Review article

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Overview of the studies on authentication of gelatin using Fourier Transform Infrared spectroscopy coupled with chemometrics

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Abstract

Background and objective: In recent years, global market of halal products has been greatly developed. Sign of "Halal" on foods' label refers to their appropriateness for Muslim and Jewish buyers, which are banned from consumption of non-halal products such as porcine derived gelatin. Pig derivatives are marketed frequently in the world because they are relatively cheap and available. Therefore, development of simple and fast methods for authentication of halal foods and raw materials has been required. At this review, we studied one of common authentication approaches for qualification of gelatin by Fourier transform infrared spectroscopy (FT-IR).

Results and conclusion: Several analytical methods were conducted for evaluation of gelatin in foods. Among them, FT-IR was relatively interested because it is a simple, fast and reliable method and does not require complicated sample preparation. In some cases, it was superior to the molecular method of polymerase chain reactions (PCR) in detection of gelatin's origin by least analytical uncertainties. Although, analysis of FT-IR results by modeling approaches such as chemometrics classification increases effectiveness of the developed method. In this review, we will show that FT-IR can be used routinely as alternative or complementary method to identify the source of gelatin with high precision.

Keywords: Authentication, chemometrics, FT-IR, gelatin, Halal

1. Introduction

Today, trade issues ahead of Muslim and non-Muslim countries in commercialization of halal products are controversial. Indeed, Halal certification is necessary to ensure quality of the products including foodstuffs, pharmaceuticals, and cosmetics, especially for Islamic communities. In 2013, global trade of Halal was estimated as 580-660 billion US dollars [1]. Due to the approved health impact of halal foods on human, its further growth is expected. Halal food market was accounted as 12% of the world trade in agricultural products in recent years [2].

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Following the advancement of science and technology, some foods may be adulterated with non-halal components. With regard, strict pressure has been taken on labelling of the foods containing additives or ingredients may be originated from non-halal sources in the whole food supply chain [3,4,5]. However, it is necessary to identify and determine non-halal components. Several analytical methods have been developed in this regard including chromatography, spectroscopy, and molecular biotechnology techniques. Among them, vibrational spectroscopy (VS) is a common method for analysis of non-halal components due to its simplicity and ease of operation [3].

Among food additives, traceability of gelatin to find out its origin (pig or cow) is important for traders. Characteristics of porcine and bovine gelatin are similar and can not be easily distinguished by conventional methods. Therefore, the scientists have tried to identify their origin by experimental techniques. Some of these methods are real time-polymerase chain reaction (RT-PCR), enzyme-linked immunosorbent assay (ELISA), high performance liquid chromatography (HPLC), and Fourier transform infrared spectroscopy (FT-IR). In addition, Hidaka and Liu reported that bovine and porcine gelatin might be detected by pH reduction after calcium phosphate deposition [6]. Most of the analytical methods require complicated and difficult sample preparation [7,8,9]. In this paper, we briefly review the studies done on authentication of gelatin as food ingredient by FT-IR method which in simpler, cheaper and feasible than most of the other techniques.

2. Molecular spectroscopy coupled with chemometrics

Molecular spectroscopy deals with reaction of electromagnetic radiation (EMR) at specific regions (200-800 nm for UV spectroscopy and 800-25000 nm for infrared spectroscopy) in samples at molecular level [10,11,12]. Non-destructive, simple sample preparation, ease of operation and fast sample analysis are considered as benefits of molecular spectroscopy [13].

VS technology refers to interaction of electromagnetic waves with molecules at infrared region. Indeed, vibration at specific region is highly selective and is associated with the chemical bonds contained in the sample [14]. The VS technology can simultaneously determine different functional groups in the sample [3,15].

VS-based technologies have succeeded in analysis of the samples along with chemometrics methods. Chemometrics studies include statistical and mathematical concepts to convert the experimental data to graphical results and models [16]. In VS, three chemometrics approaches are commonly used. Firstly, data processing (e.g. derivatization, normalization, baseline correction, standard normal variate, mean centering, derivatives, and multiplicative correction) is applied. Secondly, classification techniques included to supervised pattern recognition (e.g. partial least square-discrimination analysis (PLS-DA)) and unsupervised pattern recognition (e.g. principal component analysis (PCA) and cluster analysis) are conducted. Thirdly, regression methods, in order to correlate the vibrational spectra with concentration of the chemicals, known as principle component regression (PCR) and partial least square (PLS) are used [3,17]. By these approaches, real samples are well discriminated from the adulterated products in the market in a cost-effectiveness way conducted by cheap and feasible analysis followed by professional statistical and chemometrics methods.

2.1. Fourier transform infrared spectroscopy

Infrared spectroscopy is used for qualitative identification of sample's ingredients. The infrared spectra is considered as fingerprint of a sample by representation of specific peak at each wave number for the atoms presented in a sample [18,19]. In recent years, the scientists have developed FT-IR technique for evaluation of halal status of food products. For example, porcine meat contains higher amount of non-

saturated fat than bovine meat, which is observed as sharper peaks in FT-IR spectra [20,21].

Porcine products have usually less price than those produced from sheep or cow especially for gelatin. Therefore, producers may prefer to use pig derivatives instead of other animals despite their commitment to halal assurance. Hashim et al. in 2010 developed a spectroscopy method by using attenuated total reflectance (ATR) technique to identify the source of gelatin. They used PCA method for analysis of the spectra observed within the wave numbers of 3280-3290 and 1200-1660 cm^{-1}. Their study was confirmed by "Cooman" graph, which presented a distinct area between bovine and porcine gelatin [22]. Wave numbers in which the main peaks are observed are depicted in Figure 1. Porcine gelatin shows similar spectra with bovine gelatin. The four specific regions are 2300-3600 cm^{-1} (Amide-A), 1644-1656 cm^{-1} (Amide I), 1335-1560 cm^{-1} (Amide II), and 670-1240 cm^{-1} (Amide III) [20,23]. According to Figure 1, typical spectra of gelatin includes low intensity of amide II and III. It is consistent with the changes occurred through conversion of collagen to gelatin by denaturation of triple helix in the structure at high temperature [23].

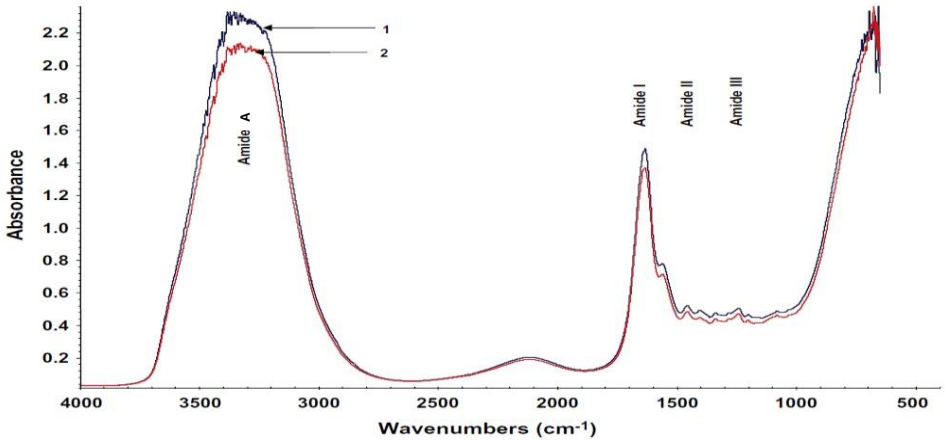

Figure 1- FT-IR spectra of 1) bovine gelatin and 2) porcine gelatin; Reproduced with permission of Hashim et al. [22]

The peak observed at 3280-3290 cm^{-1} is related to stretching N-H in amide groups. When the strength of hydrogen bonds increases, the peaks may be observed at lower frequency. The carbonyl group (C=O) is observed in the same region of bending N-H and N-C (1620-1660 cm^{-1}) that is specific to amide I. In addition, frequencies of 1650-1660 cm^{-1} and 1620-1640 cm^{-1} are referred to α-helix and β sheet structures. In the case of amide II, frequencies of 1520-1550 cm^{-1} are divided to 1540-1550 cm^{-1} specific to alpha-winding structure and 1520-1525 cm^{-1} specific to β-sheet structure. Vibration of amide II is due to the variation of N-H band deformation [22]. In this regard, the functional groups of both bovine and porcine gelatins show different intensities in their spectra, which are distinguishable in the analysis.

Nur cebi et al. in 2015 investigated effectiveness of FTIR-ATR technique in the range of 600-4000 cm^{-1} coupled with chemometrics for discrimination of porcine, bovine, and fish gelatins. Standard solutions of fish, porcine, and bovine gelatins in deionized water were separately prepared at different concentrations (4-20% w/v). Moreover, mixtures of bovine and porcine gelatin were also prepared at ratio of 3:1, 1:1 and 1:3 to achieve final gelatin concentration of 10% (w/v). As depicted in Figure 2, combination of FT-IR and multivariate analysis of PCA and cluster

analysis was successfully able to differentiate the samples [24].

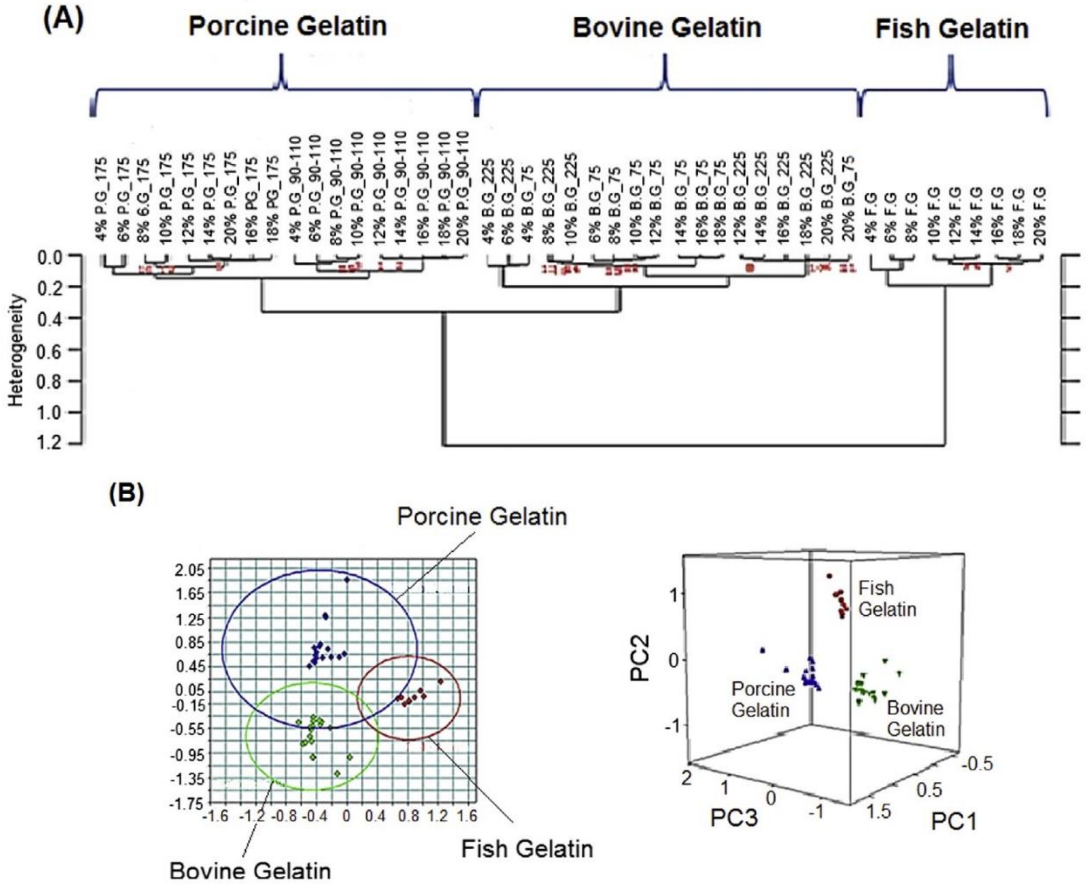

Figure 2- A) Cluster analysis of the FT-IR spectra achieved from bovine, porcine and fish gelatins, B) 2D and 3D PCA graphs of the FT-IR spectra achieved from bovine, porcine and fish gelatins; P.G: porcine gelatin, B.G: bovine gelatin, F.G: fish gelatin; Reproduced with permission of Nur cebi et al. [24]

In the study of Nur cebi et al., similar FT-IR spectra were observed for bovine, porcine, and fish gelatins (Figure 3). They include three large peaks in 1600-1700 cm^{-1} (amide I), 1520-1565 cm^{-1} (amide II), and 670-1240 cm^{-1} (amide III). Similar results were observed in other studies of Nagarajan et al. [25] and Hashim et al. [22]. The indicator peaks of the samples were correlated with the secondary structure of protein found in gelatins. Absorbance of amide I was largely induced by stretching C=O and a minor contribution of stretching C-N. Absorbance of amide II was due to bending N-H and tensile C-N. Amid III shows tensile vibration at C-N bond, flexural vibration at N-H bond, and a poor vibration at stretching C-C and C=O [24,26].

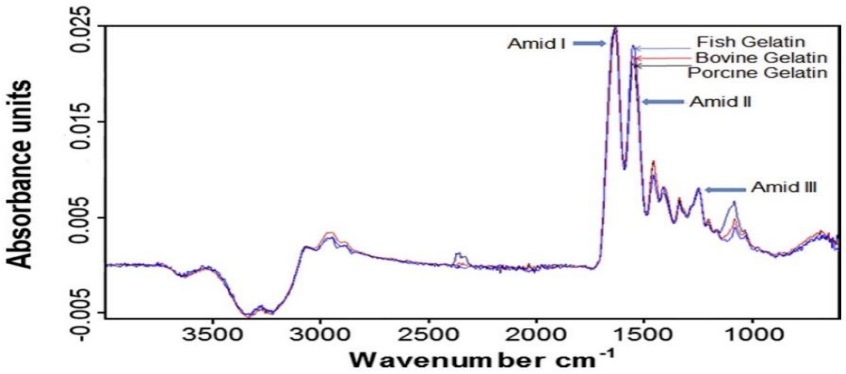

Figure 3- FT-IR spectra of fish, bovine and porcine gelatins; Reproduced with permission of Nur cebi et al. [24]

The spectra observed between 1000 and 1100 cm^{-1} are related to vibration of v(C-O) and v(C-O-C) of the carbohydrate parts. Furthermore, the peak in range of 1031-1083 cm^{-1} is related to stretching C-O in carbohydrate residue of collagen and represents amide I in proteoglycan [23,27,28]. As shown in Figure 2, significant differences were observed among fish, bovine, and porcine gelatins especially for fish gelatin that predominantly observed in the range of 1000-1100 cm^{-1} [24].

In the study of Nur Cebi et al., the mixture of bovine and porcine gelatins was separated from pure porcine and bovine gelatins. They concluded that FTIR-ATR technique as a simple and cost-effective approach can be used for tracing of gelatin [24]. Rohman et al. in 2016 investigated pig derivatives in food matrices by VS and similar results of traceability and halal authenticity were reported in their study [3].

Nur Cebi et al. further compared the results of FTIR-ATR spectroscopy with PCR and found that FTIR-ATR was more reliable than real time-PCR to discriminate the gelatins by different sources in jelly candy (Table 1).

Table 1- Comparison of FTIR-ATR spectroscopy and PCR results achieved from analysis of gummy candies (n = 30); Reproduced with permission of Nur cebi et al. [29]

Test Sample Number	FTIR results [a]	PCR results [b]
1	Bovine gelatin	Porcine negative
2	Bovine gelatin	Porcine negative
3	Bovine gelatin	Porcine negative
4	Bovine gelatin	Porcine negative
5	Bovine gelatin	Porcine negative
6	Bovine gelatin	Porcine negative
7	Bovine gelatin	Porcine negative
8	Porcine gelatin	Porcine positive
9	Porcine gelatin	Porcine positive
10	Porcine gelatin	Porcine positive
11	Bovine gelatin	Porcine negative
12	Bovine gelatin	Porcine negative
13	Bovine gelatin	Porcine negative
14	Bovine gelatin	Porcine negative
15	Bovine gelatin	Porcine negative
16	Bovine gelatin	Porcine negative
17	Bovine gelatin	Porcine negative
18	Bovine gelatin	Porcine negative
19	Bovine gelatin	Porcine negative
20	Bovine gelatin	Porcine negative

[a] HCA analysis test results of gummy candies. [b] RT-PCR porcine positive test samples.

They analyzed the peaks of FT-IR by chemometrics methods of PCA, HCA, and PLS-DA. Interestingly, the all three methods were able to significantly differentiate the candy samples. The spectral range of 1734-1528 cm^{-1} was used for HCA and PLS-DA analyses. Comparison of the results of HCA and real time-PCR of 20 samples are presented in Table 1. As observed, the gummy candies containing porcine gelatin are marked with red line. Three out of 20 commercial samples contained porcine gelatin that were truly determined by FT-IR. In comparison, all of 20 samples were determined to contain porcine gelatin by real time-PCR, which showed the lower sensitivity of this technique in this regard [29]. HCA and PCA classifications of gummy candies are presented in Figure 4 and Table 2.

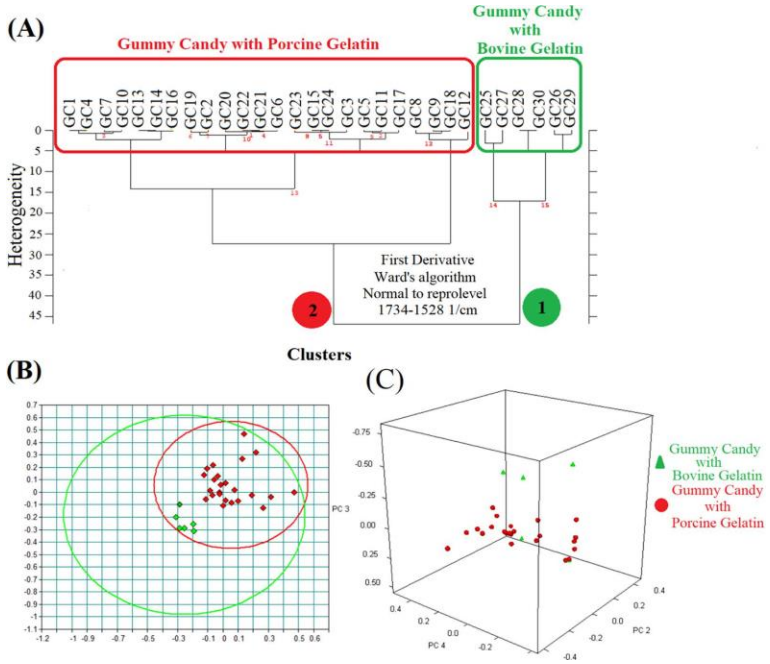

Figure 4- Chemometrics analysis of FT-IR spectra achieved from gummy candies (n = 30); A) Dendrogram of HCA (Ward's algorithm), B) 2D PCA, C) 3D PCA; Reproduced with permission of Nur cebi et al. [29].

Table 2- Report of chemometrics analysis (HCA and PCA) of FT-IR spectra achieved from gummy candies (n = 30); Reproduced with permission of Nur cebi et al. [29].

Diagnosis Number	1. Class		2. Class	
Diagnosis for gummy candy samples(produced in laboratory) Data preprocessing: First derivative Ward's algorithm, normal to reprolevel Frequency Ranges=1734-1528 cm^{-1} Standart (Euclidian Distance)	Gummy Candy 1	Gummy candy with porcine gelatin	Gummy Candy 25	Gummy candy with bovine gelatin
	Gummy Candy 2		Gummy Candy 26	
	Gummy Candy 3		Gummy Candy 27	
	Gummy Candy 4		Gummy Candy 28	
	Gummy Candy 5		Gummy Candy 29	
	Gummy Candy 6		Gummy Candy 30	
	Gummy Candy 7			
	Gummy Candy 8			
	Gummy Candy 9			
	Gummy Candy 10			
	Gummy Candy 11			
	Gummy Candy 12			
	Gummy Candy 13			
	Gummy Candy 14			
	Gummy Candy 15			
	Gummy Candy 16			
	Gummy Candy 17			
	Gummy Candy 18			
	Gummy Candy 19			
	Gummy Candy 20			
	Gummy Candy 21			
	Gummy Candy 22			
	Gummy Candy 23			
	Gummy Candy 24			

As it seen in the figure, two clusters were well separated. It showed the strength of Ward's algorithm in analysis of the results achieved from gummy candies as complex matrix needed no pretreatment. At the end, the authors introduced FTIR-ATR method as "green analytic technique" in which no solvent or reagent is required [29].

3. Conclusion

FT-IR spectroscopy is a feasible technique able to identify the source of gelatin. It also is a cost-effective and efficient method in quality assurance and verification of halal status in foods that is important for Muslim consumers. FT-IR spectroscopy is introduced as a green analytical method neither needs solvent for analysis nor harms the environment. Although, analysis of its results by chemometrics methods strengths validity of the observations through which differentiation and authentication of the marketed samples is possible. The combined method can be used routinely for verification of gelatin containing foods in the market of Islamic countries.

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5. Conflict of interest

The authors declare that there is no conflict of interest.

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