Handbook of Halal Food Production

Mian N. Riaz, Muhammad M. Chaudry

Testing Non-Halal Materials

Publication details
Winai Dahlan, Mian N. Riaz, Munir M. Chaudry
Published online on: 04 Sep 2018


PLEASE SCROLL DOWN FOR DOCUMENT

Full terms and conditions of use: https://www.routledgehandbooks.com/legal-notices/terms

This Document PDF may be used for research, teaching and private study purposes. Any substantial or systematic reproductions, re-distribution, re-selling, loan or sub-licensing, systematic supply or distribution in any form to anyone is expressly forbidden.

The publisher does not give any warranty express or implied or make any representation that the contents will be complete or accurate or up to date. The publisher shall not be liable for an loss, actions, claims, proceedings, demand or costs or damages whatsoever or howsoever caused arising directly or indirectly in connection with or arising out of the use of this material.
27 Testing Non-Halal Materials

Winai Dahlan, Mian N. Riaz, and Munir M. Chaudry

CONTENTS

The Halal Forensic Science Laboratory ................................................................. 291
Testing of Gelatin ................................................................................................... 292
  Introduction ....................................................................................................... 292
Presence of Alcohol ............................................................................................... 292
  Introduction ....................................................................................................... 292
Fatty Acid Composition ......................................................................................... 293
  Introduction ....................................................................................................... 293
DNA for Species Identification .............................................................................. 294
  Detection of Porcine DNA in Food Products .................................................... 294
References .............................................................................................................. 296
Gelatin .................................................................................................................... 297
Alcohol ................................................................................................................... 297
Fatty Acids ............................................................................................................. 297
DNA .................................................................................................................. 297

THE HALAL FORENSIC SCIENCE LABORATORY

Nowadays, raw materials used for producing halal food could be contaminated with najis (filth), mashbooh, or haram substances at any point in the food chain, either intentionally or accidentally. When small amounts of haram substances are in contact with food products, these cannot be detected with the naked eyes, or by smell or taste (Syahariza et al., 2005). This is where laboratory testing can strengthen halal compliance. Laboratory analysis will also support the preparation of a more powerful raw materials database. Of the 348 E-numbers, 92% are raw materials that are widely used by the halal food industry. Only 59%, however, are considered halal by all authorities. The remaining 33% of E-numbers (or 106 compounds) either are mashbooh or can be obtained from both halal and haram sources.

The testing procedures will depend on what are the concerns, for example, porcine DNA, source of proteins, source of the lipids, ethyl alcohol, low molecular weight compounds, or hormones (Cordella et al., 2002). Four non-halal materials, that is, animal gelatin, fatty acids, ethyl alcohol, and porcine DNA will be described in some detail.
TESTING OF GELATIN

INTRODUCTION

Gelatin, the partially hydrolyzed collagen tissue of various animal parts, is a multifunctional ingredient widely used in many food products. The crucial point for halal scientific laboratory analysis is that gelatin can be derived from halal or haram sources. There are two main types of gelatin. Type A gelatin is made exclusively from pork skins and is haram. Type B gelatin is made either from cattle and calf skins or demineralized cattle bones and is halal unless the animal slaughtering process did not comply with Islamic regulation. Fish skins are an alternative source of gelatin, which is halal for all Muslims if the fish are in category 1 (Chapter 10). Gelatin is deficient in tryptophan and has a small amount of methionine. It also has the unique amino acid hydroxyproline found almost exclusively in gelatin/collagen.

There are two general approaches used to quantitate gelatin in food products (Shabani et al., 2015). First, the picric acid test is used to detect gelatin in milk and can also be used with sour, fermented, cultured, or very old samples of milk, cream, or buttermilk. Precipitates produced using picric acid in the absence of gelatin flocculate, separate readily and do not adhere to the walls of a container and are easily removed by rinsing with water. When gelatin is present, the precipitate will remain in suspension long after the flocculent has settled, but on standing overnight, the characteristic sticky deposits will be found adhering to the bottom and sides of the test vessel (Hermanto and Fatimah, 2013).

Second, the Woessner hydroxylproline assay can be used both qualitatively and quantitatively. The hydrolysis of products with acid, oxidizing agent, and colorimetric reagents gives a red purple color in the solution when gelatin is the present. However, neither of these tests can identify which animal the gelatin was obtained from. Thus, future work will need to be done to attempt to do species identification (AOAC, 1996a). Given that gelatin is initially a hydrolysate and that many laboratories are studying the use of materials that are further hydrolyzed, this will remain a difficult problem. However, the presence of gelatin, particularly if not indicated on the ingredient statement, that is, used as a processing aid such as in clarifying juices, may alert a halal certifying agency that it needs to do more inspection of the ingredients and the process (Figure 27.1).

PRESENCE OF ALCOHOL

INTRODUCTION

Alcohol is haram. The FAO/WHO Codex Alimentarius regulations for food labeling on the use of the term “halal” mentions that producers should avoid using liquor in their products. Alcohol, that is, ethyl alcohol or ethanol, has the chemical formula of CH₃CH₂OH. Alcohols are a class of chemicals but only ethanol is of concern with respect to halal. The concentration of ethyl alcohol in various liquors can vary generally from 3.5% to 40% ethanol. The “proof” of alcoholic beverages is double the percentage alcohol. Ethanol is produced by yeast fermentation of sugar. Often the starch must first be broken down to sugar. This can be done by various microbes. However, in some foods containing sugar and other carbohydrates such as fruit juice, natural fermentations such as soy sauce, or other sauces, ethanol in small amounts may be unavoidable (AOAC, 1996b). Vinegar,
although normally prepared from ethanol is halal as almost all of the ethanol has been converted almost completely to acetic acid. Low amounts, that is, less than 0.5% may remain. This level of natural ethanol is not considered harmful to the body so that less than that amount is allowed in most countries. In addition to its presence in food, ethanol is used as a disinfectant, both to sanitize the workers’ hands, and utensil and equipment surfaces. Ethanol (70%; v/v) used for this purpose is allowed.

If the alcohol is obtained from a liquor, even one drop will not be accepted in halal food products. But synthesized or extracted ethanol (industrial alcohol) used as a solvent for dissolving flavor or color before adding into the food products can be acceptable. IFANCA only permits 0.1% in final product with 0.5% in any single ingredient. Natural alcohol between 0.1% and 0.5% is acceptable. When testing for alcohol content, it is important to know something about the product being tested and also to understand the standards of the different importing countries. At this time, there is no single standard (FAO/WHO, 2001; TACFS, 2007; Yarita et al., 2002).

The current preferred test for ethyl alcohol uses gas chromatography with a flame ionization detector. The area of the peak in the proper place for alcohol would be quantitated using a calibration curve that is grounded with a carefully measured sample of n-propanol that serves as the internal standard. Because one is looking at low levels of alcohol, sample handling and good analytical technique are needed to be comfortable before “accusing” a sample of being above the required level of the halal certifying agency (ASEAN, 2005; Marikkar et al., 2005) (Figure 27.2).

**FATTY ACID COMPOSITION**

**Introduction**

Triglycerides (TG) are the main constituents of vegetable oils and animal fats. They are also referred to as triacylglycerol (TAG), that is, a chemical compound formed by one molecule of glycerol and three molecules of fatty acids.
The fatty acids esterified to the glycerol backbone usually have carbon chains of 4 to more than 20 carbon atoms. In addition, the fatty acid can also contain one or more double bonds at specific positions leading to unsaturated and polyunsaturated fatty acids.

Animal fats tend to have a larger proportion of long chain saturated fatty acids, so they are solid at room temperature. In contrast, fats from plant sources contain a higher proportion of unsaturated fatty acids and are usually liquid at room temperature (Ensminger et al., 1994).

The fatty acids are commonly analyzed using gas chromatography. The separation of fatty acids depends on the difference in their boiling point. The TG are chemically hydrolyzed to free fatty acids, which are separated from the glycerol, and then normally converted to fatty acid methyl esters (FAME). The mixture of FAME is extracted using the organic solvent hexane prior to separation in the gas chromatograph. Methyl pentadecanoic acid (C15:0) can be added as an internal standard (Kowalski, 1989).

The proportion of the different fatty acids are characteristic of their source. However, they do change within a single species depending on diet, overall health, and age of the animal or plant. However, the proportion of fatty acid in animals and plants are quite difference, so the test can generally identify whether the fat is from animals or plants. Sometimes, one can also determine the actual species, but this becomes much harder if the sample contains a mixture of sources in a large variety of ratios. So at this time, it can be used to determine if there are potential problems but again cannot replace on-site inspection and chain-of-custody records (Figure 27.3).

DNA FOR SPECIES IDENTIFICATION

DETECTION OF PORCINE DNA IN FOOD PRODUCTS

Every living organism has a unique DNA and it can in practice be used for species identification. The most important use of this technique for halal foods is to
Testing Non-Halal Materials

determine the presence of DNA from non-halal species. It cannot be used to determine if the products of a halal animal came from an animal that was or was not slaughtered according to halal requirements. The most important species of concern is the pig (Man and Mirghani, 2001; Man et al., 2005).

The polymerase chain reaction (PCR) is a relatively efficient method with high specificity and high sensitivity. The initial DNA sample will be amplified (i.e., copied to make the concentration greater so that it is detectable and analyzable). The key to this method for halal is to have a piece of DNA that is absolutely unique to the pig as the DNA template that must be amplified. However, this is a requirement that can never actually be reached with 100% certainty. In addition, other materials are needed so that the amplification reaction is possible, that is, more strands of the DNA target are produced (Aida et al., 2005).

The DNA of interest will be amplified continuously for a total of 30 to 50 cycles leading to millions of copies. These are then normally observed using gel electrophoresis that can be difficult to quantitate but which clearly shows the presence of the amplified gene in the appropriate position on the gel.

The PCR technique has been used to detect pork in the presence of other meats in both raw and cooked products. Obviously, any purified material, for example, lard, which does not have any DNA, cannot be tested using this method. In conclusion, the PCR technique is very useful for detecting contamination with pig products, although there have been some cases of false positives, that is, the test is positive, but the product is unlikely to have been contaminated. Careful laboratory technique and confirmatory testing should be used before a company’s reputation is harmed.

A further development has been the development of “real-time” PCR, which allows the initial amount of DNA (or cDNA or RNA depending on the primer) to be quantitatively measured. It is based on the measurement of the fluorescence produced by a “reporter” molecule that increases as the reaction proceeds so that the initial amount of DNA can be obtained by extrapolation back to the sample. The
fluorescent compounds include material such as SYBR® Green, which is non-specific and binds to any DNA or sequence specific probes such as Molecular Beacons, TaqMan®, FRET Hybridization Probes, and Scorpion® Primers.

Real-time PCR is now replacing classical PCR and is being automated. Using reverse transcription, the less stable RNA molecules can be converted to the more stable DNA analog. Further developments in DNA/RNA detection for the benefit of halal compliance testing can be expected (Figure 27.4).

REFERENCES


GELATIN


ALCOHOL


FATTY ACIDS


DNA