INTISARI

Umat Islam untuk mengkonsumsi pangan halal terutama di negara dengan penduduk mayoritas beragama Islam terhadap daging yang dikonsumsi semakin meningkat. Indonesia membuat peraturan bahwa semua produk daging di pasaran harus halal. Salah satu dukungan dalam melaksanakan aturan halal adalah autentifikasi melalui pengujian bahan. Penelitian deteksi spesies hewan mayoritas dikembangkan untuk deteksi umumnya babi, sementara untuk daging anjing masih jarang dilakukan. Tujuan penelitian ini adalah untuk melakukan pengembangan metode analisis untuk identifikasi lemak daging anjing dalam produk makanan, memakai metode GC-MS, FTIR, LC-MS dikombinasi dengan kemometrik serta identifikasi DNA daging anjing memakai RT-PCR dengan menggunakan primer spesifik untuk DNA daging anjing.

Lemak dari daging anjing, ayam, babi, kambing, sapi, dan tikus diekstraksi dengan metode Blight & Dyer memakai kloroform: metanol (1:2)/air sebagai pelarut. Lemak hasil ekstraksi digunakan untuk analisis dengan metode GC-MS, FTIR dan LC-MS. Lemak hasil ekstraksi dihidrolisis menjadi asam lemak dan derivatisasi menjadi *fatyy acid methyl ester* (FAME) memakai BF3. Lemak hasil esktraksi sebagian dianalisis dengan metode FTIR. Lemak anjing, sapi, babi, ayam dan kambing diekstraksi dengan metode Blight and Dyer, kemudian dianalisis dengan LC-MS.

Data kromatogram dari GC-MS dianalisis memakai metode kemometrik terutama PCA untuk membedakan lemak anjing dan lemak hewan lainnya. Pita spektrum FTIR yang berhubungan dengan lemak sapi, anjing, babi, kambing, tikus, dan ayam dihitung, diinterpretasikan, dan dianalisa secara kualitatif. Variasi kecil diantara spektrum dianalisis sebagai dasar untuk membedakan lemak anjing dengan lemak hewan lainnya. Semua kromatogram TAG dari LC-MS diolah menggunakan metode kemometrik terutama PCA untuk membedakan lemak anjing dengan lemak hewan lainnya.

Primer kandidat diuji untuk studi spesifisitas menggunakan beberapa DNA dari daging segar dari beberapa spesies. Metode RT-PCR yang dikembangkan juga divalidasi dengan menentukan beberapa parameter linearitas, kepekaan, presisi dan efisiensi.

Lemak anjing yang dianalisis secara *Gas Chromatography-Mass Spectrometry* GC-MS) menunjukkan hasil bahwa lemak anjing mengandung asam lemak: oleat, palmitat, linoleat, stearat dan palmitoleat. Kandungan asam lemak tidak jenuh yang tertinggi dalam lemak anjing adalah asam oleat sebesar 37,71%. Asam lemak jenuh tertinggi berupa asam palmitat sebesar 22,90%. Pengelompokkan lemak hewani dengan *principal component analysis* (PCA) dengan asam lemak sebagai variabel menunjukkan bahwa lemak anjing dapat dibedakan dengan lemak hewan lain.

Analisis lemak anjing secara spektrofotometri *Fourier Transform Infrared* (FTIR) yang dikombinasikan kemometrika *partial least square* (PLS) pada bilangan gelombang 1700-700 cm^{-1} dapat digunakan untuk analisis kuantitatif

lemak anjing dalam bakso sapi dengan validitas metode yang ditunjukkan dengan nilai R^2 sebesar 0,99 untuk metode Folch dan 0,98 untuk metode Blight & Dyer, nilai RMSEC sebesar 2,0% dan 1,8%. Spektrofotometri FTIR yang dikombinasikan dengan kemometrika PCA pada bilangan gelombang 1700-700 cm^{-1} berhasil mengelompokkan lemak anjing dan lemak sapi pada produk bakso di pasaran.

Komposisi TAG lemak anjing yang dianalisis dengan *Liquid Chromatography-Mass Spectrometry* terdiri dari MOMo dan PLP. Metode LC-MS yang dikombinasi dengan kemometrik PCA berhasil membedakan lemak anjing dengan lemak hewan lain berdasarkan komposisi TAG.

Primer COI forward:5'CCTCCAACATTTCCTTAGGTTTAT-3', dan *reverse*: 5'CCTATAGAGGAGACGGTATTT-3' mampu mengamplikasi DNA daging anjing dengan suhu penempelan 56,6°C, batas deteksi 5 ng yang setara dengan 0,1% daging anjing dalam bakso, uji linieritas 100% daging anjing diperoleh nilai E= 95%, dengan R^2= 0,984. Uji linieritas sampel sampel bakso referensi anjing-sapi diperoleh nilai E=67,1% dan nilai R^2 = 0,982. Uji *repeatibility* dengan 100% daging anjing menunjukkan nilai RSD 11,51%, Primer COI spesifik untuk DNA anjing.

Kata kunci: anjing, GC-MS, FTIR, LC-MS dan RT-PCR.

ABSTRACT

The halal meat plays a key role among the International Muslim community. Muslim society need to realize a significant and quickly identification of the halal confirmation. Halal certification for meat is mandatory in Indonesia according to the Indonesian Law No. 33/2014. In order to implement this law, authentication of meats used in products needs to be conducted. Most of the available data is derived from pork detection studies, while those of other species is limited. The objectives of this research were to assess the suitability of FTIR spectroscopy, GC-MS, LC-MS coupled with chemometric principle component analysis (PCA) for rapid quantitative and qualitative (identification) analysis of dog meat, as well as RT-PCR for identification of DNA of the dog using a dog-spesific primer.

The fats of dog, chicken, pork, goat, beef, and rat were extracted with the Blight and Dyer method using chloroform: methanol (1: 2)/ water. The extracted fats were further used for analysis with GC-MS, FTIR and LC-MS methods. A part of the extracted fat was hydrolyzed into fatty acids and derivatized into fatty acid methyl ester (FAME) using BF3, while another part was directly analyzed by FTIR method. The fats of dog, beef, pork, chicken, and goats were extracted using the Blight and Dyer method and then analyzed by LC-MS method.

All chromatogram data from GC-MS were processed using chemometrics method, especially PCA, for discriminating fats from the dog with those from other species. Infra red spectral bands correlated with fats of beef, dogs, pork, goat, rat, and chicken were measured, interpreted, and qualitatively analyzed. The small variations among spectra were utilized as a basis tool to differentiate between fat from the dog with those from other species. All chromatogram data from LC-MS were processed using chemometrics method, especially PCA, for discriminating fats from the dog with those from other species.

The dog specific primers were designed using IDT software and subjected to NCBI BLAST procedure. The candidate primers were tested for specificity study using DNAs from fresh meat of some species. The developed method was validated by determining several parameters, including linearity, sensitivity, precision, and efficiency.

Gas Chromatography-Mass Spectrometry analysis demonstrated that oleic acid, palmitic acid, linoleic acid, stearic acid, and palmitoleic acid were the major fatty acid components in fats of dog. The highest unsaturated and saturated fatty acid in fats of the dog were oleic acid (37.71%) and palmitic acid (22.90%), respectively. Analysis of PCA from GCMS chromatogram was capable of identifying fats from dog, chicken, beef, pork and goat.

Principal Component Analysis of FTIR spectra at wavenumber regions of 1700-700 cm^{-1} was able to identify dog meat in meatballs. Hence, PCA was succesfully applied for classification of meatballs containing dog meat and other meats. These wavenumbers were also successfully used for quantitative analysis of dog meat in meatballs using PLSR model. Based on statistical parameters used, $R^2$and RMSEC, Folch extraction method resulted in higher R^2(0.9906>0.9856) and lower RMSEC than that of Bligh and Dyer.

The results of LC-MS analysis showed that the TAG contents in fats of dog were including MOMo and PLP. Liquid Chromatography-Mass Spectrometry spectra-based PCA was succesfully applied for classification between meatbals containing dog meat and other meats.

Primer COI forward: 5'CCTCCAACATTTCCTTAGGTTTAT-3', and reverse: 5'CCTATAGAGGAGACGGTATTT-3' was able to amplify specific DNA target at the optimized annealing temperature of 56.6˚C. Limit of detection (LOD) was 5 ng of DNA, which corresponded to 0.1% of dog meat in meatballs. The repeatability, expressed with relative standard deviation (RSD), and efficiency evaluation of the method were in the acceptable range. Hence, the method was successfully used for the analysis of marketed meatball samples.

Keywords: dog meat, GC-MS, FTIR, LC-MS and RT-PCR