Y A Y A S A N P E R G U R U A N C I K I N I
I N S T I T U T S A I N S D A N T E K N O L O G I N A S I O N A L

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Untuk melaksanakan tugas sebagai berikut:

Bidang	Perincian Kegiatan	Tempat	Jam/ Minggu	Kredit (SKS)	Keterangan
I	MENGAJAR DI KELAS (KULIAH/RESPONSI DAN LABORATORIUM)				
PENDIDIKAN	**Program Studi S1 Farmasi**				
DAN	Analisis Sediaan Farmasi (K)	Ruang A-3 Lab Kimia Dasar		1	Sabtu, 08:00 - 10:30 Sabtu, 10:30 - 13:00
PENGAJARAN	Kimia Klinis (B)(A)	Ruang A-2		1	Senin, 13:00 - 14:40
	Kimia Klinis (B)(K)	Ruang A-3		1	Senin, 19:00 - 20:40
	Bimbingan Skripsi		3 Jam/Minggu	1	
	Menguji Tugas Akhir		3 Jam/Minggu	1	
	Program Studi Profesi Apoteker				
	Pengetahuan Dasar Keprofesian di Farmasi Komunitas dan Kewirausahaan (C)	Online		0,875	Sabtu-Kamis/10.00-14.40
	Pengetahuan Dasar Keprofesian di Farmasi Komunitas dan Kewirausahaan (D)	Online		0,875	Sabtu-Kamis/10.00-14.40
	Membimbing PKPA Industri			3,5	
	Membimbing PKPA Apotek			3	
	Membimbing PKPA Puskesmas			1	
	Membimbing PKPA PBF			0,5	
II	Penulisan Karya Ilmiah		3 Jam/Minggu	1	
PENELITIAN					
III	Pelatihan dan Penyuluhan		3 Jam/Minggu	1	
PENGABDIAN Dan					
MASYARAKAT					
IV	Pertemuan Ilmiah		3 Jam/Minggu	1	
UNSUR UNSUR					
PENUNJANG					
	Jumlah Total			16	

Kepada yang bersangkutan akan diberikan gaji/honorarium sesuai dengan peraturan penggajian yang berlaku di Institut Sains dan Teknologi Nasional

Penugasan ini berlaku dari tanggal 2 Maret 2026 sampai dengan tanggal 31 Agustus 2026

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1. Wakil Rektor Bidang Akademik - ISTN
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3. Ka. Biro Sumber Daya Manusia - ISTN
4. Kepala Program Studi Farmasi Fak. Farmasi
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ORIGINAL ARTICLE

Analysis of Porcine Gelatin in Commercial Hard Capsule Shells Using FTIR Spectroscopy Combined with Principal Component Analysis

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ABSTRACT

Commercial hard capsule shell are pharmaceutical preparations that have a critical point in terms of halal certification. They contain gelatin as a main component. This study aims to detect the source of commercial hard capsule shells using FTIR Spectroscopy combined with Principal Component Analysis (PCA). The study was conducted by extracting gelatin from commercial hard capsule shells containing drugs (which marked as A, B, C, D, E) and then measuring their absorbance using FTIR. As a comparison, bovine or procine gelatin extracted from a simulated hard capsule shell was used. The FTIR spectrum data was analyzed by using PCA. The results of the analysis showed that the FTIR spectra of bovine and porcine gelatin were very similar, but PCA could classify the differences. The PCA analysis showed that samples A and D had properties similar to porcine gelatin, sample C had properties similar to hard capsule shells made from porcine gelatin, sample B had properties similar to bovine gelatin, and sample E had properties similar to hard capsule shells from bovine gelatin. These results indicate that bovine gelatin and porcine gelatin in simulated hard capsule shells can be distinguished based on the FTIR spectrum combined with PCA. Commercial hard capsule shells can be classified based on their similar properties to bovine and porcine gelatin. However, this research could not confirm the exact source of the gelatin.

Keywords: Porcine gelatin, bovine gelatin, halal analysis, FTIR, Hard Shell capsules

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Introduction

Gelatin is a natural polymer made from the hydrolysis of animal collagen protein and has many uses. It is generally used as an additive in foods, beverages, and medicines, acting as a stabilizer, emulsifier, and gelling agent. Gelatin is generally obtained from mammals, particularly porcine and bovine skin. Porcine skin gelatin is readily available and has favorable characteristics [1,2]. However, porcine gelatin poses some issues for consumers. First, it concerns religious beliefs as Muslims and Jews prohibit the consumption of pork and its derivatives. Second, several diseases, including bovine spongiform encephalopathy (BSE) and swine flu, have affected pigs and cows, raising concerns about consuming these vertebrates. Third, pork and cow derivatives can cause allergic reactions in some consumers. Fourth, the presence of vegetarian consumers makes the use of animal protein problematic in the food industry [3,4].

In the food and pharmaceutical industries, the protein source is often not disclosed, leading consumers to be wary of consuming gelatin products. Consequently, some consumers avoid gelatin products until they are certain about the source of the gelatin used. One product that uses gelatin in high concentrations is the hard capsule shell [4].

Hard capsules are one of the oldest pharmaceutical dosage forms, dating back to ancient Egypt. Despite their long history, the use of hard capsules as a pharmaceutical dosage form continues to increase. Hard capsules are preferred because they mask the taste and odor of drugs, have an attractive color, and disintegrate rapidly in

gastrointestinal fluids [5,6].

.The increase in hard capsule use is greater than that of tablets. The growth of the hard capsule trade in Europe between 1970 and 1975 was 8% to 21%. In 1996, the growth reached 34% [7]. The global empty capsule market was valued at $2,382.7 million in 2020 and is projected to reach $5,230.4 million by 2030, registering a Compound Annual Growth Rate (CAGR) of 8.1% from 2021 to 2030 [8].

Various studies have been conducted using varied analytical methods to differentiate between bovine and porcine gelatin [9], such as by using the Polymerase Chain Reaction (PCR) [10,11], Liquid Chromatography-Mass Spectrophotometry (LCMS) [12], High Performance Liquid Chromatography (HPLC) [13,14], and Gas Chromatography [15].

FTIR (Fourier Transform Infrared) is an IR spectroscopy method widely used for halal certification analyses. FTIR analysis has been widely developed because it is considered easier, faster, cheaper, and environmentally friendly. Analysis of differences between porcine and bovine gelatin has also been conducted by using FTIR and chemometric techniques. The results of these studies indicate that the FTIR method and principal component analysis chemometric techniques can classify both gelatin sources [2,16,17].

PCA (Principal Component Analysis), a chemometric tool, is a data projection technique. PCA reduces complex data and identifies significant data points, which are then used to create score plots for components 1 and 2. The resulting score plots are displayed in quadrants, making them very helpful in classifying an object [18].

In this study, bovine and porcine gelatin was analyzed from commercial hard capsule shell by using FTIR combined with PCA as there are only few publications on differentiating bovine and porcine gelatin in commercial hard capsule shell products. The combination of these two methods is expected to provide functional group data from bovine and porcine gelatin in hard capsule shell to allow the use of this method for halal analysis of drug and cosmetic preparations, especially hard capsule shells.

METHODS

Preparation of Simulated Hard Capsule Shells (SHCS)

Simulated hard capsule shells were made from two types of gelatin: bovine gelatin and porcine gelatin. The gelatin solution in distilled water was heated at 60 °C until a clear solution was formed. Glycerin, aqueous, titanium dioxide solution, and tartrazine were added to the resulting solution. The solution was stirred homogeneously and poured into molds and left to set until a hard capsule shell sheet was obtained [16]. The formula for the hard capsule shell can be seen in **Table 1**.

Table 1. The formula for the hard capsule shell

Ingredient	Percentage
Gelatin	50%
Glyserin	10%
Titanium dioxde	1,25%
Tartrazine	0,05%
Aquadest	Ad 100%

Preparation of Test Samples

Five hard capsule shells containing vitamins from different manufacturers were purchased from the Ciputat market, each with BPOM approval. Each sample is marked as A, B, C, D and E.

Gelatin Extraction from Hard Capsule Shells

A total of 0.3 g of simulated hard capsule shells from bovine and porcine gelatin and commercial hard capsule shells (test samples) were dissolved in 2 mL of hot distilled water at 60 °C. The mixture was placed in a microtube and centrifuged for 30 minutes at 10,000 rpm. Two mL of supernatant was transferred to a microcentrifuge and added to 8 mL of cold acetone in a 1:4 ratio. The supernatant was vortexed for 5 minutes until homogeneous. It was incubated at -20 °C overnight and then centrifuged for 25 minutes at 6,000 rpm. The supernatant was discarded, and the resulting precipitate was washed with acetone three times. The precipitate was then weighed [20,21].

Gelatin Analysis by Fourier Transform Infrared Spectroscopy

As much as 0.2 g of precipitate obtained from the extraction of simulated hard capsule shell sheets and hard capsule shell test samples was dissolved in 600 µL of distilled water at 60 °C until homogeneous and then

placed in an Attenuated Total Reflectance (ATR) tube. Sample scanning was performed by using FTIR spectroscopy at a wavelength of 4000-750 cm^{-1} [21].

Data Analysis by PCA

The functional group data obtained were then analyzed by using PCA techniques by entering the absorbance data from the wavenumbers of both bovine and porcine gelatin standards, simulated bovine and porcine hard capsule shell sheets, and hard capsule shell test samples into Minitab Software to differentiate the functional groups in the gelatin standards, simulated hard capsule shells, and hard capsule shell test samples [22].

RESULT AND DISCUSSION

The initial stage of the research was the creation of simulated hard capsule shells, which served as a comparison for determining the gelatin content of commercial hard capsule shells. Hard capsule shells are made from gelatin (porcine or bovine) and water, with the addition of glycerin, tartrazine dye, and titanium dioxide. Titanium dioxide acts as an opaque agent, making the hard capsule shells opaque [19].

The resulting simulated hard capsule shells were thin, yellow, opaque, and rollable. Organoleptically, simulated hard capsule shells made from bovine gelatin are pale yellow or brownish-yellow, while hard capsule shells made from porcine gelatin are bright yellow. The color of the resulting hard capsule shells matched the color of the gelatin powder used, with porcine gelatin being pure white and bovine gelatin being more yellow-brown.

To analyze the gelatin spectrum from hard capsule shells, the titanium dioxide must be separated. Titanium dioxide has nonspecific IR absorption but exhibits several peaks at wavenumbers around 1450 nm and 1950 nm, which are suspected of interfering with FTIR analysis. Gelatin extraction was performed by using cold acetone, which denatured the protein or gelatin. Acetone can precipitate more protein than other organic solvents such as methanol and chloroform [20]. An overnight incubation process aimed to complete the gelatin precipitation process.

FTIR spectroscopy is a rapid analytical method and has the potential to differentiate the spectra between two samples [21]. This analysis aims to identify the amino acid functional groups of two gelatin sources. FTIR identification is based on the characteristics of the amino acid functional groups that make up the gelatin. In this study, sample scanning was performed at a wavenumbers of 4000-750 cm^{-1} with a resolution of 4 cm^{-1}.

The difference in the spectra between porcine and bovine gelatin can be seen in **Figure 1**. The figure shows that the spectra of porcine and bovine gelatin have very similar patterns. The difference is only seen in the absorbance of each peak of the spectrum. The spectra of porcine and bovine gelatin have characteristics in specific regions, namely at 3600-2300 cm^{-1} (Amide A), 1656-1644 cm^{-1} (Amide I), 1560-1335 cm^{-1} (Amide II), and 1240-670 cm^{-1} (Amide III) [18]. The spectra of bovine and porcine gelatin are similar to those of the gelatin in the study by Hasyim et al, 2010 [21].

The same is also seen in the spectra of bovine and porcine gelatin from simulated capsule shells as shown in **Figure 2**. The gelatin spectrum from the simulated hard capsule shell extraction has distinctive characteristics in the specific regions of 3600-2300 cm^{-1} (Amide A), 1656-1644 cm^{-1} (Amide I), 1560-1335 cm^{-1} (Amide II), and 1240-670 cm^{-1} (Amide III). **Figures 1 and 2** indicate that the gelatin from the simulated capsule

Absorption in the 3290-3280 cm^{-1} region is associated with N-H bond stretching and intramolecular hydrogen bonding in the amine groups within the amino

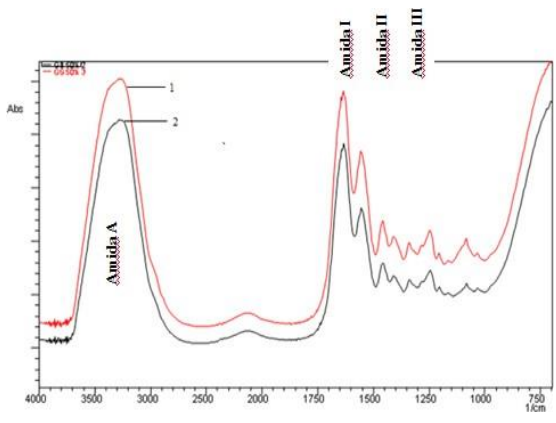

Figure 1. The FTIR spectra of porcine and bovine gelatin. 1: Bovine Gelatin. 2: Porcine Gelatin

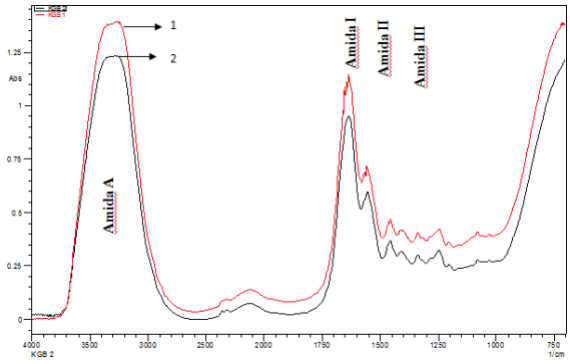

Figure 2. The spectra of bovine and porcine gelatin from simulated capsule shells. 1: bovine gelatin, 2: porcine gelatin

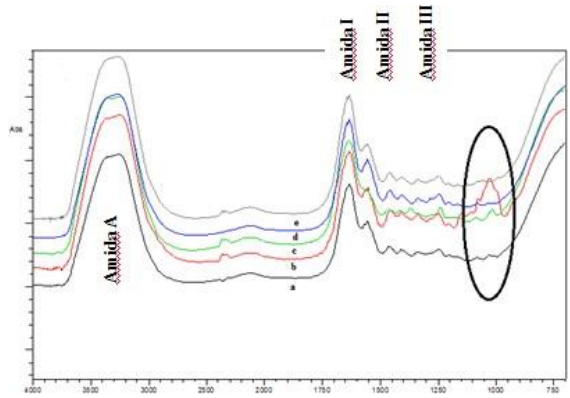

Figure 3. The spectra of gelatin from a commercial hard capsule shell. a: sample A, b: sample B, c: sample C, d: sample D, e: sample E

acid chain. The absorption is parallel-polarized to the N-H bond, indicating the presence of hydrogen bonding interactions within the alpha helical structure of gelatin. The resulting peak can shift to lower frequencies as the hydrogen bond strength increases [21]. The stretching double bond in the carbonyl group C=O interacts with the N-H group of the peptide bond (C-N), appearing in the 1660-1620 cm^{-1} region, which is often referred to as the amide I region. The frequency range of 1660-1650 cm^{-1} represents the alpha helix structure and 1640-1620 cm^{-1} shows the beta sheet structure. The frequency in the amide II region, namely 1550-1520 cm^{-1}, shows the deformation of the N-H group from the alpha helix structure (1550-1540 cm^{-1}) and the beta sheet structure (1525-1520 cm^{-1}) [21]. While the frequency at 1500-1200 cm^{-1} is a representation of CH2 deformation, this region is also specific and becomes a characteristic of several hydrocarbon groups found in some macromolecular compounds, such as fatty acids, proteins, and polysaccharides. The peptide group is a repeating structure of proteins that provides 9 characteristic bonds called amides A, B, and I-VII. The IR absorption characteristics of the peptide chain are shown in **Table 2.**

A key part of this research was measuring commercial hard capsule shells. The spectrum results of the commercial hard capsule shell gelatin are shown in **Figure 3**. Although the spectrum has the same pattern as the simulated hard capsule shells, differences are visible in the amide I, amide II, and amide III regions. To group these differences, the Minitab software used the Principal Component Analysis (PCA) menu. The wavenumber and absorbance values of each peak served as the primary data for the PCA. The results of this grouping are shown in **Figure 4**.

Based on the score plot curve in **Figure 4**, it appears that bovine gelatin (quadrant I) and the simulated capsule shells made from bovine gelatin (quadrant IV) are not in the same quadrant, but both have positive PC1 values. Porcine gelatin (quadrant II) and the hard capsule shells made from porcine gelatin (quadrant III) are in different quadrants, but both have negative PC1 values. This may be due to heating, additives, and the cooling process during production. Furthermore, the extraction process can affect amino acid composition.

Table 2. The IR absorption characteristics of the peptide [5]

Peptide Chain	Wave number (cm-1)	Information
Amide A	3300	NH stretching
Amide B	3100	NH stretching
Amide I	1600-1690	C=O stretching
Amide II	1480-1575	CN stretching, NH bending
Amide III	1229-1301	CN stretching, NH bending
Amide IV	625-767	OCN bending
Amide V	640-800	Out-of-plane NH bending
Amide VI	537-606	Out-of-plane C=O bending
Amide VII	200	Skeletal torsion

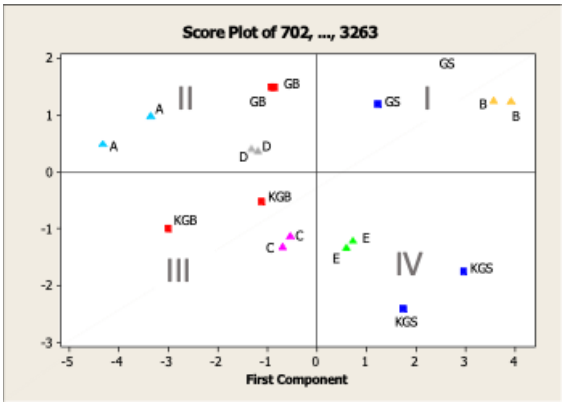

Figure 4. The result of the PCA score plot. GS: Bovine gelatin, GB: Porcine gelatin, KGB: Porcine gelatin from simulated capsule shells, KGS: Bovine gelatin from simulated capsule shells, and A, B, C, D, E: gelatin from commercial hard capsule

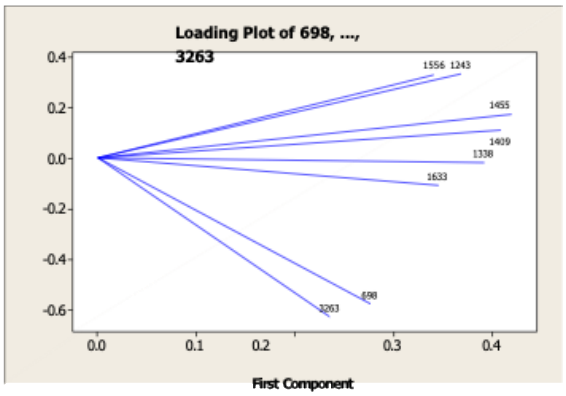

Figure 5. Loading plot curve to determine the most influential wavenumbers in differentiating bovine and porcine gelatin

Test samples A and D were in the same quadrant as porcine gelatin, namely quadrant 2. Test sample C was in the same quadrant as the capsule shells made from porcine gelatin. The results of the quadrant analysis indicate that test samples A, C, and D share similar physicochemical properties with porcine gelatin. Meanwhile, test sample B was in the same quadrant as bovine gelatin, namely quadrant 3. Test sample E was in the same quadrant as capsule shells made from bovine gelatin, indicating that test samples B and E share similar physicochemical properties with bovine gelatin.

To determine the most influential wavenumbers in differentiating bovine and porcine gelatin, a loading plot curve was used, as shown in **Figure 5**. The loading plot curve indicates the wavenumbers that contribute most to the formation of principal component values. The further a variable is from the origin (0,0), the greater its contribution to the PCA process [19].

From the loading plot curve, it is known that the wavenumbers 1455 cm^{-1}, 1409 cm^{-1}, and 1338 cm^{-1} had the furthest horizontal distance from the x = 0 line. This means that these variables have the greatest contribution to the formation of PC1 values with coefficient values of 0.420, 0.408, and 0.392, respectively. Meanwhile, the variables that contributed most to the formation of PC2 had the furthest vertical distance from the y = 0 line. These wavenumbers were 1556 cm^{-1}, 698 cm^{-1}, and 3263 cm^{-1} with coefficient values of 0.341, 0.276, and 0.235, respectively. Other variables with smaller coefficient values still affect the PC1 and PC2 values and play a role in the results of differentiating bovine gelatin and porcine gelatin, but their contribution is not as large as the main variable.

CONCLUSION

The results of the FTIR analysis showed that bovine gelatin and porcine gelatin had almost the similar spectrum, but the two types of gelatin could be distinguished by using a combination of FTIR and chemometric techniques in the Principal Component Analysis (PCA) menu. The most influential spectrum peaks were at wavenumbers 1455 cm^{-1} and 1409 cm^{-1}. The PCA analysis showed that samples A and D had similar properties to those of porcine gelatin, sample C had similar properties to those of simulated hard capsule shells made from porcine gelatin, sample B had similar properties to those of bovine gelatin, and sample E had similar properties to those of hard capsule shells from bovine gelatin.

CONFLICT OF INTEREST

The authors declare that there is no conflicts of interest regarding this investigation.

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