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*Inspiring Innovation with Integrity
in Agriculture, Ocean and Bioscience for a sustainable World*

Autentikasi Halal melalui Pendekatan Metabolomik

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*Disampaikan pada Seri Kuliah Daring Falsafah Sains Halal
PUI – PT Institute for Halal Industry and System, Universitas Gajah Mada
Sabtu 31 Oktober 2020*



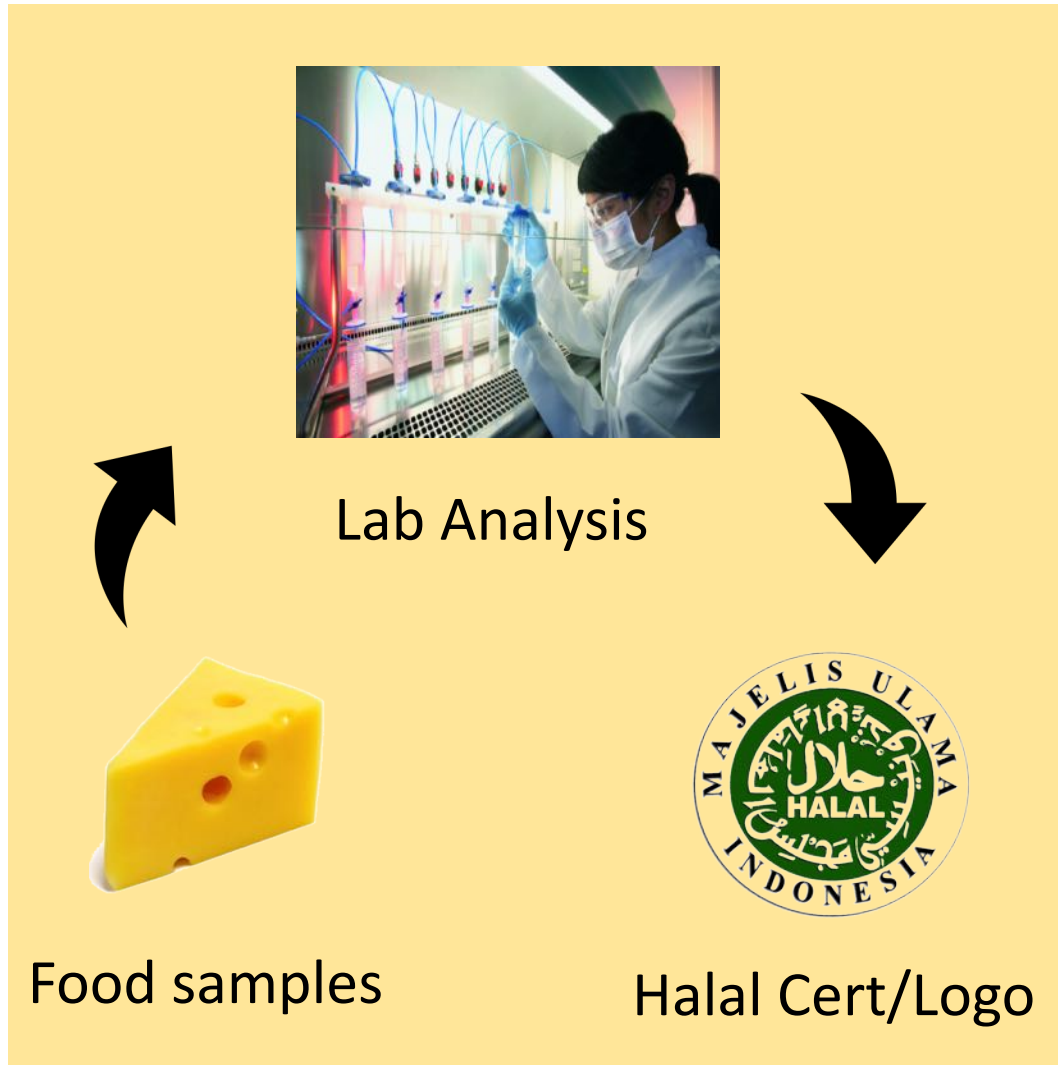
- Halal Status Determination : Halal Audit vs Laboratory Analysis
- Omics Approach in Halal Food Authentication
- Conclusion/Perspectives



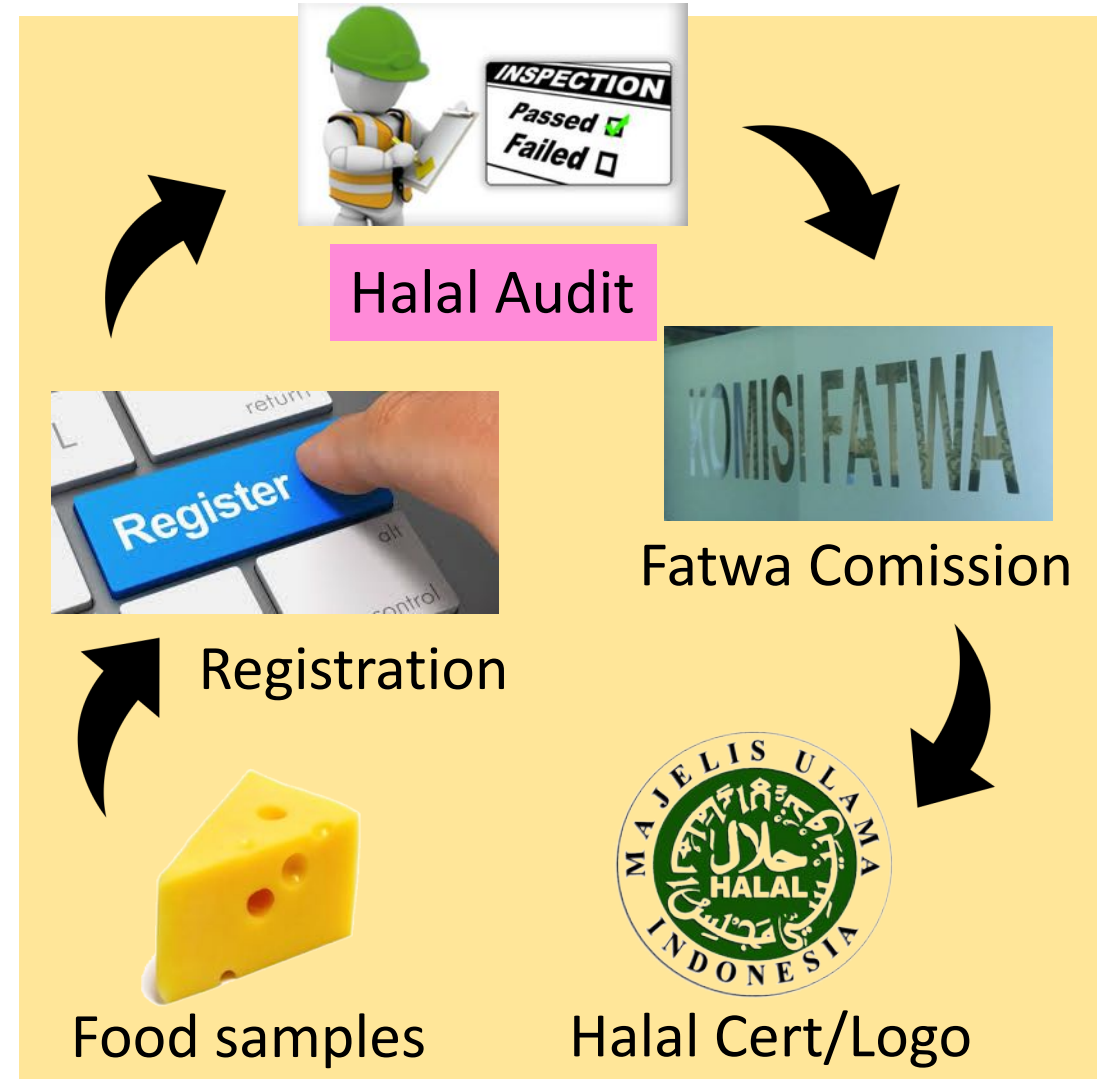
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Halal Certification: Halal Audit vs Laboratory Analysis



?



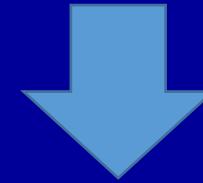
1. Some products must be analyzed in laboratory at the time of certification process by LPPOM MUI. Sampling shall be performed on final products and / or raw materials (particularly new raw material) at the time of audit. The following are the list of products and/or raw materials to be obligatorily analyzed in laboratory:

No.	Product	Test Target	Sample Taken
1	Processed meats or any products using meat ingredients, excluding seasoning products, fats and fatty acids.	Pork Protein	Final product
2	Seasoning products using animal ingredients, e.g: meats.	Pork DNA	Final product
3	Items using animal ingredients, e.g: animal skins, bones, furs, etc.	Pork DNA	Raw material or final product
4	Restaurant / catering / kitchen menus using fresh meats or processed meats, excluding seasoning products, fats and fatty acids.	Pork Protein	Raw material
5	Restaurant / catering / kitchen menus using seasoning products from animal ingredients, c.g: meats not having MUI <i>halal</i> certificate.	Pork DNA	Raw material
6	Animal derivative products or any products containing animal derivative (animal extracts, gelatins, bones, etc)	Pork DNA	Final product
7	Products using gelatins, e.g: capsules, chocolates, candies, cakes, vitamins, drugs, resins, cosmetics etc.	Pork DNA	Raw material (gelatin)
8	Enzyme products from animal sources	Pork DNA	Final product
9	Products using enzymes from animal sources	Pork DNA	Raw material
10	Drinks suspected of containing ethanol which are estimated to contain final ethanol $\geq 0.5\%$.	Ethanol residue	Final product
11	Cosmetic products classified as waterproof / water resistant and inks for general election use	Water penetration	Final product
12	Recycled water	Clean Water Quality Standard	Final product

Notes:

- Testing shall be conducted at test laboratory that has been accredited and / or by implementing SNI ISO/IEC 17025: 2017 and appointed by LPPOM MUI.

Current Lab analysis status in halal certification in Indonesia



as halal supporting documents for the respective materials. It is not equal to halal certificate (SK Direktur LPPOM-MUI 2014)



HALAL ANIMALS
WHICH MEATS COME FROM
HALAL AND NON-HALAL SLAUGHTERING?
HOW ABOUT A PROPER STUNNING?

Rules of halal method

- Uttered - "bismillahi Allahu Akbar"
- Done by - adult sensible Muslim
- **Stunning** - allowed
- Should done **quickly**
- **Casting**- laid on its back
- Neck vessels and passages (oesophagus and trachea) are severed by a **single slash of a sharp knife**
- must not - **in the sight of the beast**
- Prior to killing – should feed water

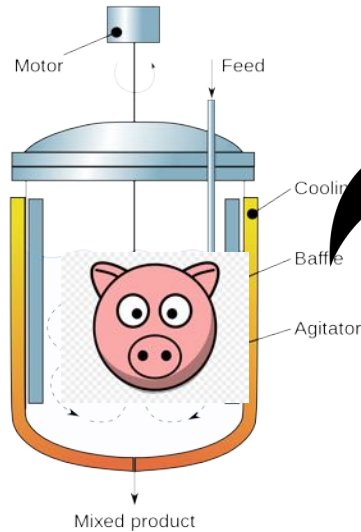
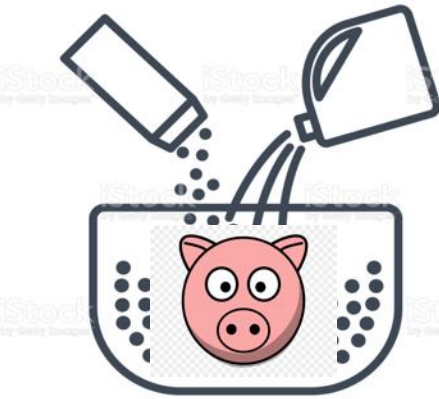
A swift, deep incision with a very sharp knife on the throat, cutting the wind pipe, jugular veins and carotid arteries of both sides but leaving the spinal cord intact. The animal is then left to bleed to death

A diagram illustrating the halal slaughter method. It shows a cross-section of an animal's head and neck. A sharp knife is shown making a single, deep incision on the throat, severing the windpipe, jugular veins, and carotid arteries. The diagram is labeled with 'A' in a circle in the top right corner.

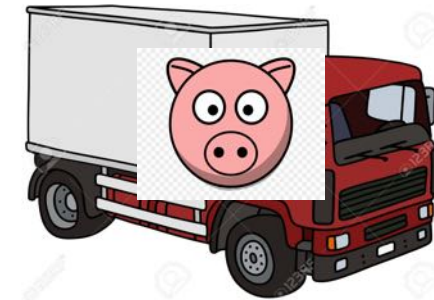
Food Production Process

Minor ingredients

Major ingredients



Production Facility



*Processing aids : materials important to make the product but not present in the final product, e.g. enzyme, active carbon, water to wash, brush to clean, etc

Bone Derived Activated Carbon

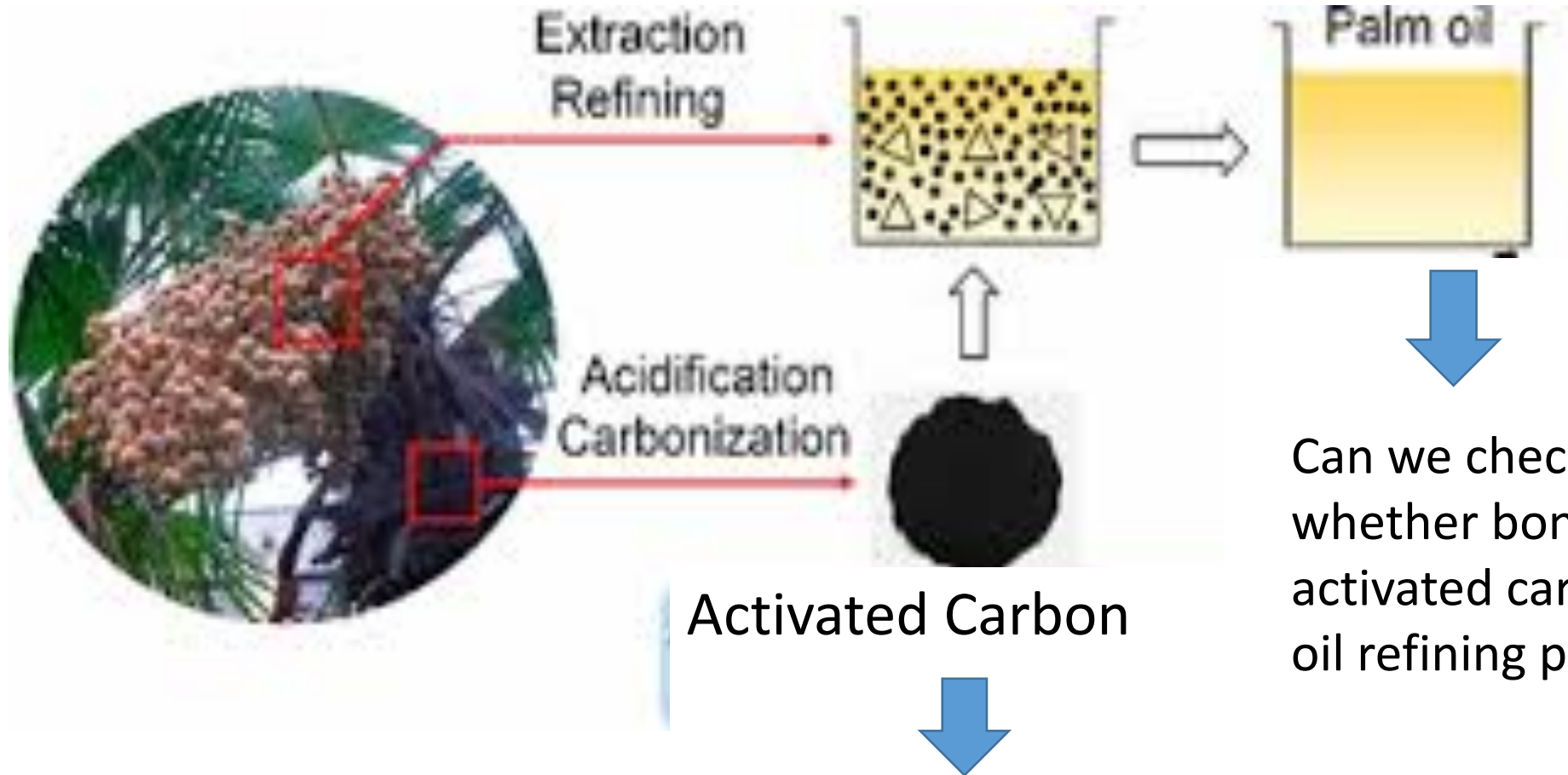
Activated carbon/bone char:

- Produced by burning of bone and converting it into carbon.
- It is used as bleaching agent (in sugar refinery, glucose syrup refinery, drinking water treatment, oil refinery, and other bleaching processes)
 - Example: Fijafluor[®]
- Other possible source: wood, earth



FIJAFLUOR[®] is the trademark of an innovative activated carbon manufactured from cattle bones, under strict healthiness and quality controls in the process of obtaining HIDROXIAPATITA (tricalcium phosphate) and activated carbon.

FIJA FLUOR is an ionic exchanger. Carbon is obtained through the activation process of cattle bones specifically designed to remove fluorine and some other organic and inorganic compounds from liquids and fluids.



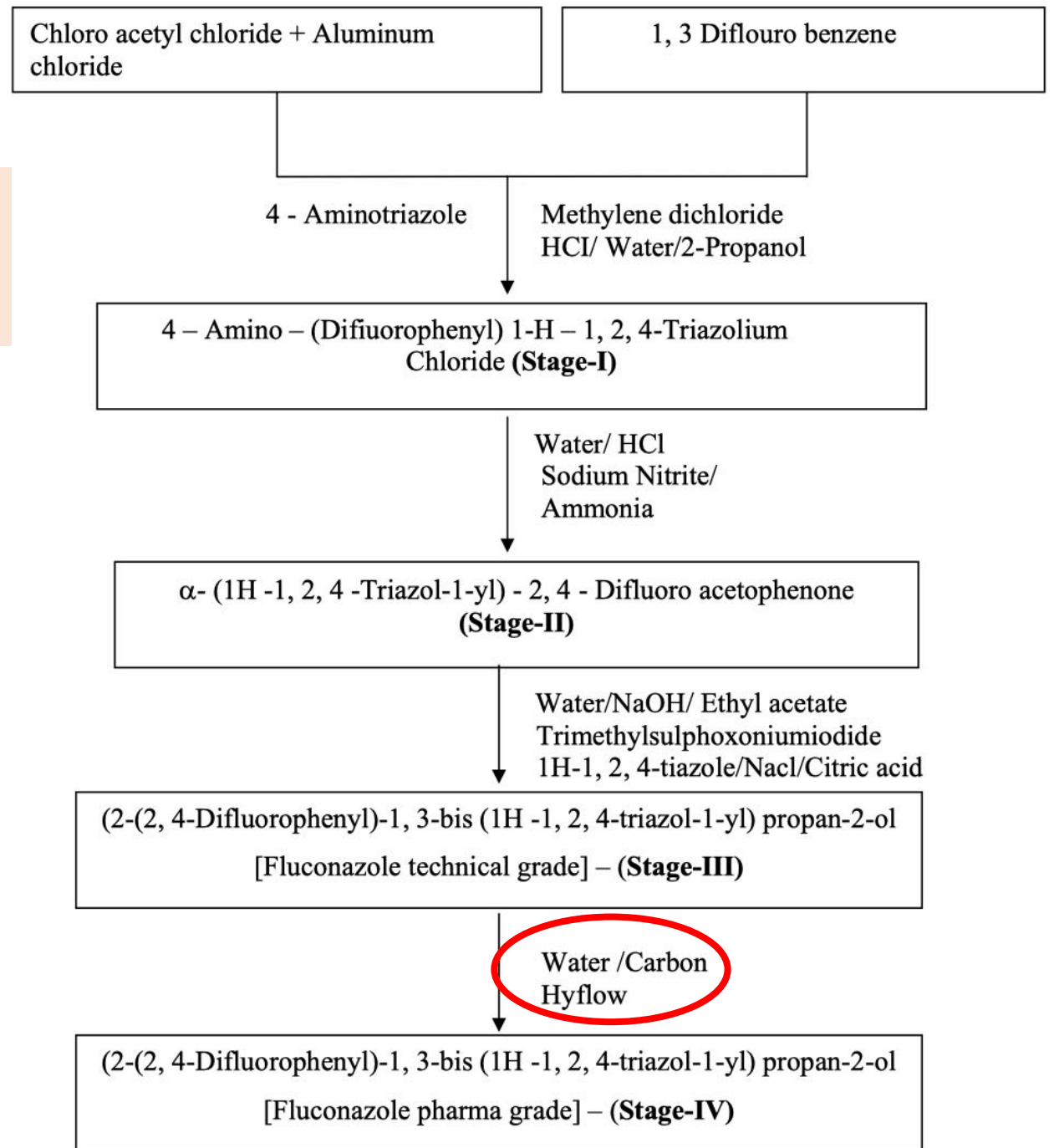
Can we check the oil in the Lab whether bone derived activated carbon was used in oil refining process?

In halal certification by audit : Documents explaining the origin of activated carbon

How about medicines?



During the audit, this flow chart was requested



Fluconazole tablet/capsules/cream
Send to lab to check the origin of activated carbon?



?



**Cysteine : component of bread
leavening agents**



BREAD



HUMAN'S HAIR??

News | Featu

Home | Coro

Is there

Society



An important food
hair, says televisio
on Thursday even

The amino acid L-
include duck featr
found. Its research
cysteine.

Production of Cysteine: Approaches, Challenges and Potential Solution

Nur Izzah Ismail¹, Yumi Zuhanis Has-Yun Hashim^{1,3,*}, Parveen Jamal^{1,3}, Rashidi Othman^{2,3}
and Ham

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**How to differentiate L-Cysteine
from human's hair or from
microbial product?**

Abstract: Cysteine has a wide application in pharmaceutical, foods and cosmetic industries. In the biological system, through its unique properties of sulfur and thiol, cysteine also plays important roles in stability, structure, catalytic activity, and regulation of numerous proteins. In nature, cysteine can be found in animal proteins, fruits, vegetables, legumes and cereal. Due to its wide application, the production of cysteine in large scale is in favour. At present, cysteine is produced from keratin of animal sources as well as through microbial bioconversion and fermentation. Each production method poses its own challenges and limitation; which includes low yield, high-cost and poor consumer acceptance. As such, alternative source for large-scale cysteine production is of interest. Plants are seen to be an attractive substrate for the extraction of cysteine.

Raspberry ice cream, vanilla ice cream, the beaver (Berang-berang). Is there any connection?



Castoreum

What is Castoreum?

- A yellowish, oily substance with a strong odor that is secreted by beavers from castor sacs located in the skin cavities between the pelvis and the base of the tail. Beavers use it to mark their territory.

Urban Legend

- Is it used as a food additive for raspberry flavoring?



International Journal of Toxicology, 26:51–55, 2007
Copyright © American College of Toxicology
ISSN: 1091-5818 print / 1092-874X online
DOI: 10.1080/10915810601120145

Safety Assessment of Castoreum Extract as a Food Ingredient

G. A. Burdock

Burdock Group, Washington, DC, USA

ECONOMIC USES

Castoreum extracts are used as flavor components in most major categories of foods (particularly vanilla flavored), including alcoholic and nonalcoholic beverages, frozen dairy desserts, candy, baked goods, gelatins and puddings, and meat and meat products (Burdock 1995).

The tincture (maximum use level of 0.4%) is mainly used in cosmetics as a fragrance or as a fixative in soaps, creams, lotions, and perfumes (especially men's and oriental perfumes) (Opdyke 1973). It blends well with other fragrances including ambra notes, calamus, canaga, cedarwood, labdanum products, isoeugenol, oakmoss products, sandalwood oil, veratraldehyde, zingerone, and others (Arctander 1960).



Castoreum and castoreum substitutes

Communication

Stereoselective Synthesis of Enantiomeric Alkaloids from Castoreum[†]

Alexander Stoye, Gabriele Quandt, Björn Brunnhöfer, Markus Weymann Dr., Horst Kunz Prof. Dr. ✉

First published: 03 March 2009 | <https://doi.org/10.1002/ange.200900000>

[†] This work was supported by the Fonds der Chemisch-Industriellen Forschung (Fonds der Chemischen Industrie) for a predoctoral fellowship.

Author ANONIS, D. P

Source *Perfumer & flavorist*. 1997, Vol 22, Num 5, pp 1-5 ; ref : 21 ref

CODEN PEFLDI **ISSN** 0272-2666 **Scientific domain** Food science technology ; Chemical industry parachemical industry

Publisher Allured, Carol Stream, IL **Publication country** United States

Document type Article **Language** English

Keyword (fr) Castoreum Composition chimique Cosmétique Dermatologie Industrie pharmaceutique Matière première Origine animale Parfum Production Produit naturel Produit synthétique Substitut Utilisation

Keyword (en) Castoreum Chemical composition Cosmetic Dermatology Pharmaceutical industry Raw materials Animal origin Perfume Production Natural product Synthetic product Substitute Use

Keyword (es) Castóreo Composición química Cosmético Dermatología Industria farmacéutica Materia prima Origen animal Perfume Producción Producto natural Producto sintético Substituto Utilización

Classification **Pascal** 001 Exact sciences and technology / 001D Applied sciences / 001D08 Chemical industry and chemicals / 001D08D Washing products. Cosmetics and toiletries. Perfumes / 001D08D03 Essential oils, perfumes

Discipline Chemical and parachemical industries

Origin Inist-CNRS **Database** PASCAL **INIST identifier** 2823508



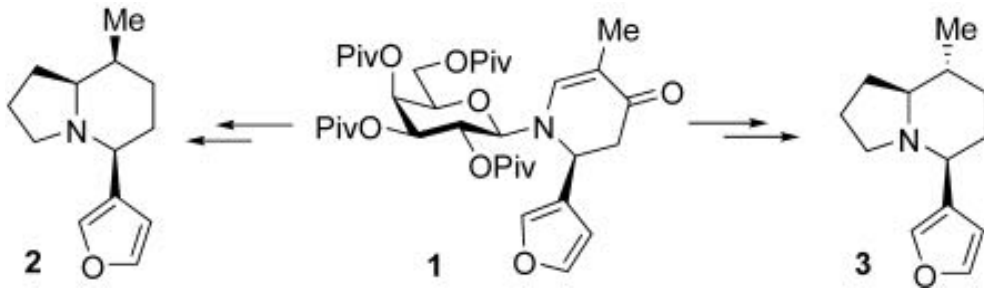
Natural Castoreum Extract

In audit for flavouring agent : the origin is confirmed by halal supporting documents (Flow chart, COA) which mentioning the origin of castoreum oil



Raspberry essence dosage average 0.05 %

OR



Synthetic Castoreum

Castoreum dosage in Artificial Raspberry flavour maximum 0.4%, might contains **22 other compounds**
<http://www.perflavory.com/demos/df1005932.html>

Ingredients	Percentage (% w/w)
Sugar	14.6
Fat	10
MSNF	11.46
Stabiliser	
Without stabiliser	0 (A)
Mixture commercial gums	0.3 (B)
Mixture of tested gums	0.3 (C)
Emulsifiers (mono-diglycerides)	0.2
Vanilla	0.073
Colour	0.16
Maltodextrin	0.16
Water	63.18
TOTAL	100



Made
sugar

University of Massachusetts Amherst

ScholarWorks@UMass Amherst

Masters Theses 1911 - February 2014

1941

Apple juice; clarification by the gela

Robert Louis Messier

University of Massachusetts Amherst

E. I. BENITEZ, J. E. LOZANO

EFFECT OF GELATIN ON APPLE JUICE TURBIDITY

E. I. BENITEZ and J. E. LOZANO

PLAPIQUI (UNS-CONICET), CC 717, (8000) Bahía Blanca, Argentina.

jlozano@plapiqui.edu.ar

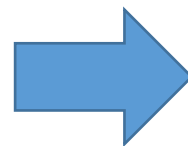
Clarification, or fining, of apple juice with gelatin and bentonite is a common industrial practice (Stocké, 1998). These fining agents work either by sticking to the particles or by using charged ions to cause particles to stick to each other, in any case making them heavy enough to sink to the bottom by the action of gravity. What is left is a transparent though not a clear juice. Subsequent filtration operations are needed to obtain a crystal clear product. Differences in the nature of ionic charges of protein, polyphenols and the fining agents, induce flocculation and sedimentation and result in the removal of these potential haze precursors from solution.



Halal Positive List



Fresh Apples



Can we check the juice in the Lab whether gelatin was used in apple juice clarification process?

Bentonite

Gelatin

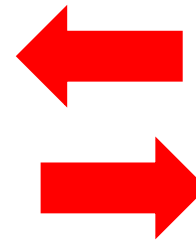
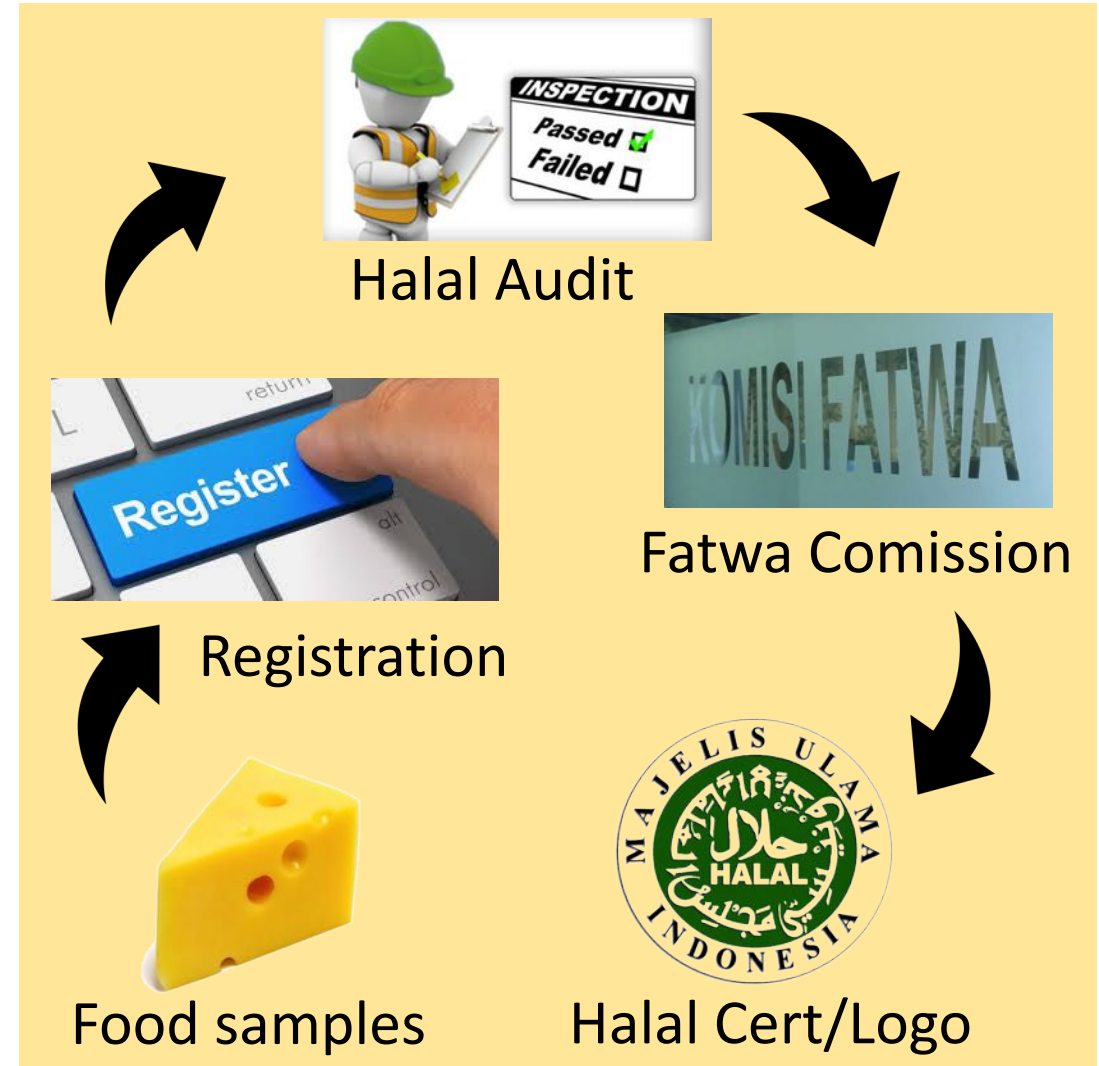
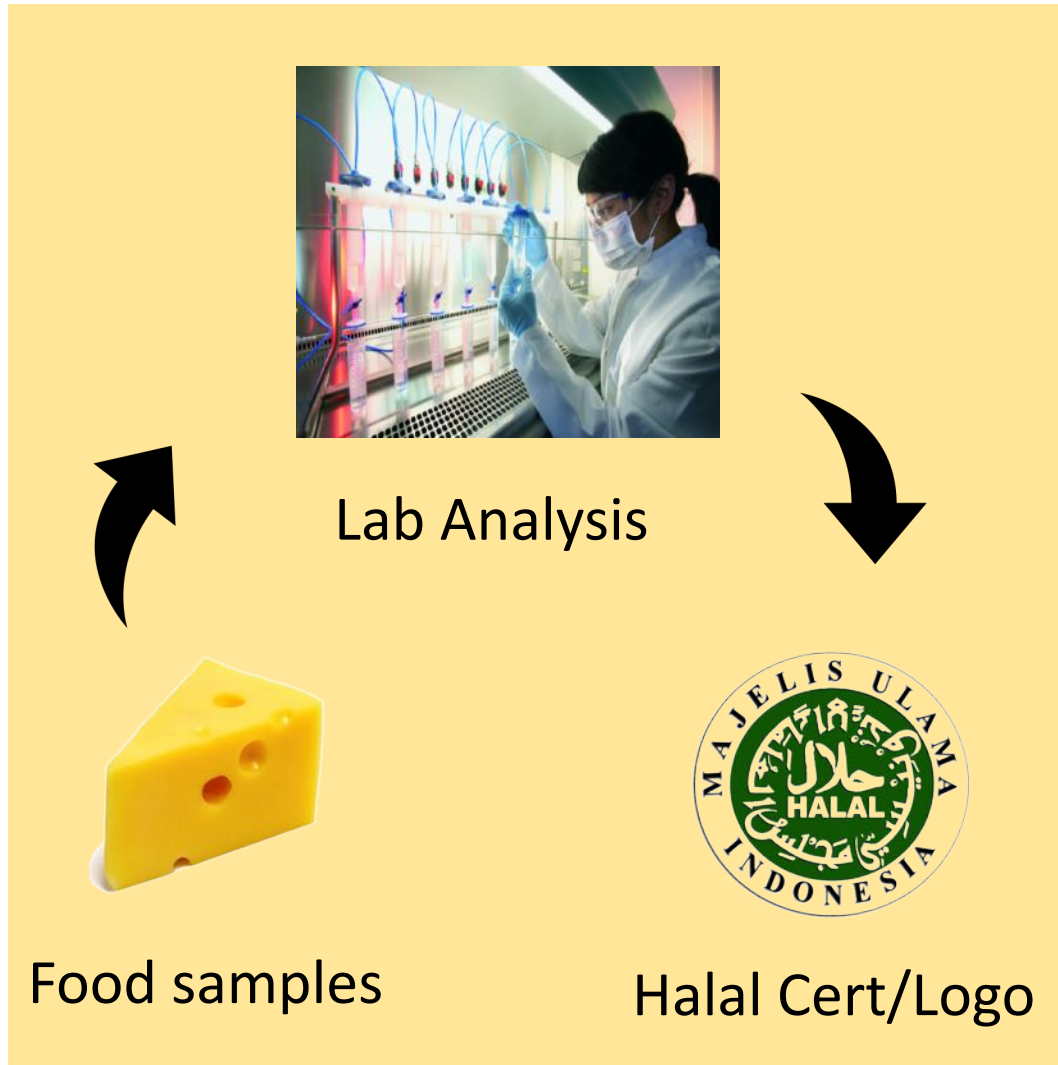


Halal Positive List

In halal certification by audit : Valid Halal Certificate required



- **Violation of halal assurance system implementation**
 - Changing halal critical materials during certification period without communication with the certifier body
 - Changing vendors/suppliers of halal critical materials during certification period without communication with the certifier body
 - Sharing facility Contamination during production, storage, distribution, and serving due to improper cleaning
- **Fake data given during the audit**
- **Violation of agreement between raw material vendors with the halal certified company**
 - Hidden ingredients : vendors do no declare all ingredients to produce material purchased by the halal certified company
 - Vendors do not give a proper raw material halal documentations
 - Vendors change the way of raw material manufacturing process without notification

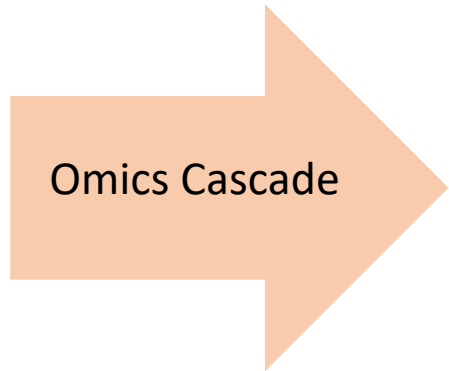




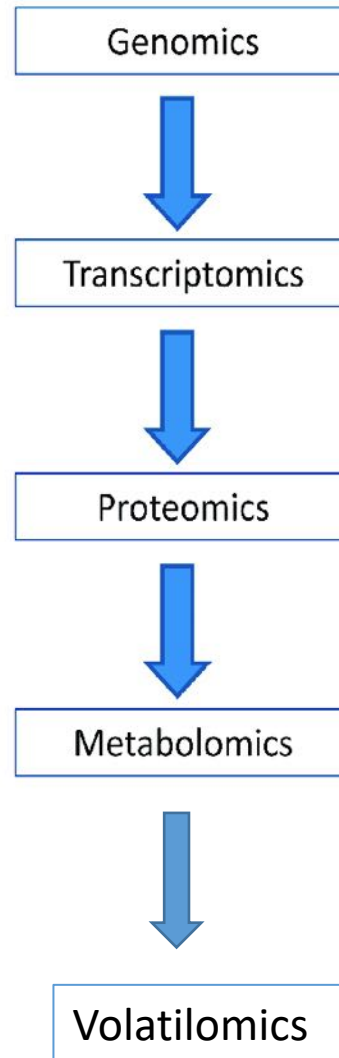
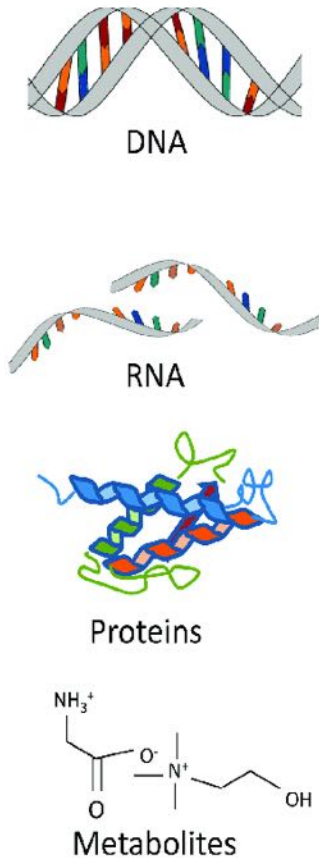
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Omics Approach In Halal Food Authentication



Haukaas et al. (2017)



Metabolomics : comprehensive and systematic study of low molecular weight compounds (metabolites), as a whole in a given sample (Fiehn, 2003; Ulrich-Merzenich et al., 2007)

Volatilomics : detection, characterization and quantification of all volatile metabolites in biological system (Lytou et al. 2019)

Omics - Advantages:

- Rapid Discovery of New Biomarkers Candidate
- High throughput
- Probably more compatible for complex matrix such as food matrix
- Volatilomics : very simple preparation

Volatilome : the totality of VOCs produced by all living organisms (plants, animals, and so forth) (Cumeras 2017)





- **Fourier Transformed Infra-Red (FTIR)**

- Mixture analysis
- Non-destructive
- Broad spectrum target
- Cheap, fast, simple sample preparation
- Only recognizes functional groups

- **Nuclear Magnetic Resonance (NMR)**

- Mixture analysis
- Molecular structure information (1D NMR + 2D NMR)
- Non-destructive
- Broad spectrum target
- Fast and simple sample preparation
- High investment cost
- Less sensitivity
- Overlapping signals
- Lack of database

- **Chromatographic technique, usually couple to Mass Spectrometry (e.g. GC-MS, LC-MS)**

- GC : volatiles or compound which can be volatilized, non-polars
- HPLC/UPLC : polars

- **Electronic Nose, SPME (couple to GC) :**

- Mixture analysis
- Volatiles
- Non-destructive
- Simple sample preparation

Suitable platforms for volatilomics



Multivariate Data Analysis (most common PCA, PLS-DA/OPLS-DA)

Principal Component Analysis (PCA)

“Enabled reduction of data dimensionality, while simultaneously retaining maximum data variability to overview relationships among observations/samples and their correlation with the measured variables (e.g. spectral data) *”

Showing the grouping/classification of samples

PCA is used as a first step to judge the quality of the model, before continue with supervised multivariate

Partial Least Square (PLS)/ Orthogonal Projections to Latent Structures (OPLS) - Discriminant Analysis

Provide insights into separations between experimental groups (2 or 3 groups)

Facilitating identification of unique variable which differentiate the groups

Extracting Information from PCA/OPLS-DA

Score plot

To observe samples position/grouping

Loading Plot and S-Plot

Correlation between sample and the measured variables

Variable Influence on Projection (VIP) value

Indicating the importance of marker variables for the separation/classification



Different type of meat has specific odor

Different meat different cooking method → different odor



Contents lists available at [ScienceDirect](https://www.sciencedirect.com)

Meat Science

journal homepage: www.elsevier.com/locate/meatsci



A volatilomics approach for off-line discrimination of minced beef and pork meat and their admixture using HS-SPME GC/MS in tandem with multivariate data analysis

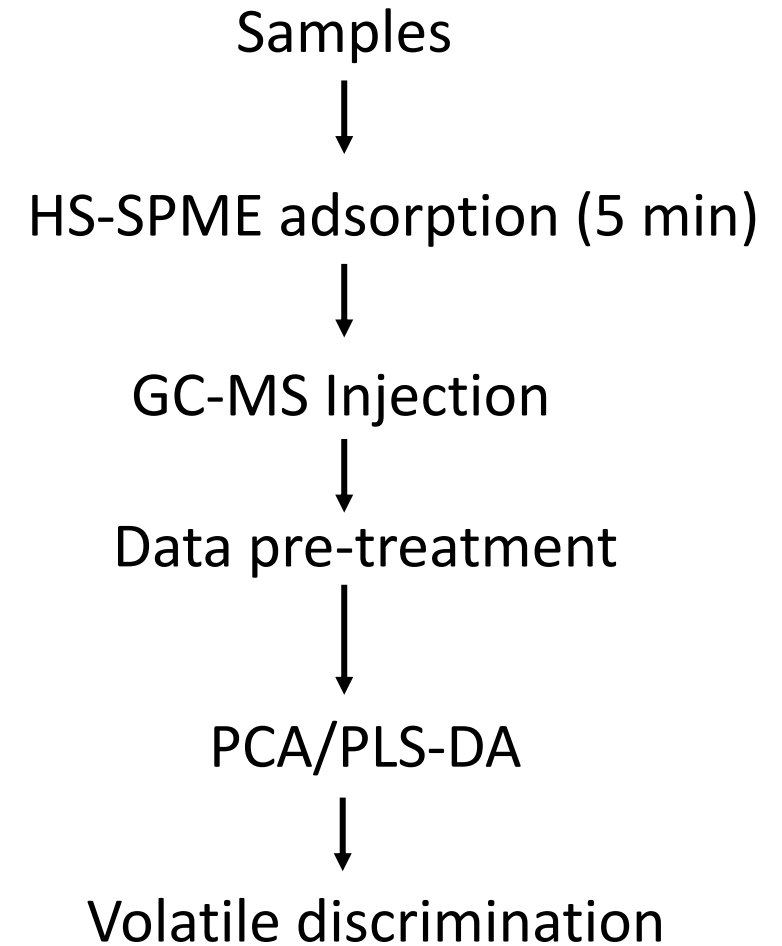
Dimitrios E. Pavlidis^a, Athanasios Mallouchos^b, Danilo Ercolini^c, Efstathios Z. Panagou^a, George-John E. Nychas^{a,*}

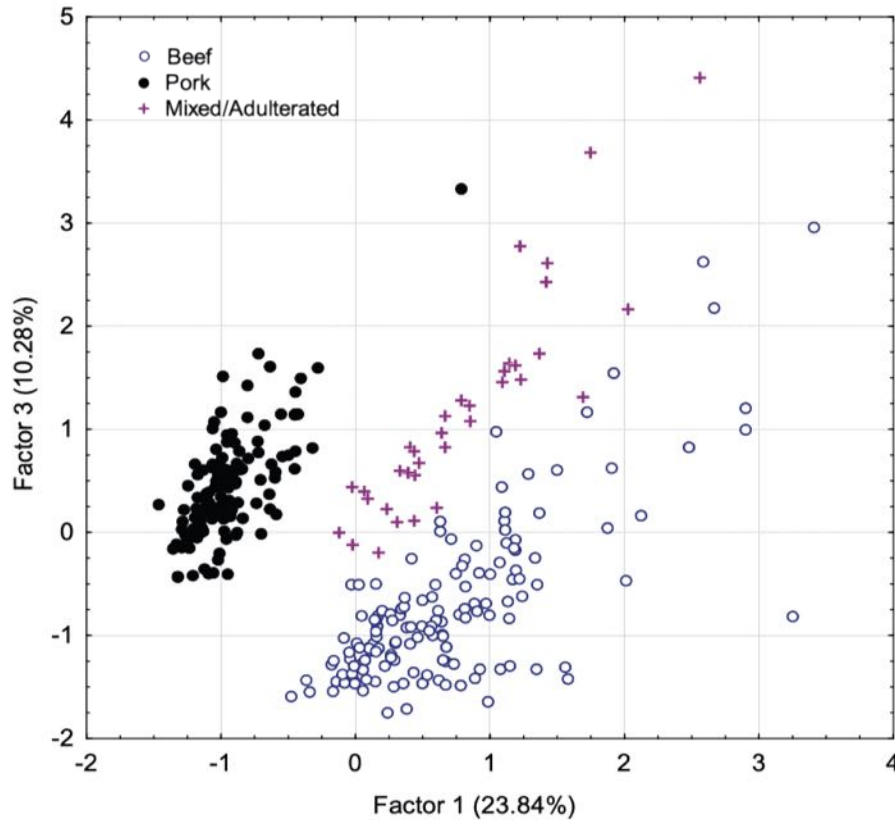
^aLaboratory of Microbiology and Biotechnology of Foods, Department of Food Science and Human Nutrition, Agricultural University of Athens, Iera Odos 75, Athens 11855, Greece

^bLaboratory of Food Chemistry and Analysis, Department of Food Science and Human Nutrition, Agricultural University of Athens, Iera Odos 75, Athens 11855, Greece

^cDivision of Microbiology, Department of Agricultural Sciences, University of Naples Federico II, Portici, Italy

- 204 Beef
- 198 Pork
- 55 Beef-pork mixed (7:3)
- Minced meat
- Collected over 2 years period
- Samples taken on day 1, 3, 5 after slaughtering





PCA Score Plot

Volatiles Positively Correlate with		
	Beef	Pork
Aldehydes	Acetaldehyde, heptanal, octanal and decanal	Pentanal, hexanal, nonanal, benzaldehyde, (E)-2-hexenal, (E)-2-heptenal, (E)-2-octenal
Alcohols	butanol, 1-penten-3-ol, pentanol, hexanol, 2-octen-1-ol and octanol	-
Ketone	3-hydroxy-2-butanone, 2-butanone, 2-heptanone and 2,5-octanedione	1-octen-3-one
Esters	ethyl acetate	methyl acetate
Furans	2-ethylfuran, 2-pentylfuran	-
Terpenes	-	δ -3-carene

Authors' Note : Validation required (e.g. quantification)

Analysis of Volatiles in Beef, wild boar, and Chicken Meatballs by SPME-GC/MS

Agy Wirabudi Panata^{1,4}, Noviyan Damawan^{3,4}, Lia Amalia^{1,4}, Nancy D Yuliana^{2,4}, *

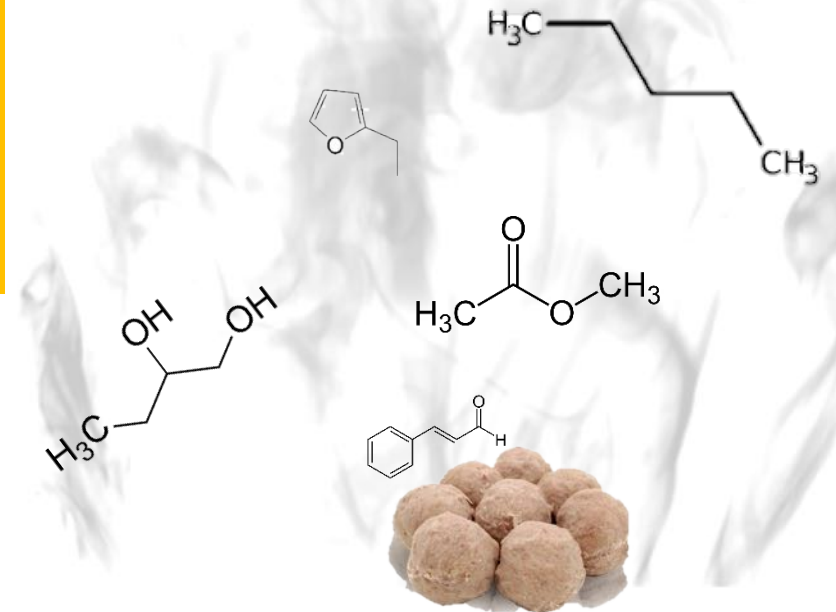
¹ Graduate School of Food Science, IPB University, Bogor, Indonesia

² Department of Food Science and Technology, IPB University, Bogor, Indonesia

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⁴ Halal Science Center, IPB University, Bogor, Indonesia

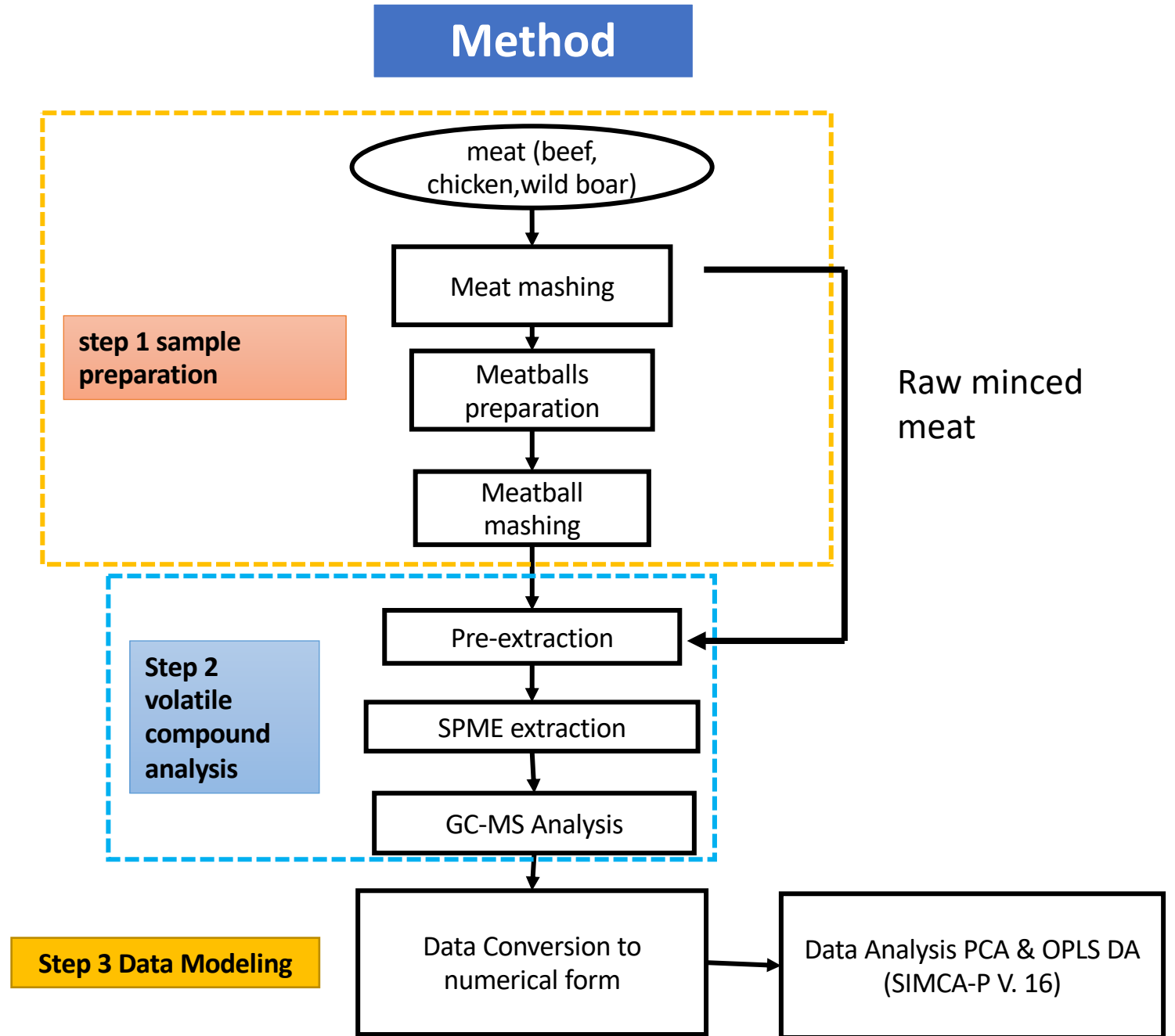
*Corresponding Author : nancy_dewi@apps.ipb.ac.id



Goals:

Discriminating meats from halal and non halal animals (raw and meatball) based on their volatiles profile using SPME-GC-MS

Working flow



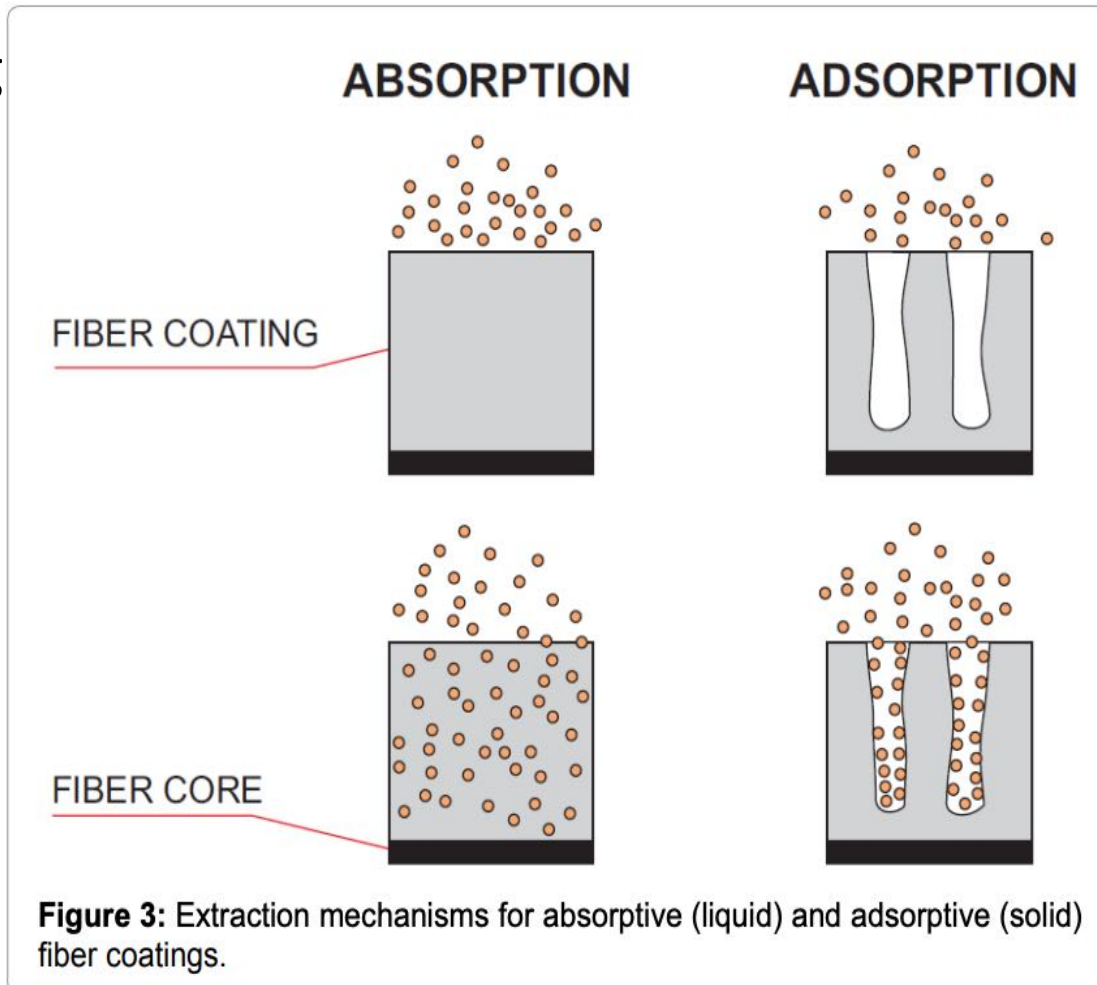
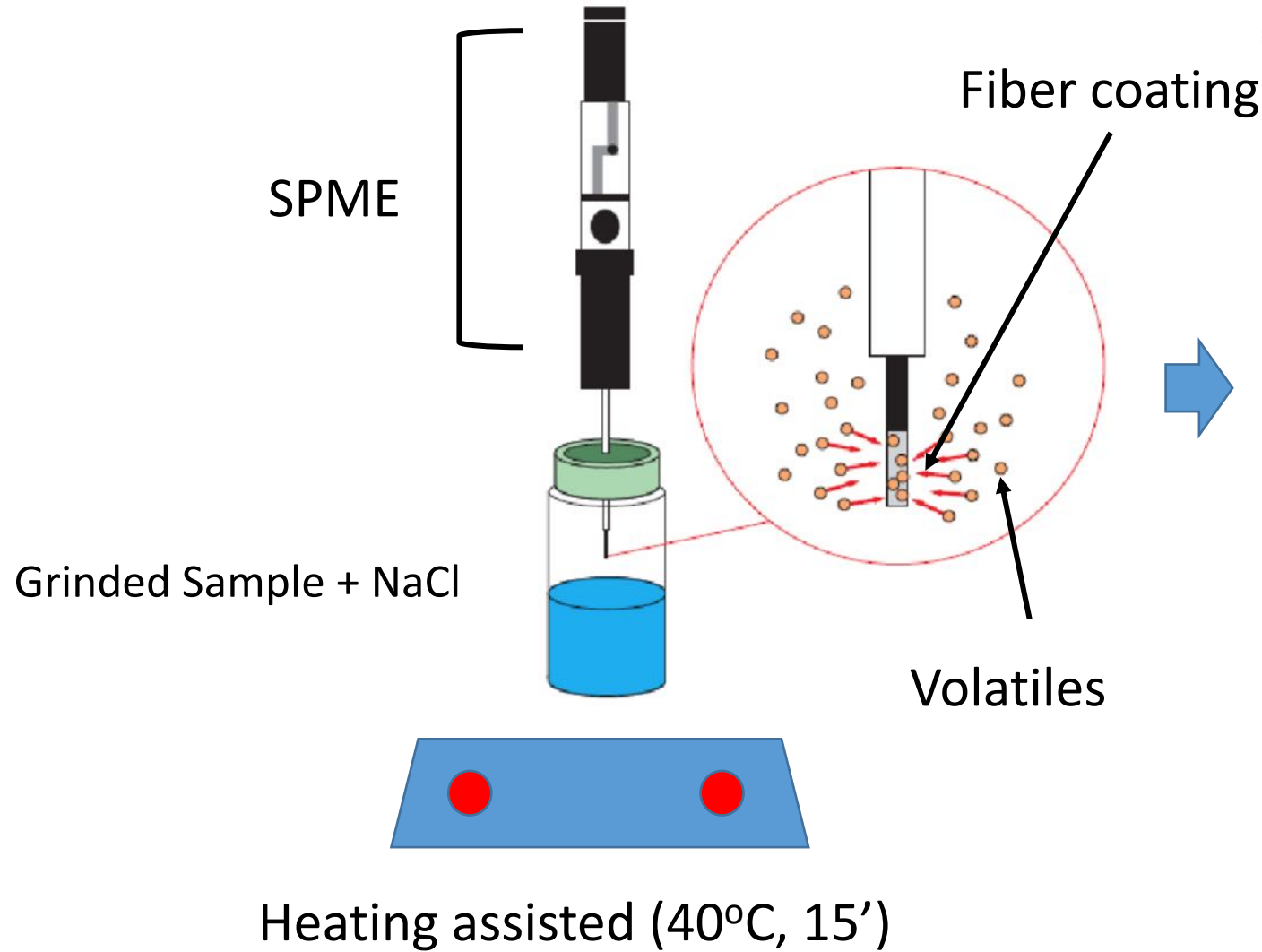
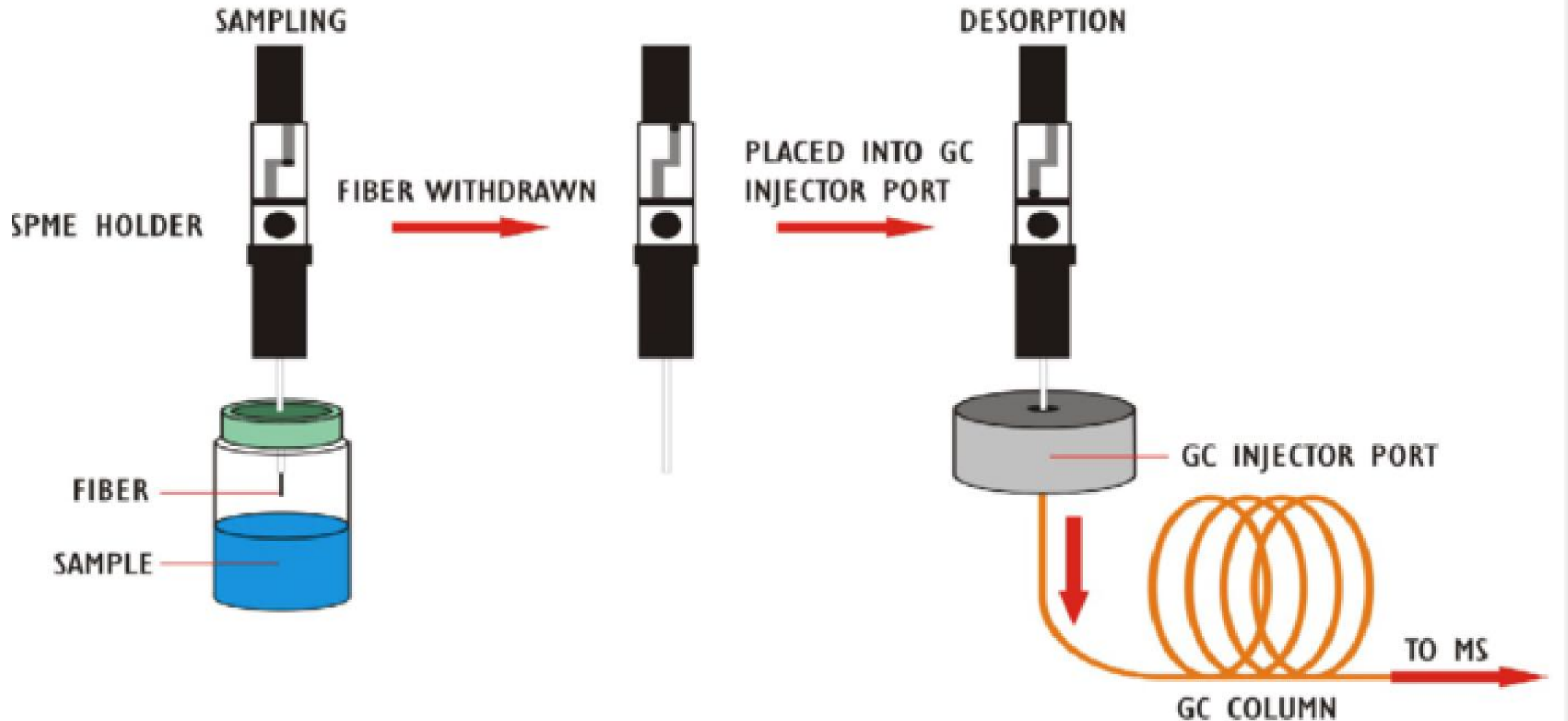


Figure 3: Extraction mechanisms for absorptive (liquid) and adsorptive (solid) fiber coatings.

Polymer coating and thickness	Recommended application	Mechanism	MW	Polarity
100 µm PDMS	Volatiles	Absorbent	60-275	Non-polar
30 µm PDMS	Non-polar semi-volatiles	Absorbent	80-500	Non-polar
7 µm PDMS	Non-polar high molecular weight compounds	Absorbent	125-600	Non-polar
60 µm PEG	Alcohols and polar compounds	Absorbent	40-275	Polar
85 µm PA	Polar semi-volatiles	Absorbent	80-300	Polar
75 µm/85 µm CAR/PDMS	Gases and low molecular weight compounds	Adsorbent	30-225	Bipolar
65 µm PDMS/DVB	Volatiles, amines and nitro-aromatic compounds	Adsorbent	50-300	Bipolar
60 µm PDMS/DVB	Amines, nitroaromatic and polar compounds (HPLC use only)	Adsorbent	50-300	Bipolar
50/30 µm DVB/CAR/PDMS on a StableFlex fiber	Flavour compounds: volatiles and semi-volatiles, C3-C20	Adsorbent	40-275	Bipolar
50/30 µm DVB/CAR/PDMS on a 2 cm StableFlex fiber	Trace compound analysis	Adsorbent	40-275	Bipolar

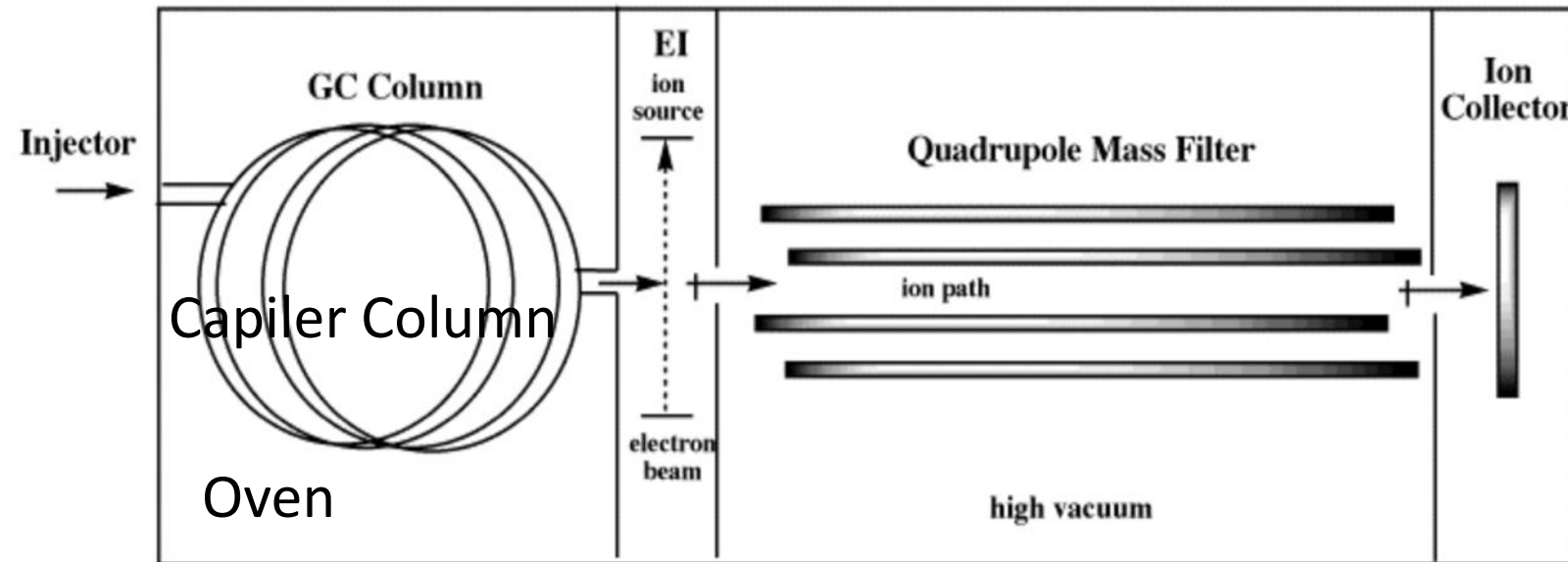
CAR: Carboxen; PDMS: Polydimethylsiloxane; DVB: Divinylbenzene; HPLC: High Performance Liquid Chromatography; PA: Polyacrylate; PEG: Carbowax-Polyethylene Glycol [19].

Table 2. Summary of commercially available SPME fibers.



1. Gas Chromatograph

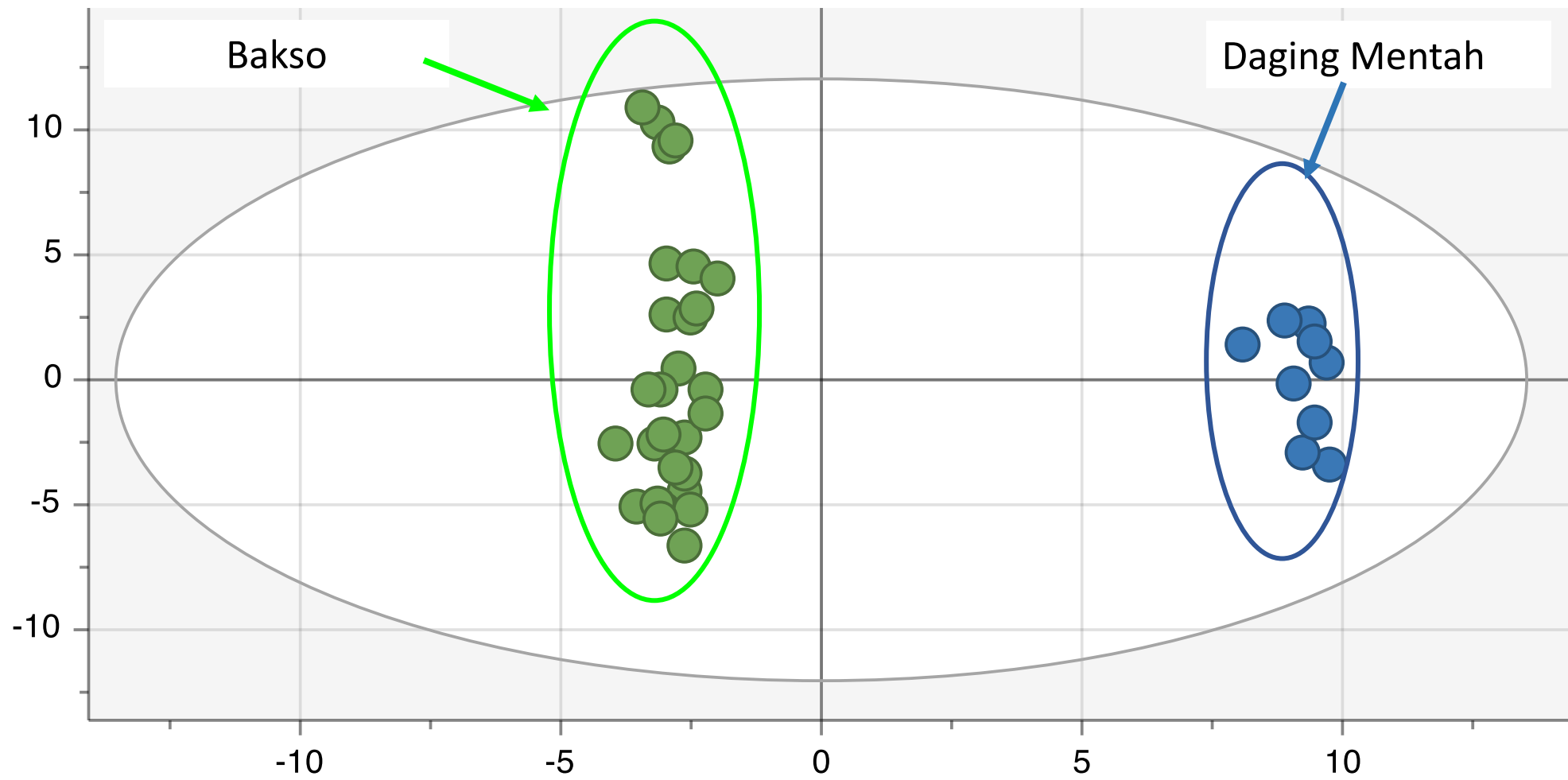
2. Mass Spectrometer



Tentative compounds identification: comparison with National Institute of Standards and Technology (NIST 14) database spectral library, and comparison between experimental and theory (literature) Linear Retention Index (LRI)

1. In GC : separation based on polarity and boiling point
2. In MS : Ion fragment selection based on electric field and m/z of ions

Score plot OPLS-DA daging babi hutan, sapi dan ayam (mentah dan bakso)





- **Halal food analytical science is important to support halal status determination**
- **Challenges**
 - Able to trace target molecules in minute amount, present in a heavily complex mixture
 - Able to trace target molecules which has been transformed into different molecules due to processing method
 - Fast, cheap, simple sample preparation: Applicable for SMEs
- **Volatilomics-based method using SMPE-GC-MS is an easy method useful for discriminating halal and non-halal meat (raw or cooked)**

Thank you!

Contact : nancy_dewi@apps.ipb.ac.id



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