IDENTIFIKASI GELATIN DARI ANJING BERDASARKAN PROFIL ASAM AMINO DAN KEMOMETRIK

IDENTIFICATION OF DOG GELATIN BASED ON AMINO ACID PROFILES AND CHEMOMETRICS

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ABSTRAK

Gelatin adalah protein yang diperoleh dengan memanaskan kulit, tendon, ligamen, dan tulang dengan air. Gelatin banyak digunakan di beberapa produk makanan dan industri farmasi. Beberapa agama seperti Islam melarang pengikutnya mengkonsumsi produk makanan yang mengandung keturunan babi dan anjing, termasuk gelatin. Deteksi adanya kandungan nonhalal dalam produk pangan telah menjadi studi penting di banyak negara. Penelitian ini bertujuan untuk membedakan gelatin anjing dari kambing, sapi dan babi berdasarkan profil asam amino yang dikombinasikan dengan kemometrik dari komponen utama analisis (*Principal Component Analysis/*PCA). Pemisahan dan penentuan asam amino menggunakan *liquid chromatography mass spectroscopy*. Hasil penelitian menunjukkan bahwa lima kandungan asam amino tertinggi dalam gelatin anjing adalah asam glutamat, glisin, alanin, arginin, dan metionin. Parameter persentase tinggi puncak masing-masing asam amino dari masing-masing sampel dianalisis dengan PCA. Berdasarkan PC1 dan PC2, gelatin dari anjing, kambing, sapi, dan babi bisa dibedakan.

Kata kunci: gelatin anjing, profil asam amino, komponen utama analisis.

ABSTRACT

Gelatin is a protein obtained by boilling skin, tendons, ligament, and bones with water. Gelatin is widely use in some food products and pharmaceutical industry. Some religions like Islam prohibited their followers to consume any food products containing pig and dog derivates, incluide gelatin. The detection of some forbidden content in food product has been an important subect of stuydy in many countries. The current study was aimed to differentiate of dog gelatin from goat, bovine, and porcine based on their amino acid profiles combined with chemometric of principal component analysis (PCA). Separation and determination of amino acid using liquid chromatography mass spectroscopy. The

results show that the fifth most amino acid content in dog gelatin were glutamic acid, glicine, alanine, arginine, and methionin. Parameters of peak height percentage of each amino acids from each samples were analyzed by PCA. Based on PC1 and PC2, gelatin from dog, goat, bovine, and porcine could be distinguished.

***Key words**: dog gelatin, amino acid profile, chemometrics.*

Introduction

Gelatin has a large application in pharmaceutical and food product. The amino acid composition and its sequence in gelatin are different from one source to another, but always consist of large amount of glycine, proline, and hydroxyproline (Gilsenan and Ross-Murphy, 2000). Dog and its derivat is classified as nonhalal animal, as followed by the majority of muslims scholar (Regenstein et al., 2003). Some unethical seller replaces source of bovine gelatin with porcine or with dog to get economical profits. Therefore, it is necessary to differentiate between dog, goat, porcine, and bovine gelatins.

Several method have been differentiate gelatins mainly involving bovine and porcine gelatins. Nemati et al. (2004), used reverse phase-high performance liquid chromatography (HPLC) in combination with fluorescence detection, while Zhang et al. (2008) have developed HPLC coupled with mass spectrometry. Demirhan et al. (2012) has developed DNA-based technique using polymerase chain reaction. Raraswati et al. (2014) used reverse phase-high performance liquid chromatography in combination with chemometrics.

In this study, liquid chromatography mass spectroscopy (LCMS) was used for profiling amino acid content present in dog, goat, porcine, and bovine gelatins. The amino acid analyzed using chemometrics of principal component analysis (PCA). PCA is a supervised data projection methode used for classification and differentiation of objects (Miler and Miler, 2005).

Material and Methods

Standards of bovine and porcine gelatins from Sigma (St Louis, USA); LCMS grade acetonitrile, formic acid, water, HCl pro analysis, NaOH pro analysis were obtained from E Merck (Darmstat, Germany). Dog and goat bones obtained from local market in Purwokerto.

Making of Gelatin

Degreasing: the dog and goat bones were washed and cleaned by removing dirt, meat residue, and fat on it. The bones were heating in boiling water for 30 minute while stirred. Furthermore, bones were drained and cut into small pieces (3-5 cm) to expand the surface. Demineralization: bones were added with HCl 5% at the acid resistant container for 2-3 weeks until

ossein was formed. The ossein bones was then washed with bidistilled water until the pH was about 6-7. Extraction: neutral ossein was added with bidistilled water which the ratio between ossein and bidistilled water was 1:3 (w/w). Furthermore it was extracted in waterbath at 80 ˚C for 7 hours, then filtered with filter paper. Drying: the filtrate was drying using an oven by pouring it into plastic plated alumunium pan at 50 ˚C for 24 hours. After gelatin sheets were obtained, then milled using a blender to get gelatin powder.

Sample Preparation

An approximately of 0,1 gram gelatin were carefully weighed, placed in glass vials containing of 5 mL of 6 N HCl. The vials were subsequently sealed with their caps and placed in an oven, which was heated to 110 ˚C. After 22 hours hydrolisis period, the samples were cooled at room temperature. The samples were diluted until 50 mL with bidistilled water and shaken smoothly. Furthermore, each sample was filtered using syringe driven filter with pore size 0.45 µm.

Analysis of Amino Acid with LCMS

The profiles of amino acid in laboratory prepared gelatin and standar gelatin were separated and determined using LCMS. The column used was C18 (2.1 mm x100 mm, particle size 2.7 µm), flow rate 0.3 mL/min, mobile phase used acetonitrile and water (1:1), total analysis time 15 minute, injection volume 10 µL.

Statistical Analysis

Principal Component Analysis (PCA) for classification and differentiation of samples was carried out using Minitab software version 16 (Pennsylvania, USA). Parameters of peak height percentage of each amino acid from each sample were used as variables.

Result and Discussion

The profiles of amino acid were analyzed with LC-ESI-MS by compare the percentage of peak height. Figure 1 shows the mass spectrum of amino acid in goat, bovine, dog, goat, and porcine gelatins.

The level of amino acid in dog, goat, porcine, and bovine gelatins were shown in Table 1. Certain amino acids like glycine, methionin and tryptophan, arginine, glutamic acid, and alanine in gelatine made from dog bones were present in higher level than in gelatin from bovine, porcine, and goat gelatin. While, the concentrations of arginine,

methionin and tryptophan, glisine, glutamic acid, and phenilalanine in goat gelatin were highest. Meanwhile, the levels of methionin and tryptophan, arginine, glutamic acid, phenilalanine and glicine in gelatin from bovine were present as highest. While, argine, glutamic acid, methionin and tryptophan, glycine, and phenylalanine were present as highest in porcine gelatine. This result in line with research conducted by Raraswati et al. (2003) which shows that the highest amino acid content in bovine and porcine gelatins were glycine and threonine.

Table 1. Amino acid composition in goat, bovine, porcine and dog gelatin

Amino Acid	Goat Gelatin	Bovine Gelatin	Porcine Gelatin	Dog Gelatin
	% w/w	% w/w	% w/w	% w/w
Aspartat acid	17,53	25,92	32,44	16,46
Glutamic acid	44,45	47,14	48,53	42,83
Asparagin	5,85	5,25	6,51	5,22
Serine	31,54	23,72	18,75	28,14
Histidin	18,35	19,99	18,19	14,41
Glycine	46,7	38,26	40,36	52,63
Alanine	31,58	30,46	26,07	42,97
Arginine	50,48	52,36	54,7	44,22
Methionine & Tryptophan	47,42	53,18	47,14	47,58
Phenilalanine	34,27	38,88	40,04	31,67
Lysine	20,83	10,41	10.41	17,47

It seems that the peak height of these amino acids can be used as simple discrimination between porcine and bovine gelatins. However, employing only these amino acid profiles for differentiation may not provide enough confidence. Therefore, the application of a multivariate statistical method could be helpful to establish the difference between bovine and porcine gelatins using amino acid profiles as variable.

PCA is a technique for reducing the amount of data when there is correlation present, and this technique is not useful if the variables are uncorrelated (Miller and Miller, 2005). In this study, PCA was used to extract the significant variables from parameter of peak height percentage for each amino acids.

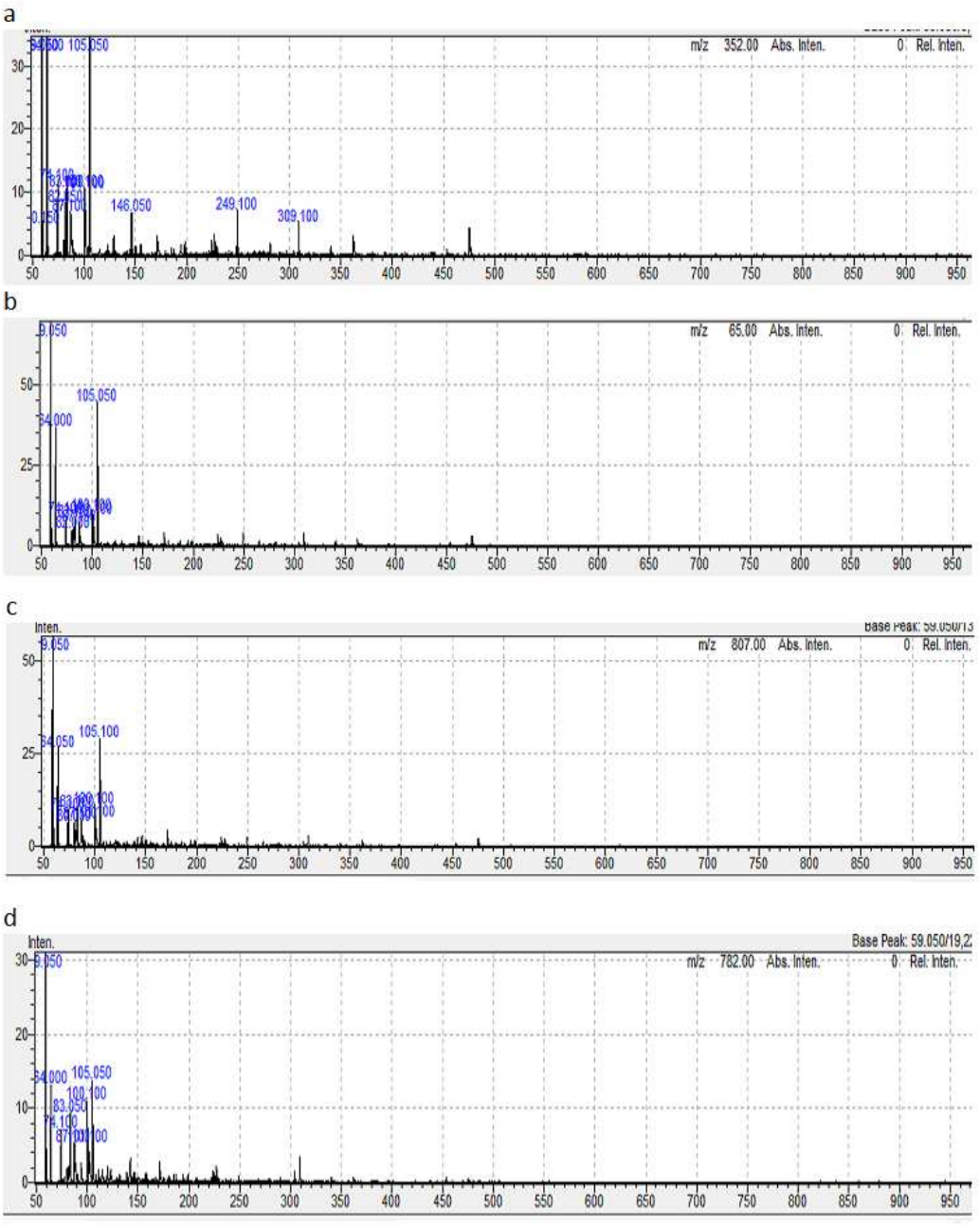

Figure 1. Mass spectrum of amino acid from dog (a), goat (b), bovine (c) and porcine (d) gelatin.

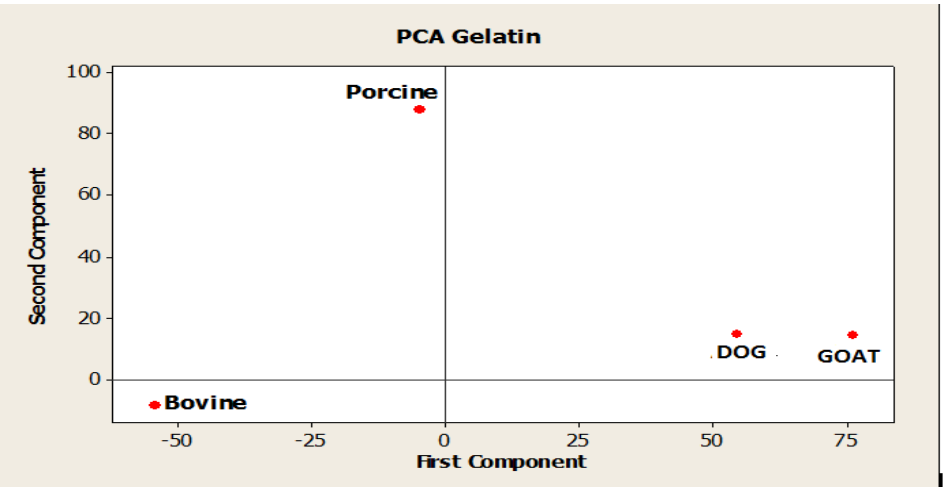

Figure 2. PCA score plot for classification of porcine, bovine, goat and dog gelatins.

Four samples (porcine and bovine gelatins coming from Sigma and dog and goat gelatin) were processed by PCA. The results of PCA were presented in a two dimensional graph. Figure 2 shows the PCA score plot of porcine, dog, goat, and bovine gelatins. The horizontal axis is the scores for the first PC, and the vertical axis for the second PC. Bovine and porcine gelatins, were clearly separated. Dog and goat gelatin were separated but in the same quadran, it shows that dog and goat gelatin has similiarity.

Conclusion

It can be concluded that amino acid profiles in combination with principal component analysis can classify and differentiate dog gelatin from goat, bovine, and porcine gelatin.

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