ANIMAL FATS DISCRIMINATION USING LASER INDUCED BREAKDOWN SPECTROSCOPY AND PRINCIPAL COMPONENT ANALYSIS

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ABSTRACT

Adulteration of lard in foods raises concerns among Muslims and Jews. To address this issue, laser induced breakdown spectroscopy (LIBS) system is used in this work to differentiate various extracted animal fats in liquid form. However, laser-liquid interaction produces splashing due to the shockwave effect thus generate poor plasma plume in LIBS emission signals. LIBS difficulties in liquid form were overcome by freezing the samples and turned into solid form using freezer and liquid nitrogen. Then, the frozen samples were ablated using Nd:YAG laser of 1064 nm wavelength, 170 mJ pulsed energy and 6 ns pulse duration to produce plasma on sample's surfaces. The plasma was captured using a spectrometer *via* optical fiber. The spectrometer was connected to a computer for displaying LIBS signals. The LIBS signals of the samples were then further evaluated using principal component analysis (PCA). PCA is a statistical analysis method for reducing the dimensionality of large data sets without any information loss. Experimental findings indicate that LIBS emission intensity of extracted chicken and lamb fats using liquid nitrogen method was 4 - 37 % and 4 - 19 % higher than freezer method, respectively. However, LIBS emission intensity of extracted beef fat and lard using freezer method was 12 - 41 % and 6 - 59 % higher than liquid nitrogen method, respectively. PCA demonstrated that the data points of extracted animal fats using liquid nitrogen method were more clustered than those frozen in the freezer. PCA also revealed that good discrimination achieved between extracted animal fats using liquid nitrogen method compared to the freezer freezing method. Therefore, LIBS system coupled with the PCA approach has high potential for detection of animal fats in food products.

ABSTRAK

Pengadukan lemak khinzir dalam makanan meningkatkan kebimbangan dalam kalangan orang Islam dan Yahudi. Untuk menangani isu ini, sistem spektroskopi leraian aruhan laser (LIBS) digunakan dalam kajian ini untuk membezakan pelbagai ekstrak lemak haiwan dalam bentuk cecair. Walau bagaimanapun, interaksi laser-cecair menghasilkan percikan disebabkan oleh kesan gelombang kejutan sehingga menghasilkan kepulan plasma yang lemah dalam isyarat pancaran LIBS. Kesukaran LIBS dalam bentuk cecair diatasi dengan membekukan sampel dan diubah ke bentuk pepejal menggunakan penyejuk beku dan cecair nitrogen. Kemudian, sampel beku diablasikan menggunakan laser Nd:YAG dengan panjang gelombang 1064 nm, tenaga denyut 170 mJ dan tempoh denyut 6 ns untuk menghasilkan plasma di atas permukaan sampel. Plasma ditangkap menggunakan spektrometer melalui gentian optik. Spektrometer telah disambungkan kepada komputer untuk memaparkan isyarat LIBS. Isyarat LIBS sampel kemudian dinilai lebih lanjut menggunakan analisis komponen utama (PCA). PCA adalah kaedah analisis statistik untuk mengurangkan dimensi set data yang besar tanpa kehilangan maklumat. Penemuan eksperimen menunjukkan bahawa keamatan pancaran LIBS bagi ekstrak lemak ayam dan kambing menggunakan kaedah cecair nitrogen masing-masing adalah 4 - 37 % dan 4 - 39 % lebih tinggi daripada kaedah penyejuk beku. Walau bagaimanapun, keamatan pancaran LIBS bagi ekstrak lemak lembu dan khinzir menggunakan kaedah penyejuk beku masing-masing adalah 12 - 41 % dan 6 - 59 % lebih tinggi daripada kaedah cecair nitrogen. PCA menunjukkan bahawa titik data bagi ekstrak lemak haiwan menggunakan kaedah cecair nitrogen lebih berkelompok berbanding yang dibekukan di dalam penyejuk beku. PCA juga mendedahkan bahawa diskriminasi yang baik telah dicapai antara ekstrak lemak haiwan menggunakan kaedah cecair nitrogen berbanding kaedah pembekuan penyejuk beku. Oleh itu, sistem LIBS diganding dengan pendekatan PCA mempunyai potensi tinggi untuk pengesanan lemak haiwan dalam produk makanan.

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LIST OF ABBREVIATIONS

GC-MS	-	Gas Chromatography Mass Spectrometry
EA-IRMS	-	Elemental Analyzer-Isotope Ratio Mass Spectrometry
FTIR	-	Fourier Transform Infrared Spectroscopy
LIBS	-	Laser Induced Breakdown Spectroscopy
PCA	-	Principal Component Analysis
PCs	-	Principal Components
PC	-	Principal Component
w/w	-	Weight per weight
AAS	-	Atomic Absorption Spectroscopy
ICP-MS	-	Inductively Coupled Plasma Mass Spectroscopy
GC	-	Gas Chromatography
PCR	-	Polymerase Chain Reaction
DSC	-	Differential Scanning Calorimetry
DNA	-	Deoxyribonucleic acid
TG	-	Triacylglycerol
VCO	-	Virgin coconut oil
GC x GC-TOF/MS	-	Two-Dimensional Gas Chromatography and Time-of-Flight Mass Spectrometry
GC-SAW	-	Gas Chromatography with Surface Acoustic Wave
PLS-DA	-	Partial least square-Discriminant analysis
CA	-	Cluster analysis
v/v	-	Volume per volume
PLS	-	Partial least square
LDA	-	Linear Discriminant Analysis
ROC	-	Receiver Operating Characteristics
LOD	-	Limit of detection
PLSR	-	Partial least square regression
ANOVA	-	Analysis of variance
Nd:YAG	-	Neodymium-doped yttrium aluminium garnet
SNR	-	Signal-to-noise ratio

LPE	-	Laser pulse energy
TD	-	Time delay
DLS	-	Distance lens to sample
NIST	-	National Institute of Standards and Technology
VALD	-	Vienna Atomic Line Databases
TEC	-	Thermoelectric cooler
PET	-	Polyethylene terephthalate
PE	-	Polyethylene
PP	-	Polypropylene
PS	-	Polystyrene
PA	-	Polyacrylate
SVD	-	Singular Value Decomposition
NIPALS	-	Nonlinear Iterative Partial Least Squares
ATR	-	Attenuated total reflectance
NIR/MIR	-	Near-infrared/Mid-infrared
DTGS	-	Deuterated triglycine sulphate
FL	-	Flashlamp
NA	-	Numerical aperture
F1	-	First group of freezer method
F2	-	Second group of freezer method
F3	-	Third group of freezer method
N1	-	First group of liquid nitrogen method
N2	-	Second group of liquid nitrogen method
N3	-	Third group of liquid nitrogen method
a.u.	-	Arbitrary unit

LIST OF SYMBOLS

nm	-	Nanometer
^{o}C	-	Degree Celsius
C18:0	-	Stearic acid
C	-	Carbon
O	-	Oxygen
H	-	Hydrogen
Mg	-	Magnesium
Na	-	Sodium
Ca	-	Calcium
K	-	Potassium
Fe	-	Iron
C18:1n9c	-	Oleic acid
C18:2n6c	-	Linoleic acid
C52, C54	-	Examples of Triglycerides
MHz	-	Megahertz
kHz	-	Kilohertz
cm^{-1}	-	Reciprocal centimeter (wavenumber)
Pb	-	Lead
µs	-	Microseconds
W	-	Watt
J	-	Joule
cm^{2}	-	Centimeter square
N	-	Nitrogen
Cl	-	Chlorine
Sr	-	Strontium
Ba	-	Barium
Cu	-	Copper
Cd	-	Cadmium
Hg	-	Mercury
Cr	-	Chromium

NaCl	-	Sodium chloride
mg/KG	-	Milligram per kilogram
mm	-	Millimeter
µl	-	Microliter
ZnSe	-	Zinc selenide
ml	-	Millilitre
s	-	Seconds
mJ	-	Millijoule
ms	-	Milliseconds
Hz	-	Hertz
mm^2	-	Millimeter square (area)
θ	-	Angle of optical fiber to direction of laser beam
µm	-	Micrometer

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CHAPTER 1

INTRODUCTION

1.1 Background of Study

Adulteration of lard in food products is a religious sensitivity to Muslim and Jew societies (Kurniawati, Rohman and Triyana, 2014). This is because they are prohibited to consume foods contaminated with pig derivatives such as lard (Kwon and Tamang, 2015). The pig derivatives are not halal and not kosher in Islamic and Jews (Regenstein, Chaudry, and Regenstein, 2003) respectively. Usually, lard is used to increase the texture, taste and profit of certain food products such as virgin coconut oil (Xu *et al*., 2015), chocolate (Suparman *et al*., 2015) and cocoa butter (Azir *et al*., 2017). According to Che Man *et al.* (2014), pure lard is prepared through heating and filtering processes of raw lard before mix with palm oil. The contamination of lard in virgin coconut oil will reduce the quality and benefits of this expensive oil (Tengku Mansor, Che Man and Rohman, 2011). The contaminated of cheaper ingredient such as lard also will lead to false labelling of food ingredients (Kim, Kim & Park, 2015). To address all these issues, detection of lard in food products using promising approaches is highly needed.

Numerous techniques have been developed for lard detection in food products. For instance, Gas Chromatography Mass Spectrometry (GCMS) (Nizar, Marikkar, and Hashim, 2013), dielectric spectroscopy (Amat Sairin *et al*., 2019) and Fourier Transform Infrared (FTIR) spectroscopy (Rohman and Che Man, 2010) are used to discriminate lard from beef, chicken and mutton fats. Nurrulhidayah *et al*. (2015) employed differential scanning calorimetry (DSC) for detection of lard in butter. Meanwhile, real-time polymerase chain reaction (PCR) is utilized to detect lard adulteration in chocolate (Rosman *et al*., 2016). However, these techniques are tedious, too laborious and costly. Therefore, Laser Induced Breakdown Spectroscopy

(LIBS) has been developed for discrimination of fat and nerve tissues in the pig heads (Mehari *et al*., 2016).

LIBS is an atomic emission spectroscopy technique which utilises laser pulses to ablate small volume of the sample. In LIBS system, high energy of laser pulses is focused on the sample surface to create plasma, containing ionize elements of the sample. LIBS has been widely applied in various fields such as biological identification (Multari *et al.*, 2013), space exploration (Knight *et al*., 2000), environmental monitoring (Kumar *et al.*, 2013) and material analysis (Hussain and Gondal, 2013) because of its versatility for analysing materials either in solid, liquid or gas phase.

In scientific research, most of LIBS applications are focused on solid rather than liquid analysis. LIBS on liquid often face difficulties to ablate or poorly ablate subsequently yield low signal intensity. This is due to high absorption of laser energy during liquid vaporisation process thus leaving only a small amount of energy for plasma formation (Lazic and Ciaffi, 2017). The interaction between laser and liquid also produced liquid splashing impacts from strong shock wave thus affecting the quality of emission signals (Markiewicz-Keszycka *et al.*, 2017).

There are two types of common LIBS system which are non-gated and gated LIBS systems. The uses of non-gated LIBS system give arises to strong continuum emission in the spectrum of the sample due to Bremsstrahlung effect (Se, Goshal and Wahab, 2019). From the literature, the researchers preferred to use gated LIBS systems to reduce the Bremsstrahlung effect (Bilge *et al*., 2016; Wang *et al*., 2019). In gated LIBS system, continuum emission in signal is reduced with the optimization of delay time between laser pulse and detection of plasma. However, the instrumentation of gated LIBS system is expensive and complicated. Alternatively, the non-gated LIBS system which relatively cheaper and simpler system coupled with partial least square regression (PLSR) has been used for prediction of calcium, sodium and magnesium in honey (Se *et al*., 2019). Different types of skin, fat, muscle and nerve tissues in pig heads were distinguished using the non-gated LIBS

system in combination with principal component analysis (PCA) (Kanawade *et al*., 2013).

Current studies indicate PCA is suitable for overcoming difficulties in analysing large measurements of LIBS data which have similar spectra for all samples (Porizka *et al.*, 2018). PCA is the multivariate analysis used to recognize the hidden pattern and to highlight main similarities and differences found in similar LIBS signals of different samples. Samples are grouped or clustered in PCA result based on their similarities. For instance, LIBS coupled with PCA has been used to discriminate different tissues in pig heads (Kanawade *et al.*, 2013) and various types of milks (Alfarraj *et al*., 2018).

Since LIBS produced weak signals on liquid samples, this study aims to improve the LIBS emission intensity of extracted animal fats in liquid form using freezing methods. The samples used in the experiments are extracted chicken fat, beef fat, lamb fat and lard freeze using both freezer and liquid nitrogen methods to transform them into solid form. The LIBS signals emission intensity of the samples from LIBS system was compared between both freezing methods. The performance of LIBS emission intensity of frozen extracted animal fats was further evaluated using PCA in the score plot. Then, LIBS system in combination with PCA was employed to differentiate various kinds of frozen extracted animal fats from both freezing methods. This coupled system also was used to discriminate the pure and adulterated frozen extracted animal fats using liquid nitrogen method.

1.2 Problem Statement

Recent techniques used for detection of lard in food products are costly, has complex procedures, tedious, require chemical reagents and high amount of samples. Therefore, LIBS is developed in order to overcome these problems. However, laser-liquid interaction in LIBS technique has several challenges due to most of the laser energy lost in vaporization of the liquid and conversion into mechanical effects such as the generation of shock wave and cavitation bubbles. These results in low LIBS

emission intensity or no LIBS signal being emitted. Besides, the shock wave effect also leads to liquid splashing on optical components of the LIBS system thus prevent the emitted signal received by optical detector. Furthermore, LIBS signals generated from non-gated LIBS system has continuum background in the signals which is caused by radiative recombination and Bremsstrahlung effect. This continuum background can interfere with the important emission lines in the signals of the samples.

Herein, this work uses two different freezing methods to solidify extracted animal fat in liquid form using freezer and liquid nitrogen methods. Through the freezing process, the liquid phase extracted animal fats is transformed into solid-phase samples to overcome liquid splashing due to the shockwave effect, thus improve the interaction between the laser beam and the samples. Then, PCA is used to further analyse signals without removing the continuum background in LIBS signals of frozen extracted animal fats. PCA also is utilized to evaluate the performance of LIBS signals of frozen extracted animal fats from both freezing methods in the score plot as the temperature of frozen samples is increased during LIBS measurements.

1.3 Objectives of Study

This study aims to achieve the following objectives:

1. To optimize LIBS parameters for laser-liquid interaction of frozen extracted animal fats.
2. To evaluate the performance of LIBS signals of frozen extracted animal fats using freezer and liquid nitrogen methods based on LIBS emission intensity comparison and PCA approach.
3. To evaluate the effect of temperature on LIBS signals of frozen extracted animal fats using PCA approach.

4. To discriminate various frozen extracted animal fats and subsequently discriminate the pure and adulterated frozen extracted animal fats using LIBS system coupled with PCA approach.

1.4 Scope of Study

Extracted chicken fat, beef fat, lamb fat and lard were prepared *via* heating and filtering processes of raw animal fats. The purity of extracted animal fats was determined using FTIR spectroscopy. The adulterated extracted animal fats were prepared by mixing the pure animal fats with 1 - 50 % of other extracted animal fats. For example, extracted chicken fat was adulterated with 1 %, 5 %, 10 %, 30 % and 50 % of extracted lard to produce the adulterated extracted chicken fats. These adulterated extracted animal fats were prepared and tested in this study to check the purity of animal fat using non-gated LIBS system. Freezer and liquid nitrogen methods were used to transform extracted animal fats in liquid form into solid phase prior to ablation process. LIBS parameters such as laser pulse energy (LPE), distance between lens and sample surface (DLS), integration time of spectrometer and angle of optical fiber to collect plasma (θ) were optimized for LIBS measurements of frozen extracted animal fats. Initial and final temperature of frozen extracted animal fats also was recorded.

The emission lines presence in LIBS spectra of frozen extracted animal fat were identified accordingly to NIST atomic spectra database (Kramida *et al*., 2019). LIBS emission intensity of each frozen extracted animal fat was compared between both freezer and liquid nitrogen methods as the temperature of frozen samples was increased. 30 LIBS spectra of each sample were then divided into three groups represented LIBS spectra collected at the early, middle and final parts of ablation process. LIBS spectra of frozen sample at each group were averaging and compared between both freezing methods. 30 LIBS spectra of each frozen extracted animal fat were then evaluated using PCA. The distribution of data points in all three groups of each frozen extracted animal fat using freezer method were compared to liquid nitrogen method.

The effect of temperature increased in frozen sample on LIBS signals was studied using PCA approach. Extracted lard was chosen and ablated with 50 laser pulses to produce 50 LIBS spectra. These 50 LIBS spectra were also divided into three groups represent first 17, second 17 and remaining 16 LIBS spectra of frozen extracted lard. These 50 LIBS spectra also were further evaluated using PCA as the temperature of frozen extracted lard was increased from 1^{st} to 50^{th} shots. Distribution of data points in the 1^{st}, 2^{nd} and 3^{rd} groups were compared between freezer and liquid nitrogen methods.

Finally, LIBS system coupled with PCA was employed to discriminate different kinds of extracted animal fats froze using both freezing methods. The combination of LIBS system, liquid nitrogen freezing method and PCA approach also used to discriminate the pure and adulterated frozen extracted animal fats in 2D and 3D score plots. Liquid nitrogen was chosen due to easier handling and is a rapid method to freeze the extracted animal fat compared to freezer method. In this study, it is not recommended to freeze extracted animal fat using liquid nitrogen for longer time because the frozen sample will turn into brittle surface. The brittle surface of frozen sample easily crack after ablated with laser pulses thus produce poor signals.

1.5 Significance of Study

The findings from this study beneficial to the application of lard detection in food products. The application of LIBS system coupled with PCA is rapid, has lower cost and easy procedures of measurements, free of chemical substance and produce promising data for detection of contamination of lard in food products. This application is important in the Halal food industry to verify the contents of food products before distribute to Muslim consumers. Furthermore, this application also can be used to identify the concentration of animal fat in food products and will benefits to the food industry in Muslim country.

In addition, this study will beneficial to other researchers in enhancing LIBS emission intensity of liquid sample using freezing method such as freezer and liquid nitrogen method. The importance of using multivariate analysis such as PCA in evaluating similar LIBS signal is revealed. The stability of LIBS signal is successfully explored using PCA approach. The enhancement of LIBS signal especially in liquid sample is important to increase the sensitivity of LIBS detection thus will improve the classification of the samples.

1.6 Outlines of Study

The thesis consists of five chapters. The introduction, problem statement, objectives of the study, scope of the study and significance of the study are included in Chapter 1. Chapter 2 includes the literature review related to this study including characteristics and processing of animal fat, authentication techniques used for detection of animal fat, basic principle and application of LIBS system and application of PCA in analyzing LIBS spectra. The research methodology in Chapter 3 describes flowchart of this study, sample preparation, experimental works and data analysis for the frozen extracted animal fats. The comparison of LIBS emission intensity and PCA results of frozen extracted animal fats using freezer and liquid nitrogen freezing methods are presented in Chapter 4. The conclusion and recommendations for future studies are written in Chapter 5.

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LIST OF PUBLICATIONS

Journal of Impact Factor

1. **Hanasil, N. S.**, Raja Ibrahim, R. K., Duralim, M., Sapingi, H. H. J. & Mahdi, M. A. (2020). EXPRESS: Signal enhancement evaluation of laser induced breakdown spectroscopy of extracted animal fats using principal component analysis approach. *Applied Spectroscopy*. (IF: 2.064)
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2. **Hanasil, N. S.**, Raja Ibrahim, R. K., Duralim, M. & Sapingi, H. H. J. (2020). Discrimination of extracted animal fats using laser-induced breakdown spectroscopy assisted with principal component analysis. *Jurnal Teknologi*, 82(4): 125-130. (IF: 0)

Non-Indexed Conference Proceeding

1. **Hanasil, N. S.** & Raja Ibrahim, R. K. (2016). Laser induced breakdown spectroscopy (LIBS) applied for analysis of fat differentiation between chicken, lamb, beef and pork. *Proceedings 4th International Science Postgraduate Conference 2016 (ISPC2016)*. 22nd - 24th February 2016. Universiti Teknologi Malaysia, pp. 420–428. (IF: 0)