale market surveillance using laboratory testing for Haram-adulterated Halal food products might be beneficial for boosting the confidence of Muslim consumers. The Halal Science Center, Chulalongkorn University (HSC-CU; Bangkok, Thailand) established the Halal forensic laboratory for testing Halal products (28). HSC-CU is also developing a multilevel management system called HAL-Q (Halal assurance and liability quality) to apply good manufacturing practices and hazard analysis critical control point principles with a Halal focus. Small amounts of Haram substances that cannot be detected by vision, smell, or taste could be in contact with food products (13). The aim of this study was to apply the Halal forensic laboratory testing method developed by HSC-CU for screening real food samples for adulteration by the major Haram substances, including porcine DNA, porcine fatty acids, gelatin, and ethanol from natural and nonnatural fermentation. The study included randomly collected food products with a recognized Halal certification logo from Thailand and some countries in Asia and the United States.

MATERIALS AND METHODS

Chemicals and solvents. The porcine species-specific primer pair, 37-component fatty acid methyl esters (FAMEs) standard, acetyl chloride, isopropanol, 70% ethanol, and sulfuric acid were purchased from Sigma-Aldrich (St. Louis, MO). Hydroxyproline, chloramine-T reagent, dimethylaminobenzaldehyde, perchloric acid, potassium chloride, potassium carbonate, methanol, absolute ethanol, n-propanol, dichloromethane, and hexane were analytical grade and purchased from Merck (Darmstadt, Germany). Deionized water was produced with a GenPure Water Purification System (Thermo Scientific, Waltham, MA).

Food samples. From 2015 to 2016, 4,829 food products with a Halal certification logo or symbol on their packaging were randomly collected from supermarkets in Thailand. All collected food samples were categorized into 10 food groups according to the Codex Alimentarius Commission (12) general standard for food additives. These food groups were dairy products and their analogs, fats and oils, meat and meat products, cereals and cereal products, fish and fish products, seasonings and condiments, beverages, syrups, ready-to-eat savories, and confectioneries. Each food group and its food samples are listed in Table 1.

Halal forensic laboratory testing methods. (i) Detection of porcine DNA by real-time PCR. Samples were ground, and 20 mg was used for DNA extraction (Wizard Genomic DNA Purification Kit, Promega Madison, WI) (31, 36). The concentration and purity of extracted DNA was estimated with a spectrophotometer (NanoDrop 2000 UV-Vis, Thermo Fisher Scientific). Two microliters of extracted DNA (20 ng) was added to 18 μL of master mix (Light Cycler 480 SYBR Green I, Roche Diagnostics, Mannheim, Germany) with 0.25 μM concentrations of each porcine species-specific primer. A total of 20 μL of the final PCR mixture was added to the thermal cycler (Light Cycler 480, Roche Diagnostics). PCR amplification was conducted as follows: initial denaturation at 95°C for 10 min and then 50 cycles of denaturation at 95°C for 10 s, an annealing step, and an extension step at 68°C for 40 s. Porcine DNA and deionized water were used as the positive and negative controls, respectively. A sample that had the same melting temperature (83.78 to 84.70°C) as the PCR product was used as a positive control in accordance with the in-house method.

(ii) Detection of porcine fatty acids by GC. Fatty acids were profiled using the Lepage and Roy (22, 23) method with some modifications. A total of 3 g of each food sample was ground with a mortar and pestle and mixed with 9 mL of dichloromethane–methanol (2:1, v/v). After 1 h of incubation at room temperature (25°C), the mixture was filtered with filter paper (No. 1, Whatman, Clifton, NJ), and 0.1 M KCl was added to the filtrate. The solution was centrifuged at 1,000 × g for 10 min (Himac CF7D2, Hitachi, Tokyo, Japan), and the lower phase of the organic solvent was collected. For FAME preparation, 200 μL of lipid extract was mixed with 2 mL of methanol-hexane (4:1, v/v). A total of 200 μL of acetyl chloride was slowly added to the mixture and then heated at 100°C for 1 h. After cooling to room temperature, 5 mL of 6% potassium carbonate was added, and the solution was centrifuged at 250 × g for 5 min. The upper phase containing FAMES was collected for gas chromatography (GC) analysis.

The fatty acid analysis was performed on a gas chromatograph with flame ionization detector (FID) (GC-2010 Plus, Shimadzu, Kyoto, Japan). A DB-23 capillary column (30 m by 0.25 mm inside diameter with 0.25-μm film thickness; Agilent Technologies, Santa Clara, CA) was used. The injector temperature was 250°C with a split ratio of 50:1. For the column temperature program, the initial temperature was set at 80°C, ramped up by 10°C/min to 180°C, held for 15 min, increased to 220°C at 4°C/min, and held for 7 min. Helium was used as the carrier gas at 62.9 mL/min. Fatty acids were identified by comparison of their retention times with a standard and expressed as a percentage of the total identified peak area. For the detection of porcine fatty acid, fatty acid profiles were compared with those of pork fat. Those with profiles similar to that of pork fat were considered positive for porcine fatty acid: (i) a palmitic acid/oleic acid ratio (C16:0/C18:1) of <0.60 (unpublished data) and (ii) the presence of 11,14-eicosadienoic acid (C20:2).

(iii) Quantitative analysis of ethanol concentration by GC. A food sample in liquid form was mixed and then centrifuged at 1,000 × g for 5 min. After centrifugation, the clear supernatant was collected and diluted with 0.02% (v/v) n-propanol as an internal standard. The mixture was then filtered with a 0.45-μm pore-size syringe filter (Macherey-Nagel, Düren, Germany) before GC injection. The analysis of ethanol concentration was carried out with the GC-2010 Plus gas chromatograph with FID. Ethanol was separated on capillary columns (30 m by 0.32 mm by 1.00 μm; DB-WAXTER, Agilent Technologies). A sample solution (1 μL) was injected into the GC system with a split ratio of 20:1. The injector temperature was 250°C, and the column temperature was kept constant at 80°C for 5 min. Helium was used as the carrier gas at 40.0 mL/min. The peak area ratio of ethanol to n-propanol was used for quantitation, and the ethanol concentration was reported as a percentage (w/v). The ethanol percentages permitted for Halal foods were defined in accordance with the Islamic legal opinion (Fatwa) of the Sheikhul Islam of Thailand (27) as 1.0% (w/v) for naturally fermented products and 0.5% (w/v) for ethanol-added products. These percentages are in accordance with those used by Department of Islamic Development in Malaysia (JAKIM),
whereas the Islamic Food and Nutrition Council of America permits 0.1% ethanol in final products (6, 27, 30). Therefore, samples with an ethanol percentage higher than the permitted percentage were defined as unacceptable.

(iv) Quantitative analysis of hydroxyproline (gelatin concentration). Quantitative analysis of hydroxyproline concentration was conducted according to the AOAC official method 990.26 (4). A 2-g aliquot of sample was homogenized in a food processor. Gelatin hydrolysis was performed by adding 15 mL of sulfuric acid to the homogenate, and the mixture was incubated at 105°C in a hot air oven (FP400, Binder, Tuttlingen, Germany). After overnight incubation, the mixture was diluted with distilled water and then filtered using glass microfiber (No. 141, Ahlstrom Corp., Helsinki, Finland). The filtrate was mixed with 1 mL of chloramine-T solution and then 2 mL of the color reagent 4-dimethylaminobenzaldehyde. The mixture was then heated at 60°C for 15 min in a water bath. After cooling, the solution was transferred to a 96-well microplate, and absorbance was measured at 558 nm with a spectrophotometer (Multiskan GO UV-Vis, Thermo Fisher Scientific, Vantaa, Finland). The hydroxyproline concentration in the sample was quantified with a standard hydroxyproline 5-point calibration curve. Samples with hydroxyproline concentrations higher than that of the negative control were defined as positive samples.

Statistical analysis. Data are expressed as the mean ± standard deviation for the ratio of C16:0/C18:1 and hydroxyproline concentration and as the median (range) for ethanol concentration. The presence of porcine DNA, porcine fatty acids, gelatin, and ethanol in analyzed samples was recorded as the number of positive samples or percentage of adulteration as appropriate. All statistical calculations were performed using Excel 2013 (Microsoft, Redmond, WA) and Statistical Package for the Social Sciences version 15 (SPSS, IBM, Armonk, NY).

RESULTS

Adulteration of Halal food samples with porcine DNA. Figure 1 shows the real-time PCR amplification and melting curve results for the porcine DNA. A melting temperature of 83.78 to 84.70°C was used as one criterion for defining a porcine DNA–positive sample. A total of 1,811 food samples from seven food groups were screened for porcine DNA adulteration (Table 2). No porcine DNA adulteration was observed in any food sample or group.

Detection of porcine fatty acid in Halal food samples. A total of 427 food samples from seven food groups were analyzed for porcine fatty acid adulteration. Figure 2 shows the representative chromatogram of porcine fatty acids obtained from the GC analysis. C18:1 and C16:0 were the two major fatty acids found in pork fat; C20:2 was also found.

Most samples in the food groups analyzed had a mean C16:0/C18:1 ratio >0.6, and some had C20:2. However, no food sample met both criteria for the presence of porcine fatty acid (Table 3).

Ethanol concentrations in Halal food samples. Table 4 shows the ethanol concentrations in 1,569 food samples from three food groups. Approximately 1.0% of all food samples had a higher percentage of ethanol than the permitted percentage. The most unacceptable samples were from the seasonings and condiments group; approximately 5.0% of total samples in this group were unacceptable. The unacceptable samples were categorized into three subgroups based on ethanol percentage: 1.0 to 1.5% (five samples), 1.5 to 2.0% (two samples), and >2.0% (four samples). In the syrups group, 1.8% of the samples (all from the concentrated syrups) were unacceptable.

No unacceptable samples were found in the beverages group. The vinegar subgroup had the highest median ethanol percentage, followed by soy sauces. The lowest median percentage was found in the fruit juices subgroup.

Presence of gelatin as estimated by hydroxyproline concentration in Halal food samples. Table 5 presents the number of gelatin-positive samples and the mean hydroxyproline concentration for 1,117 samples of Halal-certified foods with or without gelatin listed on the label. As expected, high hydroxyproline concentrations were found in the samples with gelatin on the label. However, 4.6% of the total analyzed samples that were positive for hydroxyproline test were from the group without gelatin on the label. Some of these positive samples contained meat as a major ingredient, and these meats should be further investigated to determine whether they meet Halal criteria. Among the food groups, the highest percentage of gelatin-positive samples was found in ready-to-eat savories followed by beverages.
seasonings, dairy products, and confectioneries. The mean hydroxyproline concentration was highest in samples of ready-to-eat savories.

**DISCUSSION**

This survey is the first large-scale study designed to support consumer awareness and protection and to determine the risk of Haram contamination among foods manufactured in non-Muslim countries. From 2015 to 2016, a total of 4,829 Halal-certified food samples from 10 food groups were laboratory tested for substances forbidden by Islamic law. The 62 unacceptable or suspicious samples accounted for approximately 1.3% of the total analyzed food samples. Among the four Haram substances the samples were tested for, the one most commonly found substance in the unacceptable samples was gelatin, as estimated by hydroxyproline concentration.

According to Islamic principles, pork is strictly prohibited, and Halal food must be free from any pork or pork derivatives. Testing for porcine DNA has been recognized as the best indicator of pork adulteration due to high specificity, sensitivity, accuracy, stability, and ease of testing (1, 42). In this study, real-time PCR was used to detect porcine DNA. Among 1,811 food samples analyzed, none were adulterated with pork. To the best of our knowledge, information regarding the prevalence of pork adulteration in Halal-certified food products has not been reported. Most existing studies have addressed meat adulteration and mislabeling. One small-scale study done in Saudi Arabia revealed that 9 (12%) of 75 commercial

<table>
<thead>
<tr>
<th>Food group</th>
<th>n</th>
<th>No. of samples positive for porcine DNA</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dairy products</td>
<td>33</td>
<td>0</td>
</tr>
<tr>
<td>Meat and meat products</td>
<td>119</td>
<td>0</td>
</tr>
<tr>
<td>Fish and fish products</td>
<td>325</td>
<td>0</td>
</tr>
<tr>
<td>Seasonings</td>
<td>333</td>
<td>0</td>
</tr>
<tr>
<td>Beverages</td>
<td>99</td>
<td>0</td>
</tr>
<tr>
<td>Ready-to-eat savories</td>
<td>847</td>
<td>0</td>
</tr>
<tr>
<td>Confectioneries</td>
<td>55</td>
<td>0</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td>1,811</td>
<td>0</td>
</tr>
</tbody>
</table>

**FIGURE 1.** Porcine DNA results for Halal food samples with positive and negative controls. (A) Real-time PCR amplification curve. (B) Melting curve with specific peak for porcine DNA.
food samples were PCR-positive for porcine DNA (2). In another study, 7 (11.3%) of 62 sausage samples were contaminated with pork (26).

Pork fat is another major concern with regard to adulteration of Halal food products. Because no specific fatty acid markers are available for pork fat adulteration, the evaluation was conducted based on existing parameters from previous studies. Eicosadienoic acid (C20:2) has been reported as a potential marker for lard adulteration (11, 14, 19, 39). However, small amounts of C20:2 have also been found in other animal fats, e.g., cod liver oil (11). In the present study, C20:2 was detected in canned sardines and mackerels, consistent with those previous findings. Apart from C20:2, the fatty acids C16:0, C18:0, and C18:1 also differ significantly in cocoa butter adulterated with lard. The lowest lard concentration for which all analyzed fatty acids in a sample were significantly different from those in unadulterated cocoa butter was 10% (7). Recently, fatty acid ratios have become potential parameters for indicating animal fat adulteration, including that from lard (33). In the present study, the C16:0/18:1 ratio was used as a marker, which has been significantly correlated with lard adulteration. A ratio of <0.6 significantly indicated that 20% of palm oil was adulterated with lard (unpublished data). When applying both parameters, the C16:0/18:1 ratio and the presence of C20:2, none of the samples in the present study were adulterated with pork fat or lard.

The acceptable concentration of ethanol for Halal food certification depends on the ethanol source and differs among countries (30). In Thailand, two ethanol percentages are used: 0.5% for a non–naturally fermented product and 1.0% for a naturally fermented product. In the present study, food samples from the seasonings group contained high percentages of ethanol. The subgroup of soy sauces had the median value of 0.112% (w/v) ethanol and constituted 3.7% of those samples with an ethanol concentration >1.0%. In another study of ethanol in soy sauces in which GC-FID and an electronic nose were used, similar results were obtained (29): 1 of 24 sauce samples had an ethanol concentration >1.0%. Ethanol in soy sauce production is used for

![FIGURE 2. Fatty acid profile of pork fat analyzed by gas chromatography.](image-url)

### TABLE 3. Porcine fatty acid adulteration in collected Halal-certified food samples

<table>
<thead>
<tr>
<th>Food group</th>
<th>n</th>
<th>C16:0/C18:1 (mean ± SD)</th>
<th>C20:2 (no. of samples)</th>
<th>No. of positive samples a</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dairy products</td>
<td>23</td>
<td>1.5 ± 0.8</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Fats and oils</td>
<td>15</td>
<td>0.9 ± 1.0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Cereals and cereal products</td>
<td>29</td>
<td>0.6 ± 0.4</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Fish and fish products</td>
<td>40</td>
<td>2.1 ± 2.2</td>
<td>4</td>
<td>0</td>
</tr>
<tr>
<td>Beverages</td>
<td>135</td>
<td>5.5 ± 8.6</td>
<td>1</td>
<td>0</td>
</tr>
<tr>
<td>Ready-to-eat savories</td>
<td>146</td>
<td>1.2 ± 0.8</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Confectioneries</td>
<td>39</td>
<td>1.1 ± 1.1</td>
<td>1</td>
<td>0</td>
</tr>
<tr>
<td>Total</td>
<td>427</td>
<td></td>
<td></td>
<td>6</td>
</tr>
</tbody>
</table>

a Criteria for porcine fatty acid adulteration: the ratio of C16:0/C18:1 and the presence of C20:2.

b Positive sample defined as C16:0/C18:1 < 0.6 and the presence of C20:2 fatty acid. The C16:0/C18:1 ratios for pure lard, chicken fat, and beef fat were 0.43, 0.43, and 0.53, respectively. C20:2 was detected only in lard.
stabilizing the quality and adding flavor and is generally found at various concentrations in soy sauces (20). The higher ethanol concentrations in some soy sauce products may be due to continued enzymatic activity during long-term storage (29). Similar to soy sauces, approximately 4% of all the concentrated syrup samples in the present study had a higher percentage of ethanol than permitted for Halal products. Among these samples, the highest percentage was detected in an imported product with a foreign Halal certification; other samples had only slightly higher percentages of ethanol than allowed (0.55 ± 0.07%). These slightly higher percentages may be due to batch-to-batch variation in the products. If the ethanol percentage were outside the acceptable range, the Halal status of such products would be under consideration by the Central Islamic Committee of Thailand (CICOT).

Gelatin is a commonly used ingredient with many applications in the food and pharmaceutical industries (32) and is generally produced from the skin and bones from pigs and cattle. Data from gelatin manufacturers in Europe indicate that the major source of edible gelatin is pork skin (80% of gelatin products) (40). This is in agreement with data from the Gelatin Manufacturers Institute of America indicating that pork skin is a significant raw material source of edible gelatin in the United States (16). According to Islamic principle, gelatin derived from pig skin, bones, or any other parts is strictly prohibited. However, gelatin derived from beef or other meats is permitted when the raw materials used for its production are from Halal animals with compliant slaughtering procedures. Some food products may contain gelatin derived from a subcomponent of the other materials used as the ingredient. Thus, the Halal status of these products is doubtful and must be confirmed. In this study, 1,117 of Halal-certified food samples with or without gelatin on the label were screened for gelatin, as estimated by hydroxyproline concentration. As expected, products with gelatin on the label were positive for hydroxyproline. The gelatin used in such products had Halal certificates issued by recognized Halal certification bodies. However, 47 Halal-certified food samples without gelatin on the label also were positive for hydroxyproline. Among these samples, 23.4% were imported food products with a foreign Halal certification.

The remaining samples with positive hydroxyproline results were from the ready-to-eat savories group, mainly fish-based products. In general, hydroxyproline is one of the

### TABLE 4. Number of unacceptable samples and ethanol concentration in Halal-certified food samples

<table>
<thead>
<tr>
<th>Food group</th>
<th>No. of samples</th>
<th>Ethanol not detected</th>
<th>Unacceptable (range)</th>
<th>Ethanol concn (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Seasonings</td>
<td>221</td>
<td>157</td>
<td>64</td>
<td>0.136 (0.007–0.305)</td>
</tr>
<tr>
<td>Vinegars</td>
<td>65</td>
<td>2</td>
<td>6 (1.755–3.050)</td>
<td>0.112 (0.010–1.383)</td>
</tr>
<tr>
<td>Soy sauces</td>
<td>135</td>
<td>2</td>
<td>5 (1.060–1.383)</td>
<td>0.112 (0.010–1.383)</td>
</tr>
<tr>
<td>Other sauces</td>
<td>21</td>
<td>0</td>
<td>0</td>
<td>0.040 (0.006–0.241)</td>
</tr>
<tr>
<td>Beverages</td>
<td>1,130</td>
<td>975</td>
<td>15</td>
<td>0.026 (0.004–0.305)</td>
</tr>
<tr>
<td>Fruit juices</td>
<td>797</td>
<td>217</td>
<td>0</td>
<td>0.016 (0.004–0.191)</td>
</tr>
<tr>
<td>Energy drink</td>
<td>101</td>
<td>101</td>
<td>0</td>
<td>0.033 (0.004–0.335)</td>
</tr>
<tr>
<td>Syrups</td>
<td>218</td>
<td>355</td>
<td>4 (0.502–1.190)</td>
<td>0.019 (0.004–1.190)</td>
</tr>
<tr>
<td>Concentrated syrup</td>
<td>101</td>
<td>31</td>
<td>4 (0.502–1.190)</td>
<td>0.019 (0.004–1.190)</td>
</tr>
<tr>
<td>Syrup from canned fruit</td>
<td>117</td>
<td>2</td>
<td>0</td>
<td>0.035 (0.004–0.114)</td>
</tr>
<tr>
<td>Total</td>
<td>1,569</td>
<td>952</td>
<td>47</td>
<td>0.019 (0.004–1.190)</td>
</tr>
</tbody>
</table>

a Unacceptable sample was defined as ethanol concentration >0.5% (w/v) in non-naturally fermented products (fruit juices, energy drink, and all syrups) or >1.0% (w/v) in naturally fermented products (vines and all sauces).

b Median (range) for all samples containing ethanol.

### TABLE 5. Gelatin-positive samples and hydroxyproline concentrations in collected Halal-certified food samples

<table>
<thead>
<tr>
<th>Food group</th>
<th>No. of samples</th>
<th>Hydroxyproline concn (mg/mL)</th>
<th>No. of samples</th>
<th>Hydroxyproline concn (mg/mL)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dairy products</td>
<td>67</td>
<td>0.012 ± 0.002</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Seasonings</td>
<td>100</td>
<td>0.014 ± 0.003</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Beverages</td>
<td>402</td>
<td>0.012 ± 0.001</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Ready-to-eat savories</td>
<td>442</td>
<td>0.025 ± 0.017</td>
<td>0</td>
<td>0.288 ± 0.223</td>
</tr>
<tr>
<td>Confectioneries</td>
<td>106</td>
<td>0.0123</td>
<td>0</td>
<td>0.288 ± 0.223</td>
</tr>
<tr>
<td>Total</td>
<td>1,117</td>
<td>0.019 ± 0.014</td>
<td>0</td>
<td>0.288 ± 0.223</td>
</tr>
</tbody>
</table>

a Values are means ± standard deviations of extract solution for total positive samples except confectioneries.
b Sample with higher concentration of hydroxyproline than that of the negative control.
major amino acids found in collagen; marine products such as fish have collagen similar to that of bovine or porcine animals (8, 9). Therefore, the positive hydroxyproline results for the fish-based products could have come from collagen. Among dairy products, seasonings, and beverages, gelatin was commonly used as a stabilizing, bonding, or clarifying agent (38, 41). In this study, laboratory testing revealed the presence of hydroxyproline in some products in these groups; however, these products could not be defined as prohibited because further investigation is needed to determine whether the gelatin was from Halal sources. In a previous study, the major source of gelatin in analyzed candies was pork; however, pork-derived gelatin was not detected in candies with Halal certifications (15). Therefore, laboratory testing and official certification of Halal gelatin are needed to confirm the Halal status of gelatin-containing products. To increase Muslim consumer confidence in the Halal status of gelatin-containing products, consumers are advised to choose products with a Halal certification logo or symbol on the label.

In this study, a large-scale postmarket survey was conducted to uncover forbidden substances in Halal-certified food products. Halal-certified food samples from various food groups underwent laboratory screening for four major substances deemed forbidden or suspicious by Islamic law. Among the 4,829 food samples, 62 (1.3%) suspicious or unacceptable samples were found. Gelatin was the major suspicious substance found in these products. Further investigation is needed to determine the Halal status of the gelatin source.

In conclusion, to our knowledge this is the first report using laboratory testing for the large-scale surveillance of forbidden substances in Halal-certified food products. These results confirm the important role of laboratory testing in maintaining Halal integrity, which is an increasingly important part of consumer protection. The presence of Haram substances (substances forbidden by Islamic law) is one of the most common hazards that should be monitored in Halal food production. Apart from physical, chemical, and biological hazards, Haram or non-Halal ingredients are of great concern for Muslim consumers in terms of spiritual food safety (3). The findings of this study provide useful information for government and private sector stakeholders to increase their awareness of the principles of Halal and to encourage compliance with Halal practices in food production. These findings should contribute to increased confidence of Muslim consumers and Halal food entrepreneurs in Thailand, which should be operating under the approach of religious compliance and scientific support. This approach aims to apply science and technology to Halal food production systems and to provide scientific data to the Islamic organizations involved in the Halal certification process (5).

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