Chapter

Applications of Mass Spectrometry to the Analysis of Adulterated Food

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Abstract

Food quality and safety are the major issues in food industry around the world. With the abundance of processed food with long supply chain in the market, food fraud is always a concern. Food fraud is defined as modification of an actual labeling of food chemicals in which expensive, less accessible original ingredients are replaced by lower cost and more accessible alternatives, which is also known as food adulteration. Some of these food adulterations might only affect the public mass financially, but some adulteration might affect others more seriously. Various food authentication techniques can be utilized to ensure safety and quality of food products adhering to the standards, such as DNA-based techniques with polymerase chain reaction, vibrational spectroscopy, electronic nose, and mass spectrophotometry, which has been used widely to estimate pharmaceutical and biological samples. However, most of these techniques still require substantial sample preparation or some have very high sensitivity to adulterants and are prone to give undefined results. Complex mixtures of food adulterants can be identified using very high resolution mass spectroscopy. The chemical compounds and structure of natural and mixtures of the adulterants are examined in this chapter using advanced mass spectroscopy technique and gas chromatography time-of-flight mass spectroscopy to identify the lard biomarker.

Keywords: mass spectrometry, gas chromatography time-of-flight mass spectrometry, adulterated food, lawful food, mixture food

1. Introduction

Food authentication is a major concern in food industry around the world and significantly affects the global food market. Food fraud as defined in [1] which is alteration of the true labeling of food ingredients by substituting with cheaper and more accessible alternative could affect not only serious consequences to the human health, such as food poisoning and food allergy [2–4] but also loss trust in the confidence of food quality related to the product, company reputation, and religion views [5, 6], which consequently disturbing the global market. Halal and kosher food that are diet intake restrictions are laws for Muslims and Jews religion groups for daily food consumption and have big world market. The global halal market itself worth about $7.049 Billion in 2015, and the analyst projects the market to grow

to $1.9 Trillion by 2021 [7]. The continuous growth of such market can only happen when consumers' confidence and trust in the halal labels of the food industry are always maintained and preserved [7–9].

Food fraud as also known as food adulteration is not a recent issue where some of the fraud have been reported earlier, such as adulteration of formula milk with melamine [10–12], mixing of vehicle oil in oil for human consumption in Spain [13], and addition of sawdust to make white bread [10, 14]. The incident of 2008 affected thousands of babies when their milk powders were adulterated [15]. Another incident following that was meat adulteration when prohibited substances were added to the food [16]. The concern of food quality and safety becomes a major priority of both government ministers and the public due to potential financial loss to the state income and increase consumers' health risks that resulted from breaching the food standards.

The food adulteration related to halal and kosher laws is defined as alteration of the original food with pork and its derivatives, such as blood, fat, etc. Lard is a generic ingredient, which is commonly used as a food flavor, mixture, and fat-based blend. Lard has also been reported to be used as an alternative ingredient for adulteration and as a substitute for food-cooking oils, such as butter or margarine. Due to their belief, Muslim communities and Orthodox Jews followers are prohibited to consume lard. Mass spectroscopy (MS) can be used to provide structural details and molecular weight of compounds. Advances of different techniques of MS have emerged significantly. Such advanced techniques utilizing either high resolution mass spectroscopy, that is, GC-MS, or high performance mass spectroscopy, that is, LC-MS, are able to detect more complex compounds with higher accurate identification [17, 18]. Several developments in mass spectrometry for the analysis of the food adulteration have been reported and shown in **Figure 1**.

As shown in **Figure 1**, many food adulterations have been studied in various methods of mass spectroscopy, mainly GC and LC using rapid evaporation ionization spectrometry (REIMS) technique developed initially by Takats et al. [19]. REIMS uses an electrosurgical apparatus that generates surgical smoke after interacting with a solid sample creating ionization and desorption of molecules. Currently, REIMS-based mass spectroscopy has been widely reported for the study of food adulteration, especially for fish and meat adulteration [20, 21]. Another emerging technique is GC-TOF MS mass analyzer for the investigation of a vast number of organic impurities and residues present at the low levels for food quality and safety, surrounding environment, and biological applications [22, 23]. In the analysis of food quality and safety, GC-TOF MS has been utilized to the analysis of in animal-based food origin, such as dioxin-type micro pollutants [24] for the environmental analysis, and GC/GC-TOF MS with negative ionization has been utilized in sediment and fish samples to profile short- and medium-chain

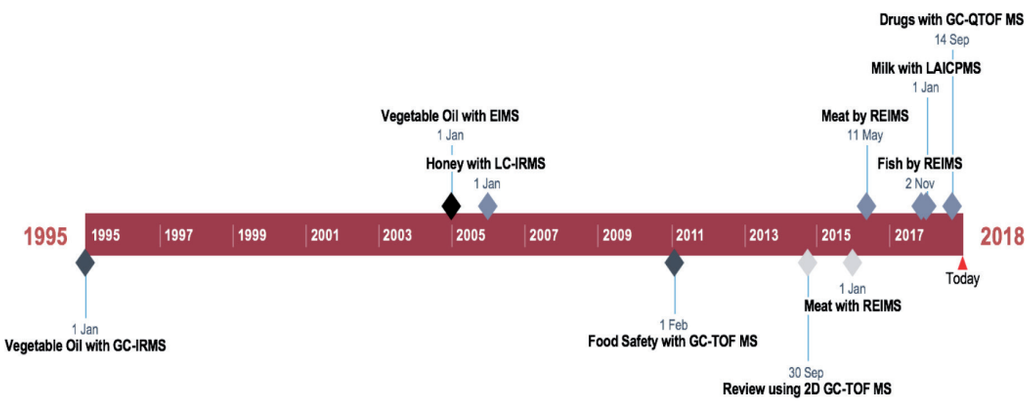

Figure 1.
Some reported work on mass spectrometry development for the investigation of food adulteration.

chlorinated paraffin [25]. In a recent report related to drug-testing investigations, this high-performance mass spectroscopy, that is, GC-TOF MS, is able to analyze doping substances [26].

The purpose of this chapter is to describe advanced mass spectroscopy applications, especially GC-TOF MS technique on investigating food adulteration on pure and mixed meat, covering pig fat, chicken, lab, and cow and to identify the possibility of recognizing a biomarker for lard chemical.

2. Food adulteration

Research communities around the world have been continually working on the food adulteration [27]. Water is a simplest and common food adulterant, especially for milk. Water mixing in milk could degrade the nutritional content, change the taste, and modify the color of milk. Other potentially dangerous adulterants, that is, melamine might be added to replicate natural milk, which seriously increase the health risk [28]. Melamine was used to increase the viscosity of the milk and to keep the composition of fat and carbohydrate to be the same as the original. Such milk adulterant had been reported to cause severe health problems, especially to the infants and young-aged children and created an unusual health outbreak in China in 2008. In some cases, expensive milk is often mixed with the cheaper milk. Reported by Calvano et al. [29], milk from unordinary animals, such as buffalo, camel, and yak, was mixed with ordinary animals, such goat or cow milk. For consumers who are very sensitive to certain types of milk, this kind of food adulteration could trigger in them very serious health problem [30].

2.1 Lawful food

It is a compulsory for most religion followers to follow specific compliances in their daily dietary meals. Such laws, for instance in Islam belief, are to avoid some foods in their dietary consumption, which contain pig meat and its derivatives like ham, bacon, sausages, pork, and lard, except in very rare situations. This requirement is referred to Halal food.

Halal food industry is currently growing significantly and is estimated to reach 20% of global food trade market as world population will consist of 30% Muslim followers by the year of 2025 [31]. Other religions also have defined dietary law; for example, Judaism has kashrut for the Jewish to follow, which also forbids the consumption of pig meat and its by-products [32]. For Hindu religion followers, the consumption of beef and its derivative is not permissible [33]. Many food manufacturers violate the requirement not to practice food fraud that is mostly due to cheap substitute materials.

Muslims and Jews are some of those religious groups that require diet intake restrictions, as they adhere to halal and kosher laws [34, 35], respectively. Although halal and kosher laws have similarities, that is, forbidding consumption of pork and derivatives, blood, etc., they have differences, such as kosher does not forbid alcohol and kosher forbids consumptions of animals that do not chew cud and have cloves, etc. [34]. Although halal and kosher are different, both laws severely forbid the consumption of pork and its derivatives such as lard [34]. Lard is pig fat derived from its adipose tissue and is often used in food production as an emulsion, shortening, or as a substitute to butter, margarine, or cooking oils. The identification of non-halal meat due to lard adulteration is of high significance. Despite many reported work that have been performed to investigate the fingerprint for non-compliance of halal food, such as lard or pig meat [6, 34, 35], the identification of biomarker for non-halal food is still in the early stages.

2.2 Mixed food

A notorious big scandal that hit Europe in 2013 related to food adulteration was the breach of true labeling due to the fraud on the beef sale that has been substituted with horsemeat [36]. The food fraud also occurred in some other part of the world when pharmaceutical preparations and chocolate were suspected to contain traces of pork in 2013 and 2014 in Malaysia [37]. In other countries, like India, it is not uncommon to sell buffalo meat adulterated with other animal meats due to financial issue and availability [38]. Such adulterated meats are very difficult to identify especially when such meats are already in the processed form. The practice of food fraud also occurs on dairy products, for example, butter is mixed with cheaper fats, such as mutton fats, chicken, and pig fats to get higher profits [39]. With these many occurrences of food adulterations around the world, ability to authenticate pure and mixed food has become a crucial aim for everybody.

2.3 Food safety and quality

Food adulteration practices not only destroy consumer trust and confidence in the products and the company reputation but also jeopardize the safety and quality of food consumed. The development of food authentication technique is necessary in food control because of the need of certain compliance in food process and the label to ensure customer confidence and trust to the food product [35, 40]. The authentication technique will also validate the food origin that includes its geographical, gene, and species source, confirming their production processes and their processing techniques [41–43].

The need for food authentication is the result from customer concerns on the food nutrition and their health as well as an assurance of the process control and food quality purposes. Such authentication techniques will also confirm the existence of food adulteration, identify the origin of the food and its ingredients, and improve the food quality and safety for pure and future mixed food.

For this purpose, mass spectroscopy has been very critical in validating and improving food quality and making us caution with any industrial and agriculture chemical to prevent harming our health, disturbing the food supply, and damaging the ecosystem that we depend on for our sustainability. The scientific finding in the environmental, agricultural, and food sciences has been significant to more resourceful and healthier food, improving our quality of life and better living in the world population that is reaching 8 billion and beyond.

3. Food authentication detection

There are several methods that can be used in food authentication process, such as electrophoretic techniques, differential scanning calorimetry (DSC), DNA-based methods (genomics, proteomics), chromatographic methods, isotopic techniques, vibrational and fluorescence spectroscopy, elemental techniques, non-chromatographics mass spectroscopy, sensory analysis, nuclear-magnetic-resonance spectroscopy, immunological techniques together with chemometrics and bioinformatics [40].

DNA-based technique with polymerase chain reaction [38, 44] is a common technique in food authentication testing to ensure halal and kosher brand food products adhere to the standards. However, most of these techniques still require substantial sample preparation or some have very high sensitivity to adulterants and prone to give undefined results if all procedures are not followed exactly.

Research on vibrational spectroscopy-based food authentication techniques is getting more popular [40, 45–52]. This is partly due to the ease of sample preparation with this technique and relatively quick result and non-destructive nature of this method. Such vibrational spectroscopy is able to discriminate with high accuracy. For instance, pork meat and lard in meatball broth [45, 47], imported chocolate [50–53], and vegetable oils [48], etc. are some of the studies. Infrared-based detection techniques, such as FTIR or Fourier transform infrared spectroscopy, are capable of identifying fingerprint of compound molecules when it is incorporated with strong chemometric techniques [47]. Some research findings of lard adulterant are reported either by mixing lard with other animal fats or adulterating lard in food [53–55]. Another work on FTIR spectroscopy by Mansor et al. [56] reported an accuracy up to 100% in performing classification of lard adulterated in virgin coconut oil when the statistical technique, such as discriminant and PLS analysis, is incorporated. However, the limitation of lard detection using FTIR spectroscopy is highlighted in Rohman and Che Man [57] when identifying meat adulteration. Basically, lard has similar IR spectrum with other animal fats and vegetable oils since they are composed with (triacylglycerol) TAG, with different lengths of the fatty acid.

Animal fats have several chemical compositions, which mostly include TAG. In fact, fats share the same fatty acid compounds but different concentrations [58]. According to Rohman and Che Man [57], analysis of fats/oils is possible by focusing on lipid components as fats which is a part biological substance group. Triglyceride is the principal constituent of animal fat, not exception of pig fat. A triglyceride is constructed from three fatty acids and one molecule of glycerol [59]. Lard predominantly consists of saturated fatty acid [59].

Another popular technique that has been continually developed for lard compound detection in food is mass spectroscopy. Several MS methods have been reported, and the important ones are liquid-based chromatography and gas-based chromatography embedded with mass spectrometry (GC-MS and LC-MS).

3.1 Genomics

One of the most popular food authentication methods is the genomics, where verification of foodstuff origin is done by analyzing the cells. Since DNA is similar in the whole somatic cells of a particular species, the original tissue of sample would not affect the results of the test. The advantage of this method is that it can amplify minute samples. Proteomics technique mainly depends on proteins acting as fingerprint of food products and therefore can be applied for a systematic search of new marker proteins. These methods are normally utilized to identify incorrect description and food labeling fraud, that is, detection of meats prohibited by Islamic laws in sausages [35].

3.2 Electronic nose

Electronic nose or e-nose is to replicate human's olfactory technique in identifying a particular substance. E-nose is commonly used metal-oxide gas sensor capable of detecting volatile organic compound (VOC) for variety detection applications including lard adulteration [60] process quality control [61, 62] and used as a formaldehyde sensor [63]. Sensing materials used in the electronic nose for metal oxide sensor are and tungsten trioxide (WO_3) and tin dioxide (SnO_2) because both materials are reported to be very sensitive to many types of volatile compounds.

The sensor selection used in e-nose was based on the chemical compounds found in lard [58]. Decanal was the chemical compound found abundantly in lard

Animal Fat	Decanal (dimensionless)
Lard	17 444
Chicken	586
Beef fat	408.5

Table 1.
Decanal profile, measured in Kovats indices [58].

but did not have significant presence in chicken fat and beef fat. **Table 1** lists the decanal content in the fats of interest in terms of Kovats indices. A set of experiments by Kohl et al. [64] revealed that both the sensing materials used in metal oxide sensors are sensitive to the presence of aldehydes. It is reported here that such sensor is expected to be more sensitive toward lard than other fats.

A scatter plot of sample dataset is shown in **Figure 2** [65]. The dataset consists of nine unique classes of three types of fat each experimented with three different temperatures. Each class consists of 10 observations. Each class is represented in the plot by a unique symbol and an abbreviation where the letter "L" represents a lard sample, "C" represents a chicken fat sample, and "B" a beef fat sample. The numbers 40, 50, and 60 after the letters represent the temperature in degree Celsius. A clear separation can be seen in the plot as except classes "L40" and "C40" where there are no overlaps. The overlap indicates the chemical structure of a chicken fat is very similar to lard, and studies conducted with other techniques have proven that as well.

Figure 3 shows the individual plot of the three classes and their responses at different temperatures [65]. Linear regression lines in the background show an upward trend in sensor response, with lard having the highest gradient out of the three. With the increase of temperatures, the density and rate at which the odor fumes are produced must increase, thus giving rise to a higher sensor response. Besides, this lard has the lowest melting point among the three fats and will therefore melt and turn to gaseous state faster. In terms of settle point values, chicken fat scored the highest above the two as more evident from **Figure 3**. However, the higher settle point values of chicken fat can be explained by the fact that chicken fat melting points are very close to that of lard.

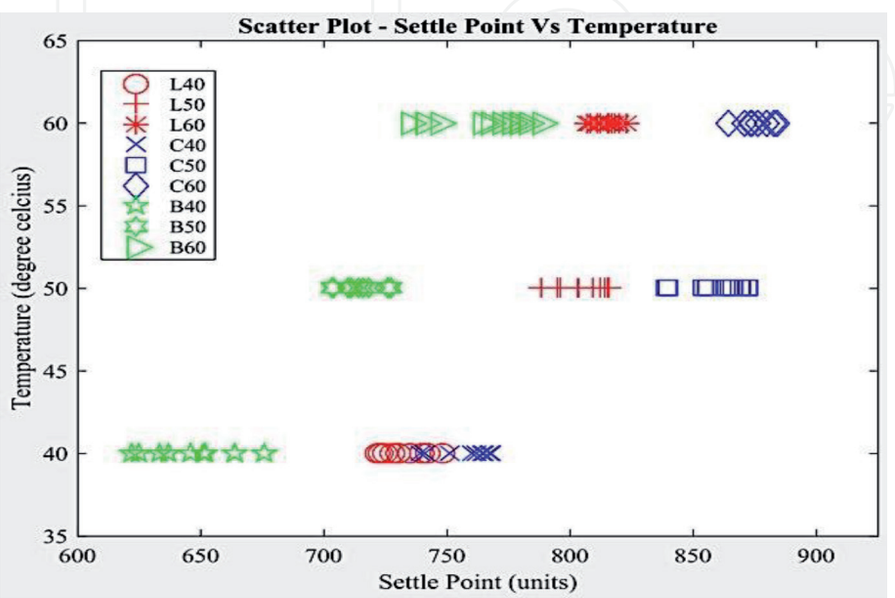

Figure 2.
Scatter plot of the entire dataset [65].

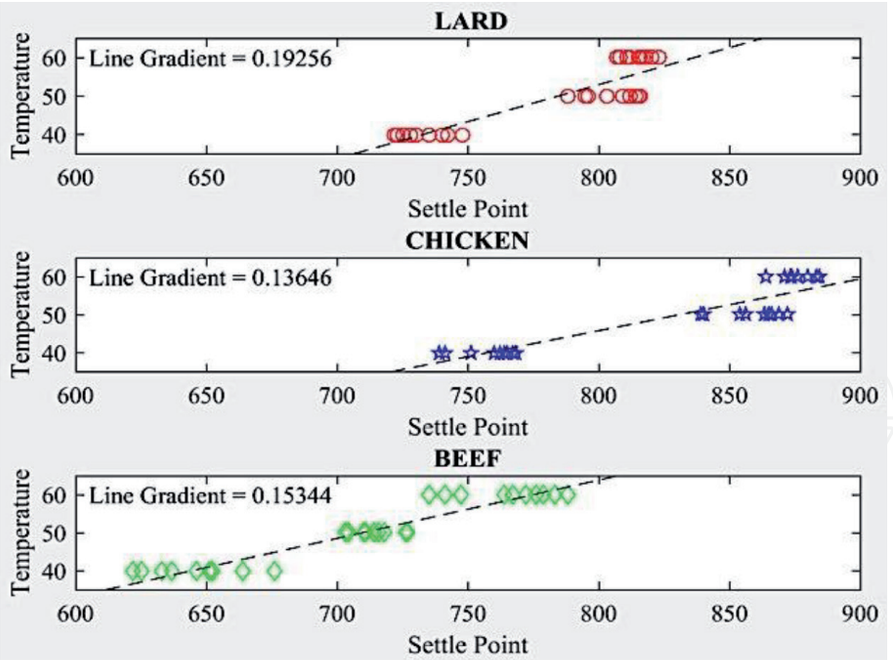

Figure 3.
Response toward change in temperature [65].

3.3 Vibrational spectroscopy

The principle of vibrational spectroscopy follows the concept that atoms in the chemical bonding within the molecule vibrate with certain frequency when it is excited. Such vibration frequency can be explained by the laws of physics and is shown in reported calculation [66]. The calculation of the lowest fundamental frequency of any two atoms that are connected by a chemical bond can be performed by assuming that the bond energy results from the vibration of diatomic harmonic oscillator and follows Hooke's Law according to Eq. (1)

$$v = \frac{1}{2\pi}\sqrt{\frac{k}{\mu}} \tag{1}$$

where, the vibrational frequency *is* v, the classical force constant is k, and the reduced mass of the two atoms is μ. In contrast to classical spring model for molecular vibration, no continuum of energy levels exists. Instead, there are levels of discrete energy that can be explained by quantum theory. Using the vibrational Hamiltonian, the time-independent Schrödinger equation can be solved for a diatomic molecule. A reduced equation of these levels can be written for the energy levels of diatomic molecules as:

$$E_v = \frac{\left(v + \frac{1}{2}\right)\hbar}{2\pi}\sqrt{\frac{k}{\mu}} \quad (v = 0, 1, 2, \ldots.) \tag{2}$$

or by using hv as the quantum term, the equation can be reduced to

$$E_v = \left(v + \frac{1}{2}\right)\hbar \quad (v = 0, 1, 2, \ldots.) \tag{3}$$

At certain extension of the stretch, the bond could eventually breakdown when the vibrational energy goes beyond the dissociation energy. **Table 2** shows the different stretching frequencies. When a fast and objective analysis is required,

	Wavenumber (cm^{-1})	Intensity
C ≡ N	2260–2220	Medium
C ≡ C	2260–2100	Medium to weak
C = C	1680–1600	Medium
C = N	1650–1550	Medium
	~1600 and ~1500–1430	Strong to weak
C = O	1780–1650	Strong
C – O	1250–1050	Strong
C – N	1230–1020	Medium
O – H (alcohol)	3650–3200	Strong, broad
O – H (carboxylic acid)	3300–2500	Strong, very broad
N – H	3500–3300	Medium, broad
N – H	3300–2700	Medium

Table 2.
Important IR stretching frequencies [67].

fluorescence and absorption spectroscopies in the range of visible to infrared region are better choice. The vibrational spectroscopy is able to provide a fingerprint of the vibrational levels of molecules in the mid-infrared (MIR) radiation (4000–400 cm^{-1}). One of the most common IR spectroscopy techniques is the Fourier transform infrared (FTIR) spectroscopy. FTIR spectroscopy utilizes the use of mid infrared spectroscopy (4000–400 cm^{-1}), which includes the fingerprint region.

3.3.1 Meat sample preparation

All meat samples were collected from a local slaughterhouse and were washed by distilled water. After that, the meat was cut by knife in pieces in the size of 1 cm^2 and stored at −20°C until it was being used. The animal fats extracted from beef, mutton, and chicken body fat as well as lard were collected by rendering the adipose tissues following the method reported by Che Man et al. [53] with little variation.

3.3.2 Post-processing analysis

Data post-processing was done using two software: Spectrograph 1.1 and MATLAB R2017b. Extracting information from spectrum results was carried out using Spectrograph 1.1, where the data are preprocessed as needed. MATLAB R2017b was used to further analyze the results from preprocessing. Principal component analysis (PCA) technique was used to analyze the quality of lard adulteration, while PLS technique was used to analyze the quantity of lard adulteration.

Figure 4 shows FTIR spectra of pure fats. These spectra consist of four regions: 1st region ranging from 4000 to 2500 cm^{-1}, 2nd region ranging from 2500 to 2000 cm^{-1}, 3rd region ranging from 2000 to 1500 cm^{-1}, and lastly the fingerprint region ranging from 1500 to 800 cm^{-1}.

3.4 Mass spectroscopy

The mass spectroscopy methods are fast becoming popular [50, 68]. This method produces unique chemical fingerprinting that can discriminate or verify

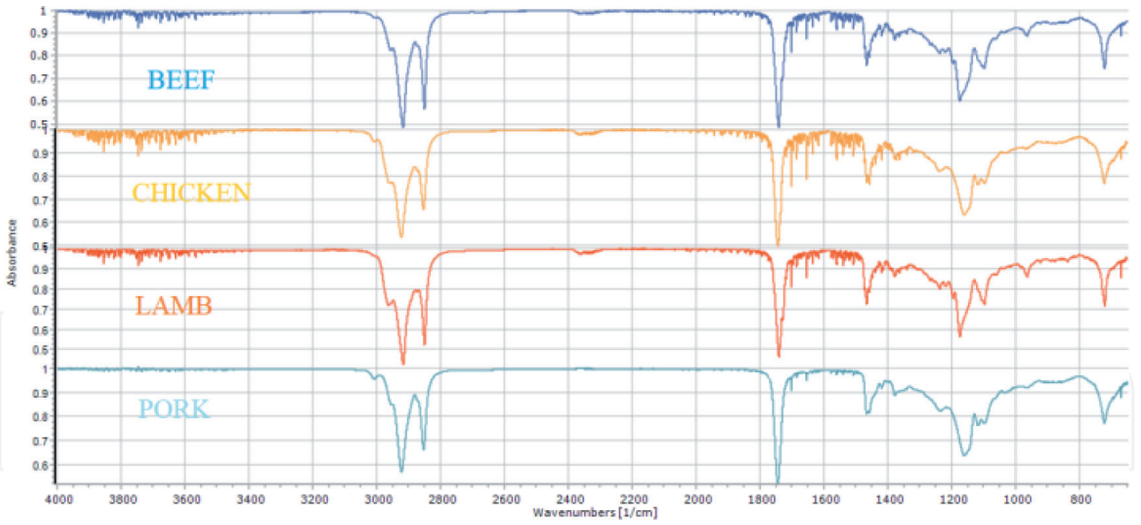

Figure 4.
Spectrogram from FTIR covering 4000–800 cm^{-1}.

foods. MS offers many advantages, such as the identification of mass spectral signal pattern and possible characterization of specific compounds coming from food adulterants. Additionally, MS does not easily react with water, which is different case for vibrational spectroscopy. MS can also provide the plant origin by measuring the specific chemical compounds. However, MS has disadvantages of direct contact requirement to the sample material and larger instrumentation. The spectral resolution of MS is more detail so it has higher possibility of finding fingerprint of food chemicals. MS also gives a higher versatility because of exchangeability of its ion sources. With different ion sources, MS can provide various ionization and is able to perform measurement of chemically different chemical compounds.

Figure 5 shows the spectrogram of different milks using electrospray ionization mass spectroscopy [69]. Obvious differences can be observed among the three milk, (a) cow milk, (b) goat milk, and (c) soy milk, by observing the number of peaks and peak intensities. The three pure milks and the two mixtures score plots are

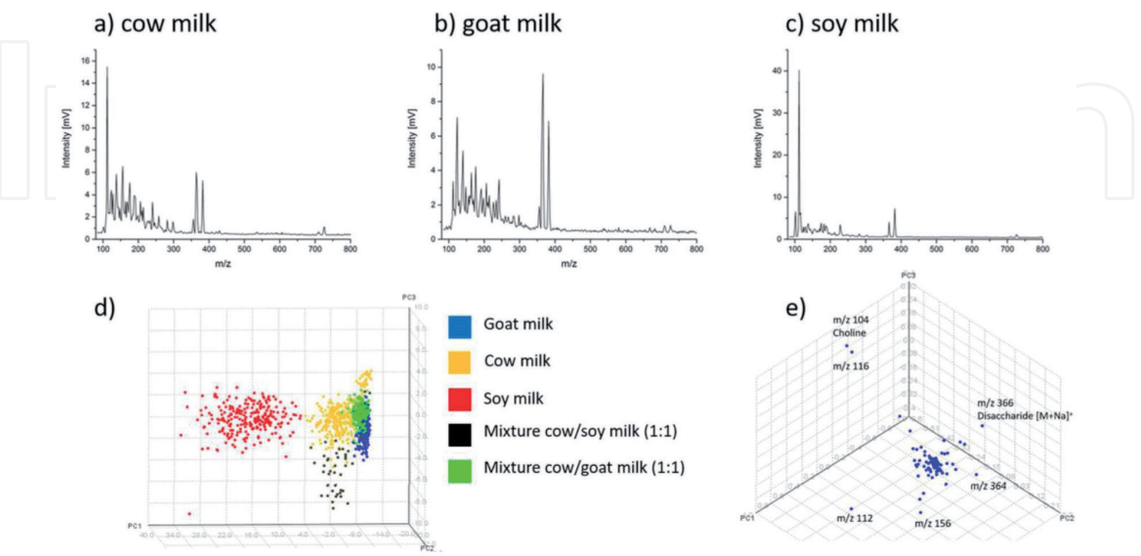

Figure 5.
ESI mass spectroscopy using ESI spectrogram of the three milk, namely cow milk (a) goat milk, (b) soy milk, (c) Plot and (d) shows PCA score plot of the milk data set from the sum of 20 spectra. Each point in plots shows a mass spectrum of goat milk (blue), cow milk (yellow), and coy milk (red), the black-color data show 1:1 mixture of cow and soy milk, and the green shows a mixture of cow and goat milk (e) PCA loading plot of the milk data set. [69].

shown in **Figure 5d**. The spectrograms of the pure milk samples are well separated in the plot, while data points for the mixture of cow and goat milk are positioned in the close proximity of those two types. The data points of cow/soy milk mixture are shown near around the data points of cow milk.

4. Advanced mass spectroscopy

The recent advanced mass spectroscopy instruments offer higher speed, better resolution, higher mass accuracy, and more sensitivity to provide comprehensive qualitative investigation, rapid profiling, and better accuracy detection and quantification of chemical compounds in complex matrices. Thus, such advanced mass spectrometries such as gas chromatography-mass spectrometry (GC-MS) or liquid chromatography-mass spectrometry (LC-MS) are able to investigate and analyze the complex adulterants. These advanced mass spectroscopies operate in scan mode at better spectrum resolution and accurate mass (HRAM).

This improved high-resolution mass spectroscopy is capable in identifying the chemical compounds and mass structure of pure and adulterated processed food, the presence of adulterants that create problems affecting food safety and quality, and the existence of natural toxin, food degradation and contaminations.

4.1 GC-MS

Gas chromatography (GC) configured with electron capture, flame photometric detection, and nitrogen-phosphorous has been used since the early 1970s for residue analysis. The confirmation of results was done with additional use of gas chromatography equipped with a different type of column or detector. Nowadays, using GC integrated with MS, it is able to simultaneously determine and confirm the chemical residues with only one instrument in one analytical run.

Following the commercial of gas chromatography (GC) 50 years ago [70], GC has been used widely in the application involving food adulterant analysis and to perform both quantitative and qualitative analysis of food ingredients, food additives, food adulterants, and contaminants in order to discover nutritional contents, improve food safety, and introduce different food varieties. Furthermore, GC has been reported to be able to identify many organic contaminants at trace levels in complex chemical compounds of food and environmental samples.

Nowadays, gas chromatography integrated to mass spectrometry (GC-MS, GC-HRMS) utilized electron impact ionization (EI) is the most often employed in GC-based MS technique for multi residue chemical analysis in food analysis because of its high selectivity and sensitivity and its ability to screen many pesticides from different chemical compound classes in very complicated matrices in a single run [71]. Advantages of electron impact ionization mass spectroscopy are insignificant influence of molecular structure on response and vast number of characteristic fragments. GC-MS is suitable for analysis of volatile chemicals. Meanwhile, the analysis with more polar compound, LC-MS is more suitable. With the absent of chemical derivatization, GC is commonly used for the analysis of sterols, low chain fatty acids, oils, aroma components and off-flavors, and many contaminants, such as toxins, industrial pollutants, and specific of drugs in foods.

4.2 LC-MS

Liquid chromatography-mass spectrometry (LC-MS) is a combined analytical chemistry technique that separates mixtures with multiple components and

provides structural identity of the individual components with high molecular specificity and detection sensitivity. Methods based on liquid chromatography (LC) were applied later after GC, because traditional UV, diode array, and fluorescence detectors are often less selective and sensitive than GC instruments. But in the last few years, the commercial availability of atmospheric pressure ionization caused a dramatic change. Compared to traditional detectors, electrospray (ESI) or atmospheric pressure chemical ionization (APCI) in combination with MS instruments has increased the sensitivity of LC detection by several orders of magnitude.

An analytical methodology using liquid chromatography-mass spectrometry has been reported by Guijarro-Díez et al. [72] for the detection of the adulteration of saffron samples with gardenia through the determination of geniposide as adulteration marker. **Figure 6** shows the MS spectra obtained for geniposide, and different MS fragments and adducts (Na^+ and $NH4^+$) were obtained for geniposide under ESI^+, whereas when the ESI^- mode was employed, the most abundant ion corresponded to the adduct $[M + HCOO]^-$ (433.1384 *m/z*), and no fragmentation was observed [72].

4.3 High resolution-mass spectroscopy

The instrument of high-resolution mass spectrometry (HRMS) provides better accuracy for the analysis of food adulteration. However, due to high instrumental complexity, HRMS has previously been limited to the most critical applications, such

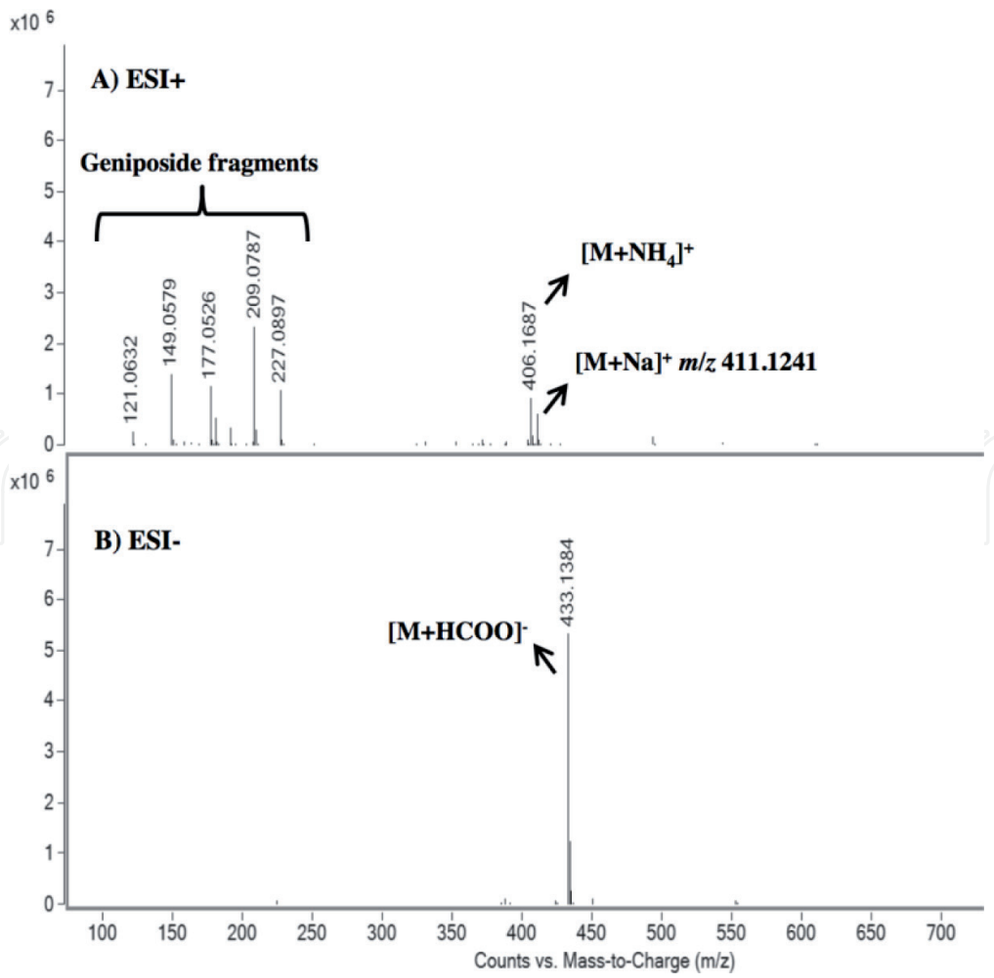

Figure 6.
Mass spectrogram geniposide spectrogram from gardenia extract investigated by LC-MS with (A) ESI^+ and (B) ESI^- [72].

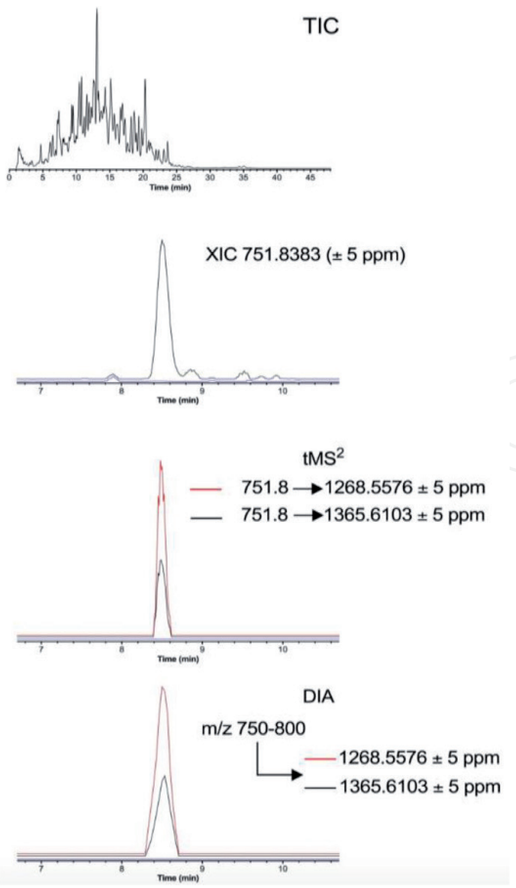

Figure 7.
The observed chromatograms (XICs) for certain signature of myoglobin proteotypic peptide-fragment pairs. The spike was noticed in the chromatograms from beef samples with 1% horse meat (blue indicates extracted blank chromatograms) [73].

as the investigation of natural organic compounds or dioxin-related chemical compounds. The existence of modern HRMS instruments such as time-of-flight (TOF) and Orbitrap instruments has significantly changed the utilization of the equipment. Therefore, high-resolution mass spectrometry (HRMS) has gotten wider acceptance in the last decades for adulterant and residue analysis in food. This positive development is because of the availability of more versatile, robust, sensitive, and advanced instrumentation. The advantages by HRMS compared to classical unit-mass-resolution are ability to provide full-scan spectra, which offers more detail and insight into the mass composition of any sample. As a result, the analyst can measure chemical compounds without the necessity of compound-specific tuning, the need of retrospective data analysis, and has a capability performing an analysis of structural elucidations of suspected chemical compounds. HRMS is still preferable compared with classical hyphenated mass spectrometry in the investigation of quantitative multi residue methods (e.g., pesticides and veterinary drugs). It is one of the most powerful tools for identifying the unknown and non-targeted samples. Improvement of the hardware and software still needs to be addressed by the equipment manufacturers for it to be superior compared to hyphenated mass spectrometry and to be a standard trace analysis tool.

HRMS technology provides proteomic research to facilitate new discovery. The recent HRMS instruments already have the sensitivity, speed, accuracy, and selectivity to deliver comprehensive qualitative analysis, rapid chemical profiling, and high-accuracy analysis and detection of proteins in complex compounds. With these advantages, HRMS-based method was suitable specifically to perform the investigation of meat speciation and to detect food adulteration [73] and is capable to identify quite specific tryptic peptides from targeted proteins.

Motivated by European scandal [74] in which the horse and pig DNA were detected in beef products sold from several retailers, HRMS method developed by Orduna et al. [73] were tested by mixing horse meat in beef meat at concentration 1% w/w. **Figure 7** shows the detection of adulteration of horse proteotypic myoglobin peptide using three different techniques of MS (140,000 FWHM), tMS or DIA [73].

5. GC-TOF MS

GC-TOF MS instrument has two operation modes, in which one mode offers very high scan rates, allowing the segregation of overlapping spectrum peaks by automatically performing deconvolution mass spectral of overlapping spectrum signals [75]. Another type of GC-TOF MS instruments provides high mass resolution, performing data evaluation with a restricted mass window of 0.02 Da [76]. For ion separation GC-TOF MS, single-quad instruments are frequently utilized used. GC-MS systems with quadrupole ion traps integrated with time-of-flight (TOF) mass spectrometers or tandem mass spectrometers are used for the analysis of pure and mixture food.

5.1 Sample preparation

The work by Witjaksono et al. [77] was conducted for total nine meat samples of three different animal meats, that is, chicken, cow, and pig. Each animal meat type is prepared to provide three different samples. The preparation of the animal meat samples and the extraction process of these animal body fats have been done using similar method mentioned before in the FTIR measurements. After obtaining the pure fats, each animal fat (approximately of 50 mg) was dissolved in 0.8 mL hexane. Later, the mixture was stirred for 1 min using an apparatus of vortex mixer and then stored in the dark at −18°C before going to GC-TOF MS analysis.

5.2 GC-TOF MS results

The analysis for this food adulteration was based on GC-TOF MS to identify and study their complex chemical compounds. The equipment used is an Agilent 7693 B GC integrated with TOF MS with hp-5ms column. The analysis was performed for all nine samples, consisting of three samples each from cow, lard, and chicken fats to investigate their aromatic hydrocarbons. The result suggests that the concentration of 1,2,3-trimethyl-benzene, indane, and undecane in lard fat are higher by 250, 14.5, and 1.28 times than chicken fat's concentrations, respectively, and higher by 91.4, 2.3, and 1.24 times higher than cow fat's concentrations, respectively. This initial result promises the possibility of finding biomarkers for non-halal food adulterants.

Table 3 provides the obtained average area covered by each hydrocarbon that is coming from three samples to represent the composition weightage for the different fat types. From **Table 1**, it is obvious that lard is distinctive from the other animal fats in several hydrocarbon compositions. Here are the resulted hydrocarbons that give bigger percentage area in lard in comparison with the other fats: benzene, 1,2,3-trimethyl-; benzene, 1-methyl-3-(1-methylethyl)-; benzene, 1-methyl-4-propyl-; hexanedioic acid, bis(2-ethylhexyl)ester; p-cymene; tridecane; undecane. By using chemometric and bioinformatics analysis techniques, these results could be further analyzed to differentiate and separate the lard fat from the other animal fats.

Hydrocarbon compound	Area %		
	Lard	Chicken fat	Cow fat
2,4-Imidazolidinedione, 5-[3,4-bis[(trimethylsilyl)oxy]phenyl]-3-methyl-5-phenyl-1-(trimethylsilyl)-	0.08299	0.04853	0.141035
Benzene, 1,2,3-trimethyl-	21.33433	0.085155	0.233378
Benzene, 1-methyl-3-(1-methylethyl)-	0.597023	0	0
Benzene, 1-methyl-4-(1-methylpropyl)-	0.013952	0	0.018403
Benzene, 1-methyl-4-propyl-	0.787343	0	0
Benzene, 2-ethyl-1,4-dimethyl-	0.374043	0.432713	0.068181
Decane	21.33433	0	21.167
Decane, 4-methyl-	1.286363	0	0.95659
Hexanedioic acid, bis(2-ethylhexyl) ester	0.583767	0	0
Indane	0.125046	0.008597	0.054052
Naphthalene, 1,2,3,4-tetrahydro-2-methyl-	0.055843	0	0
Nonane, 2-methyl-	0.098341	0	0
Octane, 2,3,7-trimethyl-	0.037965	0	0
p-Cymene	0.447551	0.116017	0
Tridecane	0.22617	0	0
Undecane	10.3596	8.062533	8.382467
Benzene, (2-methyloctyl)-	0	2.472467	2.657267
Benzene, 1-ethyl-4-methyl-	0	0.035777	0.102822
Benzene, 1-methyl-3-propyl-	0	0.680724	0
Cyclohexane, butyl-	0	0.529553	0
Naphthalene, 1,2,3,4-tetrahydro-	0	0.06464	0.138087
o-Cymene	0	0.121686	0
1-Dodecanol, 3,7,11-trimethyl-	0	0	0.222381
Benzene, 1-ethyl-2,4-dimethyl-	0	0	0.062808
Benzene, 1-ethyl-3-methyl-	0	0	0.110763
Heptacosane	0	0	0.056515
Nonane, 2-methyl-	0	0	0.062937
Squalene	0	0	0.342603
Benzene, 4-ethyl-1,2-dimethyl-	0	0.541307	0

Table 3.
Resulted composition of aromatic hydrocarbons for lard, chicken, and cow fats [77].

6. Conclusion

This chapter demonstrated the identification of lard discrimination using GC-TOF MS for cow and chicken fats. GC-TOF MS provides confirmation of lard biomarker that is different with other animal fats for their volatile hydrocarbon compounds in which complex compounds such as benzene, 1-methyl-3-(1-methylethyl)-, hexanedioic acid, bis(2-ethylhexyl)ester, and p-cymene give significant higher compositional percentage in lard fat compared to other animal fats.

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