



The Halal Science Center Chulalongkorn University

# Metabolomics-Based Profiling of Porcine and Bovine Gelatin for Halal Authentication

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Presented by...

**DR.NUREESUN MAHAMUD**

Researcher,

The Halal Science Center Chulalongkorn University, Thailand



# Outline

Introduction

Experimental design

Results

Conclusion



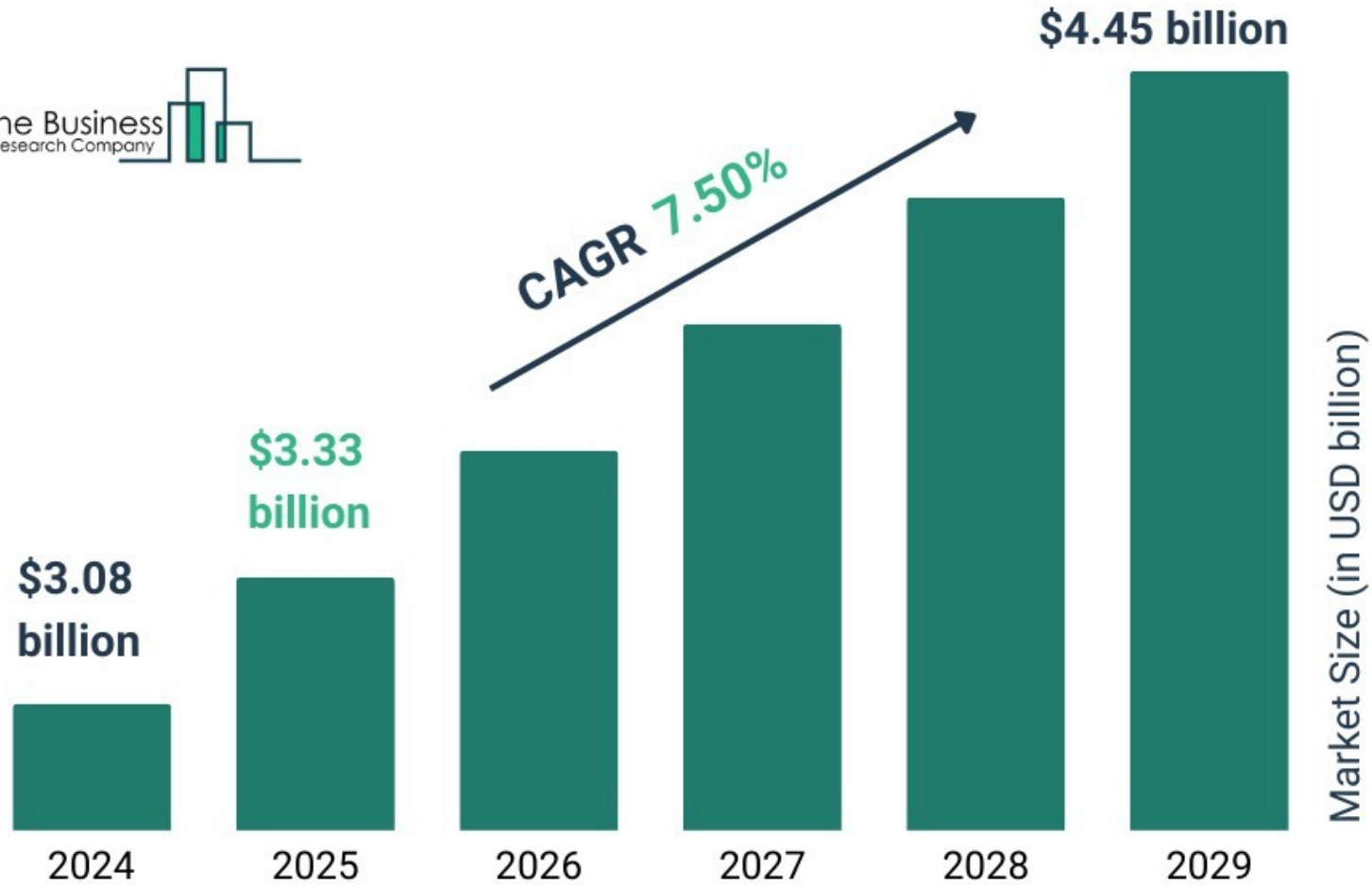
What is

gelatin?

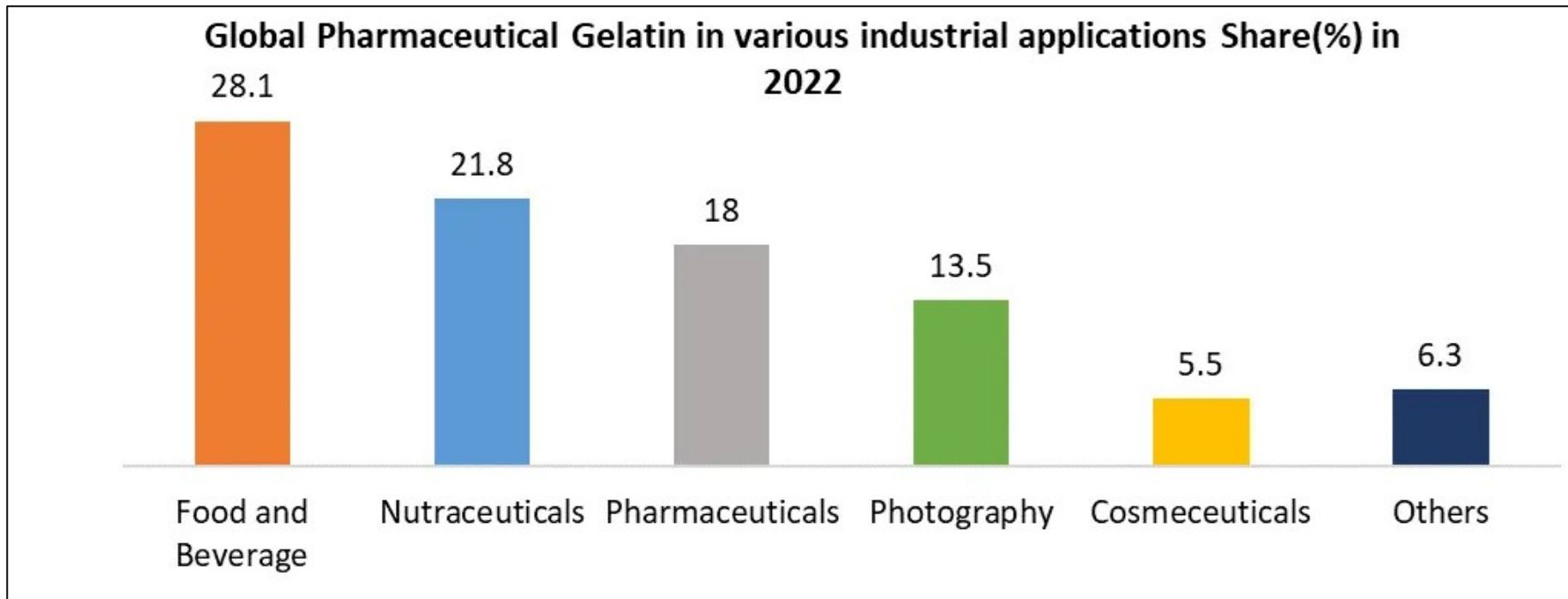
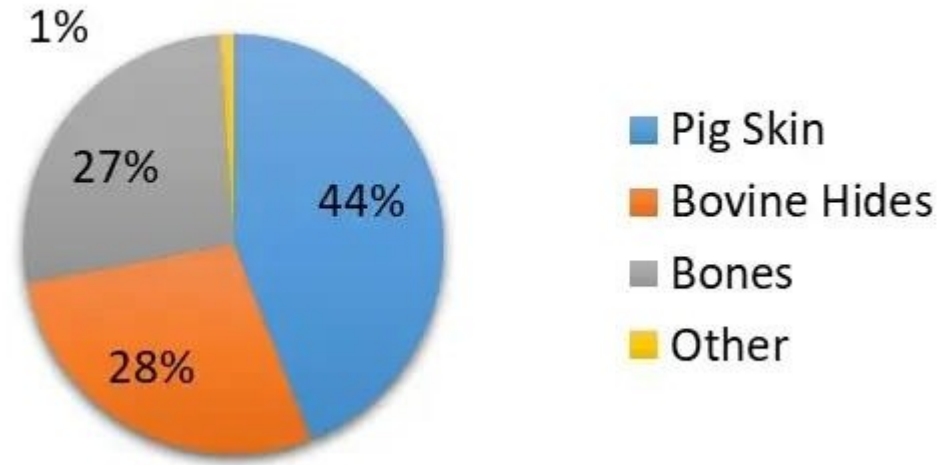
Gelatin is a protein produced by partial hydrolysis of collagen extracted from skin and connective tissue of several animals such as pig, cow and fish.



# Gelatin Global Market Report 2025



### Raw Material Used In The Gelatin Production, (%)





Article reviews

## Halal Alternative Sources of Gelatin: A Review

Nureesun Mahamud<sup>1,2</sup>, Najwa Yanya Santiworakun<sup>1</sup>, Sarin Chaovasuteeranon<sup>1</sup>,  
Firadao Boonmalert<sup>1</sup>

<sup>1</sup> The Halal Science Center, Chulalongkorn University, Bangkok, Thailand

<sup>2</sup> Department of Food and Pharmaceutical Chemistry, Faculty of Pharmaceutical Sciences,  
Chulalongkorn University, Bangkok, Thailand

### Source of Halal Gelatin

- animal-based gelatin
- plant-based alternatives gelatin
- aquatic animal-derived gelatin
- microbial and fungal sources
- synthetic and recombinant gelatin



# Development of Gelatin Analysis

2005



**Picric acid test**

2006



**Hydroxyproline test**

2011



**LC-ESI-MS/MS**

2025



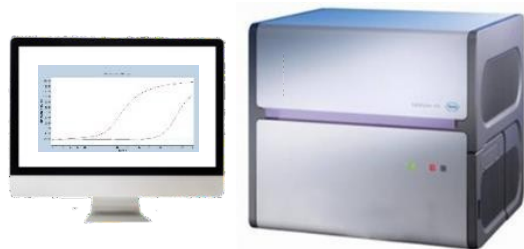
**LC-QTOF-MS/MS  
Metabolomics**

# Detection of porcine and bovine DNA in gelatin capsules by different techniques

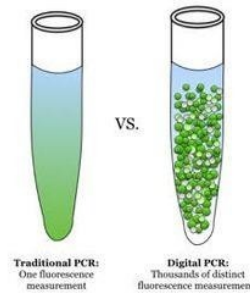


- Real-time PCR
- Digital droplet PCR (ddPCR)
- LC-QTRAP-MS/MS

## Gelatin in Soft/Hard Capsules & Pellets



Real-time PCR detection



Digital droplet PCR (ddPCR)

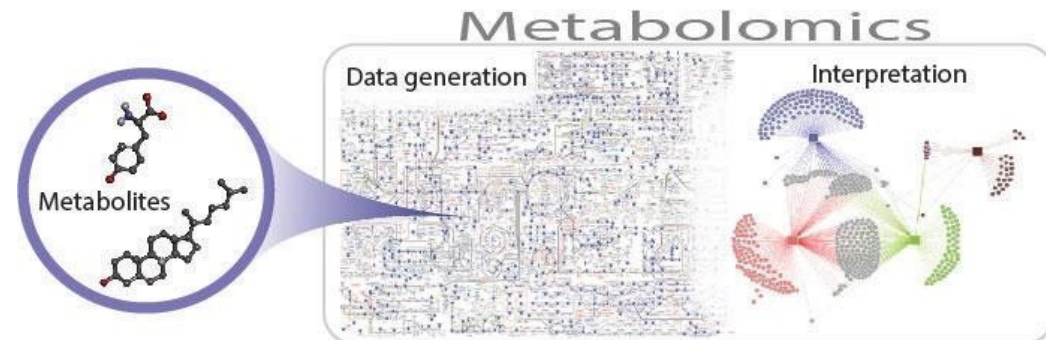


LC-MS/MS Analysis

# What is

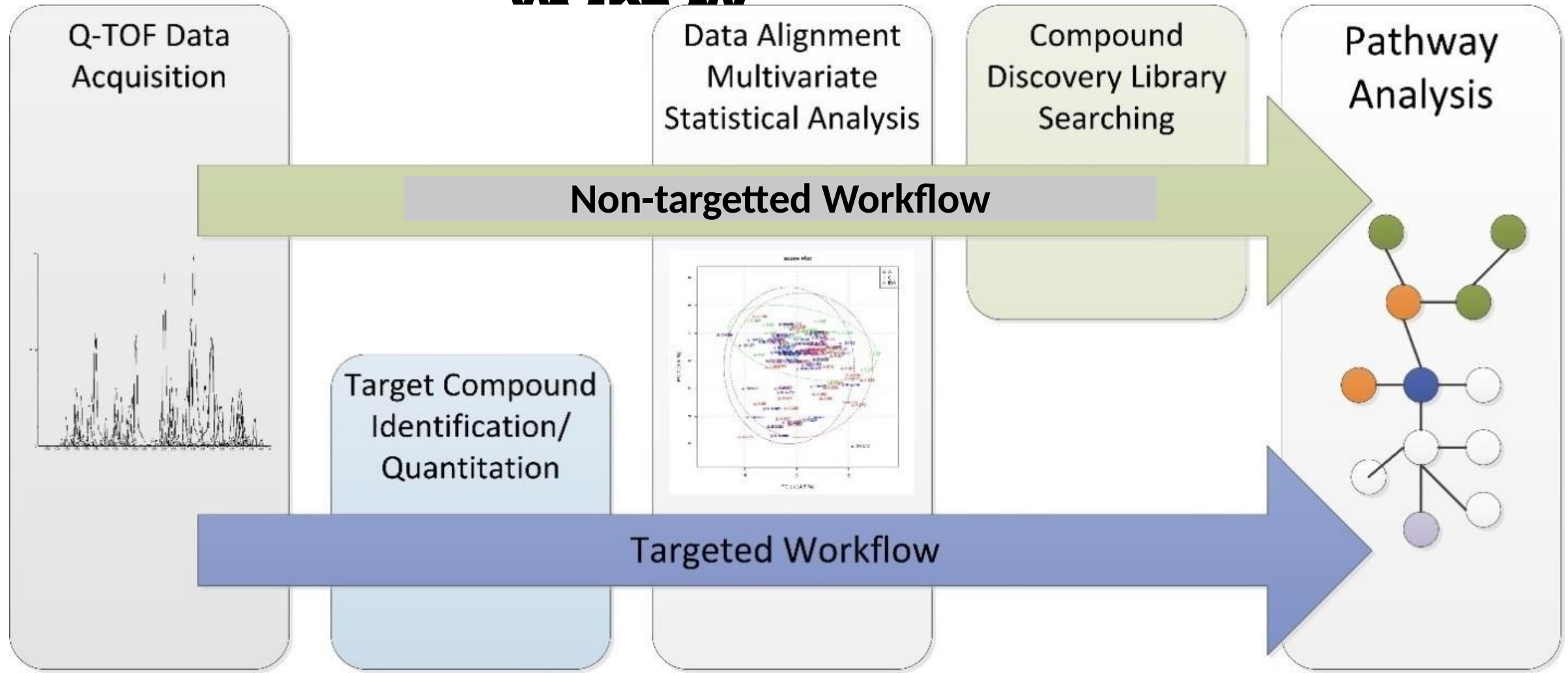
# Metabolomics

**Metabolomics** is known as the comprehensive analysis of the whole metabolome, which refers to the full complement of small molecule (< 1.5 kDa) metabolites in a cell, food, plant or biological samples.



# Metabolomics

## workflow



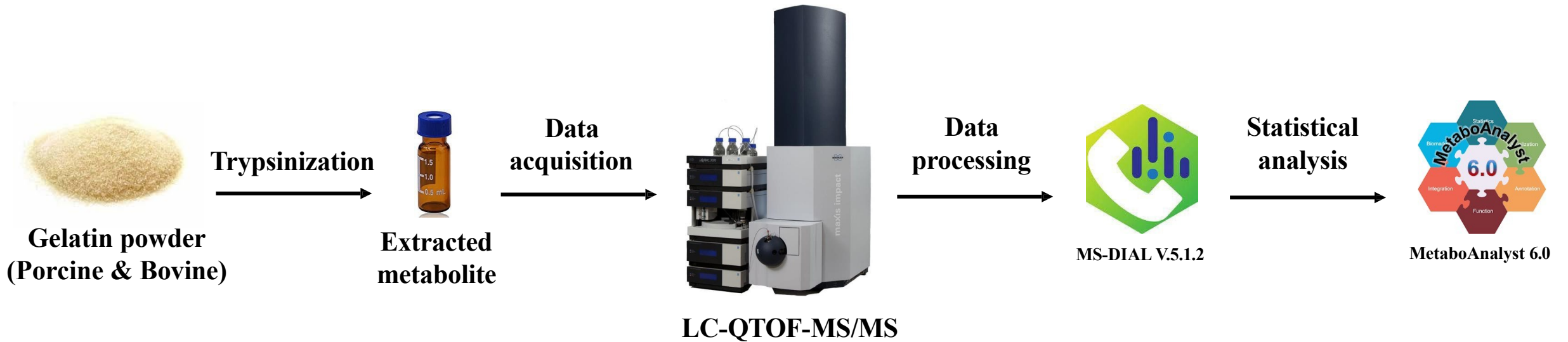


# Objectives

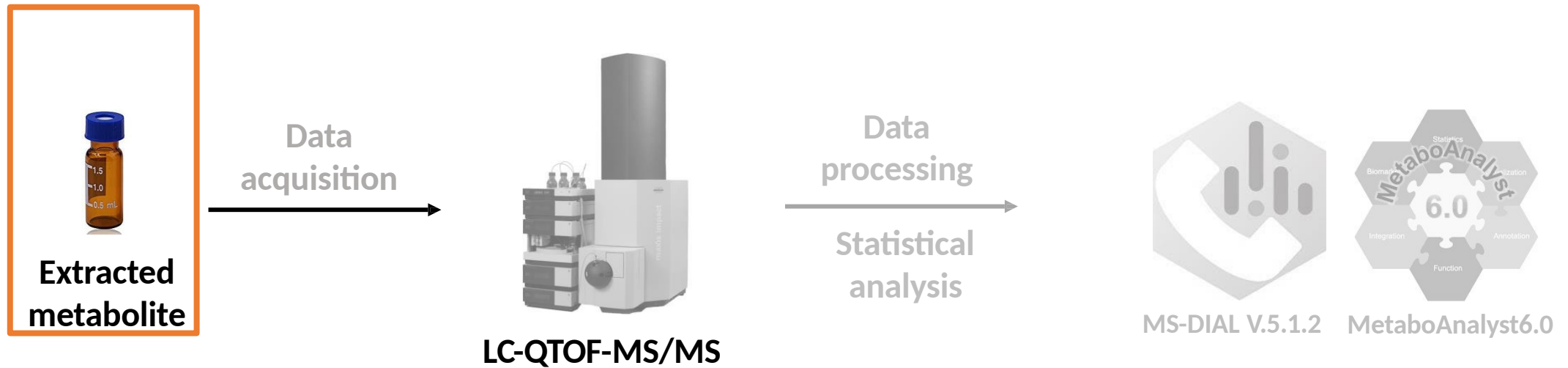
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- To develop a metabolomics-based approach using LC-QTOF-MS/MS to discriminate between porcine and bovine gelatin sources.
- To apply metabolomics technology in the context of halal authentication and applications.

# Metabolomics Workflow



# Metabolomics Analysis



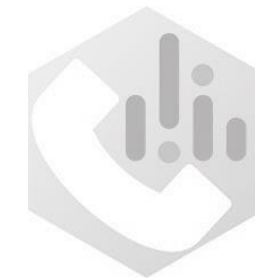
# Metabolomics Analysis



Data acquisition



Data processing  
Statistical analysis



MS-DIAL V.5.1.2



MetaboAnalyst6.0

# Metabolomics analysis by LC-QTOF-MS/MS

- **LC conditions**
  - LC system
  - Column :  
UHPLC  
C<sub>18</sub> (100  
mm x 2.1  
mm, I.D.  
1.9 μm)
  - Flow  
rate : 0.3  
ml/min
  - Mobile  
phase :  
0.1%



# Metabolomics Analysis



Data acquisition



LC-QTOF-MS/MS

Data processing  
Statistical analysis



MS-DIAL V.5.1.2

MetaboAnalyst6.0



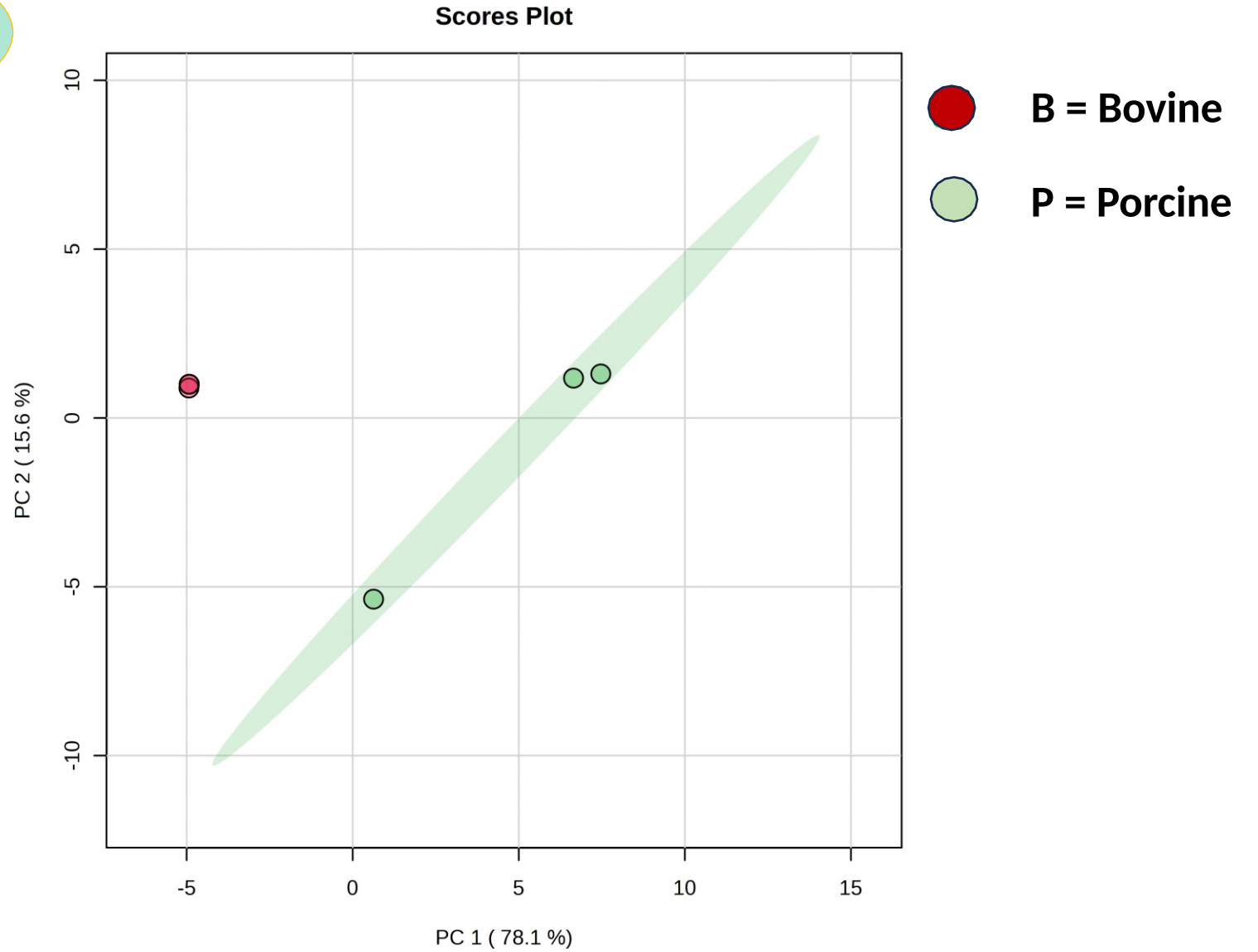
# Data Processing

	A	B	C	D	E	F	G	H	I	J	K	L	M	N	O	P	Q	R	S	T	U	V	W
4																							
5	Alignm	Average R	Average Mz	Metabolite name	Adduct type	Post curation re	Fill %	MS/MS	Reference RT	Refere	Formul	Ontology	INCHIKEY	SMILES	Annota	RT mat	m/z m	MS/MS	Comm	Manua	Manua	Isotope	Isotope
6	0	5.455	55.05423	Unknown	[M+H]+	ion correlated with	0.035	FALSE	null	null	null	null	null	null	999	FALSE	FALSE	FALSE		FALSE	FALSE	null	null
7	1	6.306	55.05438	Unknown	[M+H]+	ion correlated with	0.035	FALSE	null	null	null	null	null	null	999	FALSE	FALSE	FALSE		FALSE	FALSE	null	null
8	2	12.667	57.06876	Unknown	[M+H]+	ion correlated with	0.246	FALSE	null	null	null	null	null	null	999	FALSE	FALSE	FALSE		FALSE	FALSE	null	null
9	3	12.64	57.06899	Unknown	[M+H]+	ion correlated with	0.754	FALSE	null	null	null	null	null	null	999	FALSE	FALSE	FALSE		FALSE	FALSE	null	null
10	4	16.508	57.06987	Unknown	[M+H]+	similar chromatogr	0.439	FALSE	null	null	null	null	null	null	999	FALSE	FALSE	FALSE		FALSE	FALSE	null	null
11	5	22.051	59.06097	Unknown	[M+H]+		0.456	FALSE	null	null	null	null	null	null	999	FALSE	FALSE	FALSE		FALSE	FALSE	null	null
12	6	0.217	64.01456	Unknown	[M+H]+	similar chromatogr	0.035	FALSE	null	null	null	null	null	null	999	FALSE	FALSE	FALSE		FALSE	FALSE	null	null
13	7	6.306	67.05447	Unknown	[M+H]+	ion correlated with	0.035	FALSE	null	null	null	null	null	null	999	FALSE	FALSE	FALSE		FALSE	FALSE	null	null
14	8	6.311	69.03381	Unknown	[M+H]+	ion correlated with	0.035	FALSE	null	null	null	null	null	null	999	FALSE	FALSE	FALSE		FALSE	FALSE	null	null
15	9	5.457	69.07021	Unknown	[M+H]+	ion correlated with	0.035	FALSE	null	null	null	null	null	null	999	FALSE	FALSE	FALSE		FALSE	FALSE	null	null
16	10	16.508	71.08549	Unknown	[M+H]+	found in higher mz'	1	FALSE	null	null	null	null	null	null	999	FALSE	FALSE	FALSE		FALSE	FALSE	null	null
17	11	3.445	72.04385	Unknown	[M+H]+	ion correlated with	0.07	FALSE	null	null	null	null	null	null	999	FALSE	FALSE	FALSE		FALSE	FALSE	null	null
18	12	1.255	72.07983	w/o MS2:Pyrrolidine; CEO; RWRDLPD	[M+H]+	found in higher mz'	0.561	FALSE	7.706	72.08078	C4H9N	Pyrrolidines	RWRDLPLDKQPQOW	C1CCNC1	530	TRUE	TRUE	FALSE		FALSE	FALSE	null	null
19	13	1.309	72.08018	w/o MS2:Pyrrolidine; CEO; RWRDLPD	[M+H]+	similar chromatogr	0.333	FALSE	7.706	72.08078	C4H9N	Pyrrolidines	RWRDLPLDKQPQOW	C1CCNC1	530	TRUE	TRUE	FALSE		FALSE	FALSE	null	null
20	14	0.741	72.98607	Unknown	[M+H]+	ion correlated with	0.018	FALSE	null	null	null	null	null	null	999	FALSE	FALSE	FALSE		FALSE	FALSE	null	null
21	15	1.367	76.02094	Unknown	[M+H]+	ion correlated with	0.807	FALSE	null	null	null	null	null	null	999	FALSE	FALSE	FALSE		FALSE	FALSE	null	null
22	16	1.754	76.03819	w/o MS2:Acetohydroxamic acid	[M+H]+	similar chromatogr	0.912	FALSE	2.754	76.03874	C2H5NO2	Acetohydroxamic acids	RRUDCFGSUDOHGD	ON=S(C)C	530	TRUE	TRUE	FALSE		FALSE	FALSE	null	null
23	17	1.845	76.03832	w/o MS2:Acetohydroxamic acid	[M+H]+	similar chromatogr	0.018	FALSE	2.754	76.03874	C2H5NO2	Acetohydroxamic acids	RRUDCFGSUDOHGD	ON=S(C)C	530	TRUE	TRUE	FALSE		FALSE	FALSE	null	null
24	18	4.993	76.03888	w/o MS2:Acetohydroxamic acid	[M+H]+	ion correlated with	0.053	FALSE	2.754	76.03874	C2H5NO2	Acetohydroxamic acids	RRUDCFGSUDOHGD	ON=S(C)C	530	TRUE	TRUE	FALSE		FALSE	FALSE	null	null
25	19	0.741	78.99648	Unknown	[M+H]+	ion correlated with	0.018	FALSE	null	null	null	null	null	null	999	FALSE	FALSE	FALSE		FALSE	FALSE	null	null
26	20	2.05	79.01988	w/o MS2:Dimethyl?sulfoxide	[M+H]+		0.211	FALSE	1.582	79.02135	C2H6OS	Sulfoxides	IAZDPXIOMUYVGZ-L	O=S(C)C	530	TRUE	TRUE	FALSE		FALSE	FALSE	null	null
27	21	1.579	79.02044	w/o MS2:Dimethyl?sulfoxide	[M+H]+		0.053	FALSE	1.582	79.02135	C2H6OS	Sulfoxides	IAZDPXIOMUYVGZ-L	O=S(C)C	530	TRUE	TRUE	FALSE		FALSE	FALSE	null	null
28	22	1.536	79.02045	w/o MS2:Dimethyl?sulfoxide	[M+H]+		0.737	FALSE	1.582	79.02135	C2H6OS	Sulfoxides	IAZDPXIOMUYVGZ-L	O=S(C)C	530	TRUE	TRUE	FALSE		FALSE	FALSE	null	null
29	23	1.856	79.02046	w/o MS2:Dimethyl?sulfoxide	[M+H]+	ion correlated with	0.053	FALSE	1.582	79.02135	C2H6OS	Sulfoxides	IAZDPXIOMUYVGZ-L	O=S(C)C	530	TRUE	TRUE	FALSE		FALSE	FALSE	null	null
30	24	1.382	79.0205	w/o MS2:Dimethyl?sulfoxide	[M+H]+	found in higher mz'	0.491	FALSE	1.582	79.02135	C2H6OS	Sulfoxides	IAZDPXIOMUYVGZ-L	O=S(C)C	530	TRUE	TRUE	FALSE		FALSE	FALSE	null	null
31	25	1.254	79.02065	w/o MS2:Dimethyl?sulfoxide	[M+H]+		0.14	FALSE	1.582	79.02135	C2H6OS	Sulfoxides	IAZDPXIOMUYVGZ-L	O=S(C)C	530	TRUE	TRUE	FALSE		FALSE	FALSE	null	null
32	26	1.889	79.02072	w/o MS2:Dimethyl?sulfoxide	[M+H]+	ion correlated with	0.053	FALSE	1.582	79.02135	C2H6OS	Sulfoxides	IAZDPXIOMUYVGZ-L	O=S(C)C	530	TRUE	TRUE	FALSE		FALSE	FALSE	null	null
33	27	1.336	79.02081	w/o MS2:Dimethyl?sulfoxide	[M+H]+	similar chromatogr	0.439	FALSE	1.582	79.02135	C2H6OS	Sulfoxides	IAZDPXIOMUYVGZ-L	O=S(C)C	530	TRUE	TRUE	FALSE		FALSE	FALSE	null	null
34	28	3.161	79.05301	Unknown	[M+H]+	adduct linked to 30	0.07	FALSE	null	null	null	null	null	null	999	FALSE	FALSE	FALSE		FALSE	FALSE	null	null

# Metabolite Identification

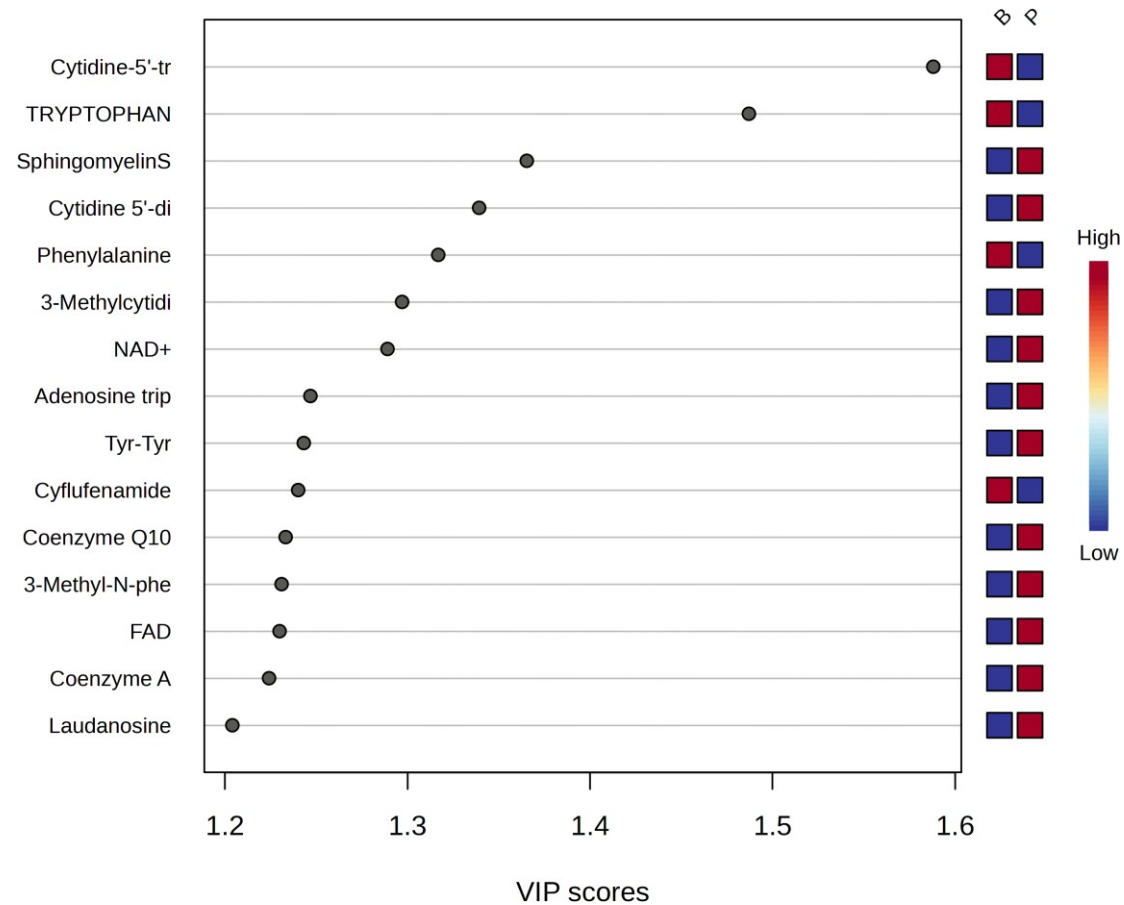
Alignm	Average Rt(m)	Average Mz	Metabolite name	Adduc	Post cu	Fill %	MS/MS	Referer	Reference	Formula	Ontology	INCHIKEY
743	1.325	345.13843	Tyr-Tyr	[M+H] <sup>+</sup>	null	0.29	TRUE	7.051	345.14481	C18H20N2O5	Dipeptides	JAQGXUEKGKTKX-F
13189	12.304	854.14709	3-hydroxybutanoyl CoA	[M+H] <sup>+</sup>	null	0.14	FALSE	-1	854.159	C25H42N7O18P3S	(R)-3-hydroxyacyl CoAs	QHHKKMYHDBRON'
357	0.988	258.10495	3-Methylcytidine	[M+H] <sup>+</sup>	null	0.43	FALSE	6.991	258.108	C10H15N3O5	Pyrimidine nucleosides	RDPUKVRQKWBSPK-
158	19.623	184.11093	3-Methyl-N-phenylaniline	[M+H] <sup>+</sup>	null	1	FALSE	18.66	184.11211	C13H13N	Aminotoluenes	TWPMMLHBHPYSMT
11647	19.563	810.12158	Acetyl-CoA	[M+H] <sup>+</sup>	null	0.14	FALSE	13.658	810.13	C23H38N7O17P3S	Acyl CoAs	ZSLZBFDCINBPY-ZS
4108	1.061	556.93024	Adenosine	[M+H] <sup>+</sup>	null	0.43	FALSE	-1	556.93256	C10H13N5O4	Purine nucleosides	OIRDTQYFTABQOQ-
4215	21.168	560.07227	ADENOSINE DIPHOSPHATE RIBOSE	[M+H] <sup>+</sup>	null	0.14	TRUE	-1	560.07898	C15H23N5O14P2	Purine nucleotide sugars	SRNWOUGRCWSEM
2982	11.634	505.98856	Adenosine triphosphate	[M-H] <sup>-</sup>	null	0.43	FALSE	6.504	505.98795	C10H16N5O13P3	Purine ribonucleoside triphosphates	ZKHQWZAMYRWXG
346	0.946	257.14325	BETAINE	[2M+Na] <sup>+</sup>	null	0.86	FALSE	-1	257.147	C5H11NO2	Alpha amino acids	KWIUHFFTVRNATP-U
10513	18.861	768.55054	Coenzyme A	[M+H] <sup>+</sup>	null	0.29	FALSE	-1	768.56	C21H36N7O16P3S	Coenzyme A and derivatives	RGJOEKWQDUBAIZ-
13640	21.447	869.68597	Coenzyme Q10	[M+Li] <sup>+</sup> *	null	0.14	FALSE	-1	869.70001	C59H90O4	Ubiquinones	ACTIUHUUMQJHFO-
202	11.016	211.13986	Cyclo(Leu-Pro)	[M+H] <sup>+</sup>	null	0.14	FALSE	1.178	211.14391	C11H18N2O2	Alpha amino acids and derivatives	SZJNCZMRZAUNQT-
78	1.061	155.07854	Cyclo-prolylglycine	[M+H] <sup>+</sup>	null	0.43	FALSE	2.06	155.0815	C7H10N2O2	Alpha amino acids and derivatives	OWOHLURDBZHNGC
1443	12.161	413.2428	Cyflufenamide	[M+H] <sup>+</sup>	null	0.29	FALSE	-1	413.23999	C20H17F5N2O2	Trifluoromethylbenzenes	ACMXQHFNODYQA'
2629	1.088	489.44208	Cytidine 5'-diphosphocholine	[M+H] <sup>+</sup>	null	0.43	FALSE	-1	489.44	C14H26N4O11P2	Pyrimidine ribonucleoside diphosph	RZZPDXZPRHQCG-
2548	9.27	484.25879	Cytidine-5'-triphosphate	[M+H] <sup>+</sup>	null	0.14	FALSE	-1	484.26001	C9H16N3O14P3	Pyrimidine ribonucleoside triphosph	PCDQPRRSZKQHHS-
94	19.612	161.09592	D-Ala-D-ala	[M+H] <sup>+</sup>	null	0.29	FALSE	-1	161.09259	C6H12N2O3	Dipeptides	DEFJQIDDEAULHB-C
278	11.051	237.12155	DETHIOBIOTIN	[M+Na] <sup>+</sup>	null	0.14	FALSE	-1	237.121	C10H18N2O3	Medium-chain fatty acids	AUTOLBMXDDTRRT-
99	1.055	163.1048	DL-5-HYDROXYLYSINE	[M+H] <sup>+</sup>	null	0.43	FALSE	5.985	163.10738	C6H14N2O3	Alpha amino acids	YSMODUONRAFBE-

# Results



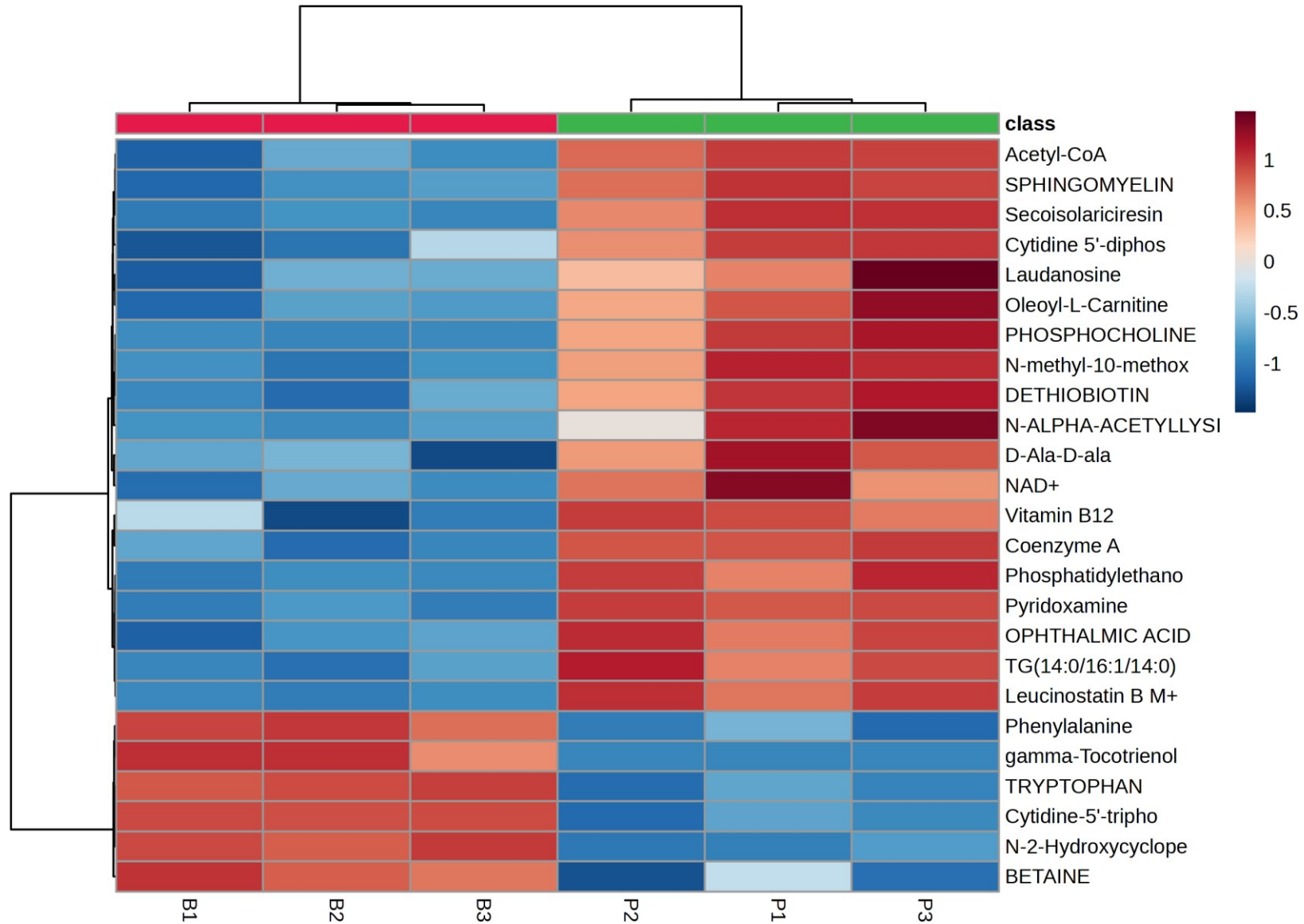
**Figure 1. PCA Analysis of gelatin**

# Figure 2. Candidate metabolites with VIP score > 1.0



**VIP = variable importance in projection**

# Figure 3. Heatmap analysis in porcine and bovine gelatin



# Conclusions

- Untargeted metabolomics approach provided the information:
  - The candidate metabolites including sphingomyelin SM(d18:0/18:1(9Z)), cytidine 5'-diphosphocholine, 3-methylcytidine, NAD<sup>+</sup>, ATP, and Tyr-Tyr, were found in higher abundance in porcine gelatin.
  - The metabolites cytidine-5'-triphosphate, tryptophan, and phenylalanine were more prevalent in bovine gelatin.
- The LC-QTOF-MS/MS-based metabolomics workflow provides high sensitivity and could also be used in halal applications.



# Further research

A comparative analysis was conducted among different animals, including pigs, cows, fish, and chickens.

Metabolite targeting analysis is conducted to confirm the potential metabolites.

# Acknowledgments



Dr. Pornpimol Mahamad  
Director



Dr. Anat Denyinghot  
Deputy Director

**This work was supported by:  
The Halal Science Center,  
Chulalongkorn University**



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# Our Research Team

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# Contact

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**Nuresun Mahamud, PhD.**

Researcher,

The Halal Science Center Chulalongkorn University

**Email:** [nuresun.hsc@gmail.com](mailto:nuresun.hsc@gmail.com)

[nuresun.s@chula.ac.th](mailto:nuresun.s@chula.ac.th)

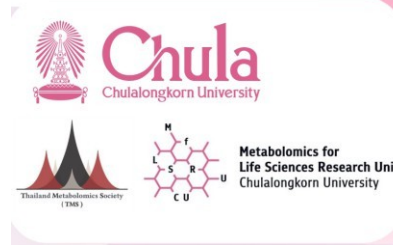
**WhatsApp:** +66873492610

# scientific reports

Check for updates

## OPEN Untargeted metabolomics reveal pathways associated with neuroprotective effect of oxyresveratrol in SH-SY5Y cells

Nureesun Mahamud<sup>1,2</sup>, Phanit Songvut<sup>3</sup>, Chawanphat Muangnoi<sup>4</sup>, Ratchanee Rodsiri<sup>5,6</sup>, Winai Dahlan<sup>2</sup> & Rossarin Tansawat<sup>1,7</sup>✉



### Comprehensive Metabolomics Approaches for Chemical Profiling of *Carica papaya* Leaf

Nalinrat Puangnil<sup>1,2</sup>, Nureesun Mahamud<sup>3</sup>, Piyawadi Khaoiam<sup>1,2</sup>, Arashaporn Uthairangsi<sup>1,2</sup>, Sreyleak Leanghy<sup>1,2</sup>, Rossarin Tansawat<sup>1,2</sup>

<sup>1</sup>Department of Food and Pharmaceutical Chemistry, Faculty of Pharmaceutical Science, Chulalongkorn University, Bangkok, Thailand

<sup>2</sup>Metabolomics for Life Sciences Research Unit, Chulalongkorn University, Bangkok, Thailand

<sup>3</sup>The Halal Science Center, Chulalongkorn University, Bangkok, Thailand

### Effect of harvest time on metabolite profiles of rice leaf using LC-QTOF MS/MS metabolomics approach



Arashaporn Uthairangsi<sup>1,2</sup>, Nureesun Mahamud<sup>3</sup>, Supat Chaiyakul<sup>4</sup>, Rossarin Tansawat<sup>1,2\*</sup>

### COMPARATIVE STUDY OF PLASMA METABOLOME DERIVED FROM DIFFERENT TYPES OF BLOOD COLLECTION TUBES

Praween Thosa<sup>a, d</sup>, Nureesun Mahamud<sup>b</sup>, Eakachai Prompetchara<sup>c</sup>, Rossarin Tansawat<sup>a, d</sup>

<sup>a</sup> Department of Food and Pharmaceutical Chemistry, Faculty of Pharmaceuticals Sciences, Chulalongkorn University, Bangkok, Thailand. <sup>b</sup> The Halal Science Center, Chulalongkorn University Bangkok, Thailand.

<sup>c</sup> Department of Laboratory Medicine, Faculty of Medicine, Chulalongkorn University Bangkok, Thailand. <sup>d</sup> Metabolomics for Life Science Research Unit, Chulalongkorn University, Bangkok, Thailand.

A metabolomics research project we conducted



thank you