New Approaches to Food Authenticity Testing

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Digital Technologies and Fingerprinting to Improve Food System Outcomes

Improving the performance of food safety system to control foodborne diseases

Improving the performance of the food system is central to reaching the Sustainable Development Goals.

Expanding digital opportunity for all, including farmers and agribusiness, will improve the food system outcomes in both rural and urban areas.

Use of digital technology can help reduce costs, help farmers make more precise decisions, and improve access to information, knowledge and markets.

The Issue

Improving the performance of the food system is critical if we are to sustainably feed nearly 10 billion people by 2050 while raising farmer incomes, protecting them from climate change and helping them to thrive.

Digital technologies have the potential to deliver significant positive impacts across food value chains.

Food systems:

more resource-efficient and

climate-resilient such as precision agriculture, gene-editing and biological-based crop protection, or technologies that improve traceability from farm to fork.

Food Authenticity

Food fraud is designed to increase the perceived value of both food and ingredients and is a growing concern in our global food supply.

As quality programs are upgraded to better address this concern, new analytical digital tools are being developed to evaluate if known and unknown adulterations or modifications have been added to foods.

The two main approaches involve: both of which are used to reduce the number of food fraud incidents

Targeted analyses-Classical (fatty acid profile of olive oil)

Non-Targeted analyses- Fingerprinting (melamine in milk powder)

Food Fraud

"A collective term encompassing the deliberate and intentional substitution, addition, tampering or misrepresentation of food, food ingredients or food packaging, labeling, product information or false or misleading statements made about a product for economic gain that could impact consumer health" (Michigan State University, Spink and Moyer 2011).

The key points in this definition are the food being intentionally changed for financial gain and disregarding the health or monetary impact on consumers.

Common Adulterated Products

1. OLIVE OIL
(origin, mixing with other oils)

2. FISH
(species, quality, fresh vs frozen-thawed, wild vs farmed)

3. ORGANIC FOODS

4. MILK
(origin, type, dilution, melamine)

5. GRAINS
(basmati rice, durum vs tender wheat in pasta, GMO)

6. HONEY & MAPLE SYRUP
(sugars added, origin)

7. COFFEE & TEA
(arabica/robusta, origin and varieties, grass cuttings)

8. SPICES
(saffron origin, chilli coloured with illegal dyes)

9. WINE
(origin-PGI)

10. CERTAIN FRUIT JUICES
(diluted, 100% declared fruit)

- The British Retail Consortium (BRC) Global Standards
- The U.S. Pharmacopeial Convention (USP)
- AOAC International

Having access to information on historical and developing threats to the supply chain.

Performing a documented vulnerability assessment on all food raw materials.

Performing appropriate assurance and/or testing to reduce uncovered risks for raw materials.

The seven principal ways a food or food ingredient can be adulterated to increase its perceived value.

1. Substitution: replacing some or all of a food with a cheaper material. Examples include extra virgin olive oil, partially or totally, substituted with lower grade olive oil.

2. Unapproved Enhancements: additives used to trick routine analytical tests. Examples include melamine added to increase perceived protein level in milks, and high fructose corn syrup added to honey.

3. Concealment: adding a material to mask the lower quality food. Examples include poultry injected with hormones to conceal disease and food coloring applied to fresh fruit to cover defects.

4. Mislabeling: when a food product's label does not accurately reflect its ingredients. Examples include labeling tilapia as yellowfin tuna and conventionally grown foods labeled as organic.

5. Dilution: adding cheaper materials to expensive ingredients. Examples include olive oil diluted with potentially toxic tea tree oil and watering down products with unsafe water.

6. Counterfeiting: replacing a food or ingredient with a "look-alike" product. Examples include dietary supplements and medications.

7. Gray Market Production/Theft/Diversion: the sale of excess or unreported product. This covers most foods and increases the risk of a product not having proper safety assurances.

Targeted Analyses TA

used if the adulterating materials are known or if the authentic food contains specific marker compounds that can be used to assess its purity.

In the TA approach, the known compounds can be analytically targeted to either determine if the food has a previously identified adulterant or if the food had been diluted or replaced by looking for compounds known to be at certain levels in the authentic product.

The range of technologies used for targeted testing is quite broad, covering everything from traditional wet chemistry to high-end liquid chromatographic mass spectrometry methods.

Targeted Analyses TA-Examples

Extra virgin olive oil is sometimes adulterated with cheaper vegetable oils-fatty acid profile that can be measured to see if it is at the expected level found in olive oil. Measuring levels of expected sterols, ECN42, and stigmastadiene. These measurements together assess both the oil's identity and the degree of virginity.

Verifying a meat or fish species using PCR and DNA sequencing-fish freshness is assessed by measuring volatile nitrogen or biogenic amines using simple chromatography.

Organic claims are monitored using multi-residue screens for pesticides, herbicides, and fungicides-using several detection technologies.

Non-Targeted Analyses-NTA Fingerprinting

Combining lab-based analytical test methods with statistical software to determine if a questioned food falls within the expected parameters.

Creating a fingerprint of the food or ingredient that is known to be genuine and then comparing it to a questioned food's fingerprint or ingredient.

Nuclear magnetic resonance (NMR), spectroscopic (such as IR, NIR, SWIR, FTIR, Raman), and other common technologies can create distinctive profile "pictures" that can be used by chemometric software that identifies regions to compare to tested foods to determine how different the profile pictures are from each other.

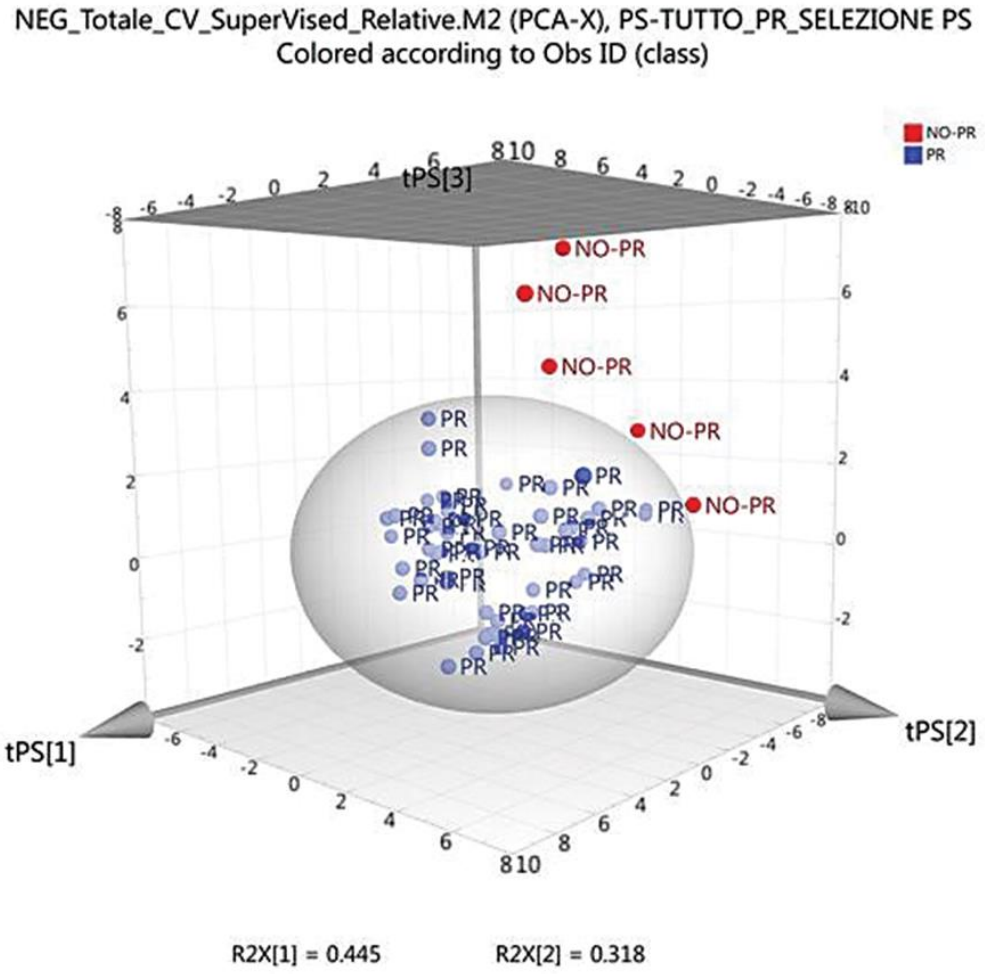
Summary comparison of the TA and NTA approaches

Subject	Targeted Analyses	Non-Targeted Analyses
Approach	Bottom-up	Top-down
Analytes	Targeted compounds	Fingerprint
Sensitivity	High sensitivity	High throughput
Sample preparation	Selective	Unselective/minimum
Data analysis	Univariate/Calibration	Multivariate/Modeling. May require chemometric software
Control limits	Publicly available	Specific reference databases
Consistency	Simple: Sample representativeness	Complex: Experimental design

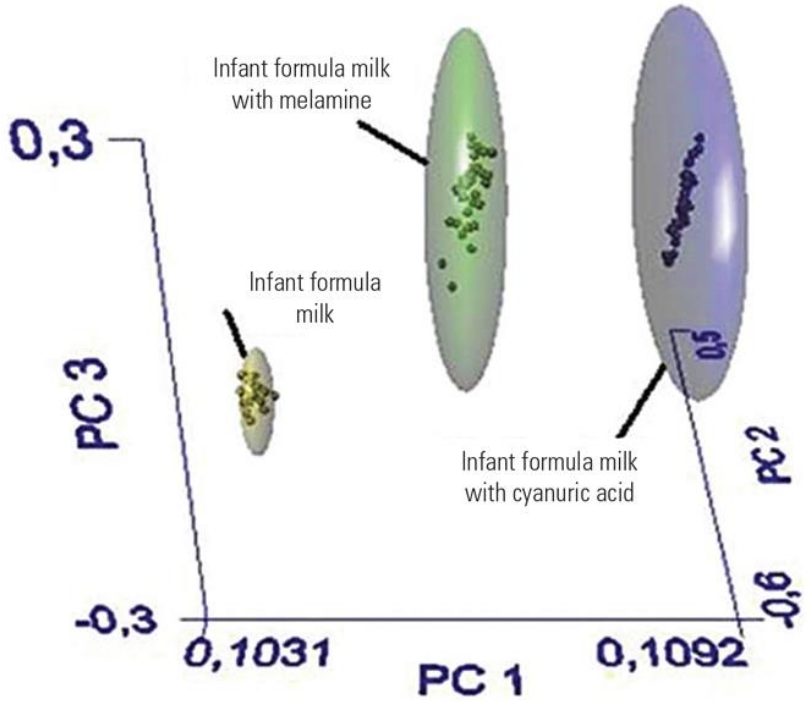
Evaluating cheese authenticity (Popping et al. 2017)- utilization a metabolomics-oriented, non-targeted method that assesses 18 compounds using liquid chromatography-high resolution mass spectrometry (LC-HRMS).

The base fingerprint was created using 52 authentic samples- the fingerprint was then verified by analysis of 32 verification samples.

This approach was able to correctly assess the cheese authenticity with 87.5% accuracy.


NEG_Totale_CV_SuperVised_Relative.M2 (PCA-X), PS-TUTTO_PR_SELEZIONE PS
Colored according to Obs ID (class)
NO-PR
PR
tPS[3]
tPS[1]
tPS[2]
R2X[1] = 0.445
R2X[2] = 0.318

Melamine/cyanuric acid in the milk-a NTA can be used to create a milk chemical fingerprint for comparison to milks that have been adulterated with different nitrogen-containing compounds.



A mid-FTIR spectroscopy with multivariate analysis (Garcia-Miguel 2018)

GARCIA-MIGUEL 201)

Rapid and quantitative detection of the microbial spoilage in food samples by diffuse reflectance spectroscopy (600–1100 nm)

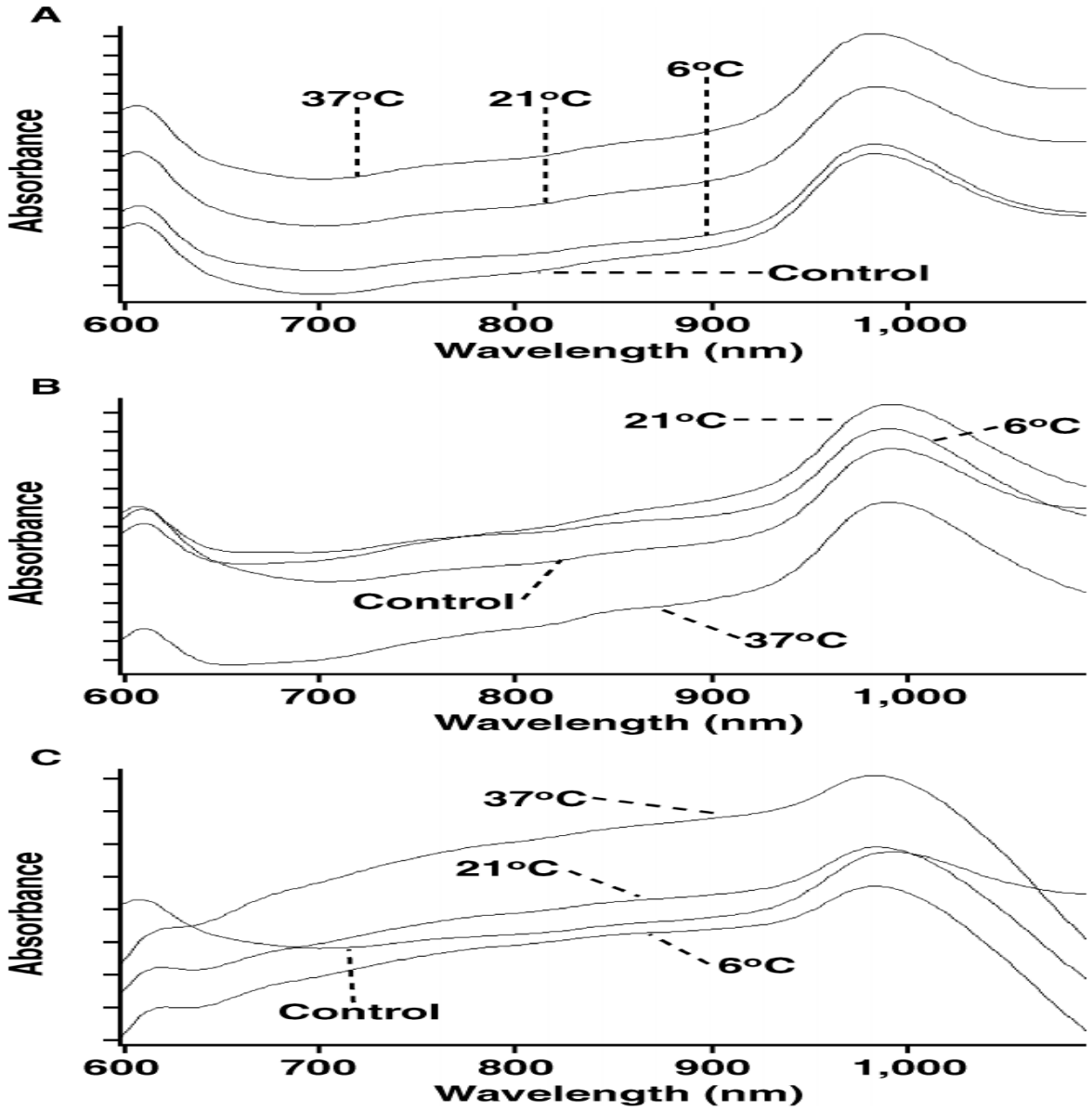
Visible and SW-NIR spectroscopy is a technique with a considerable potential for monitoring food safety and food spoilage.

Visible and SW-NIR combined with PCA is capable of perceiving the change of the microbial loads in food samples once the APC increases slightly above 1 log cycle.

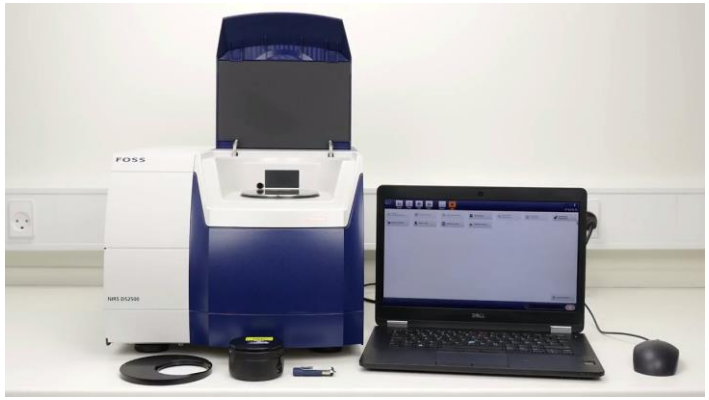
Accurate quantification of the bacterial loads can be calculated from the PLS-based prediction method.

Visible and SW-NIR can acquire a metabolic snapshot and quantify the microbial loads of food samples rapidly, accurately, and noninvasively. This method would allow for more expeditious applications of quality control in food industries.

Monitoring Quality Loss of Pasteurized Skim Milk Using Visible and Short Wavelength Near-Infrared Spectroscopy and Multivariate Analysis



Representative visible and short wavelength near-infra-red spectral patterns (600 to 1,100 nm) of control (t = 0 h) and milk samples stored at 6, 21, and 37°C for t = 3 (A), 8 (B), and 30 h (C).



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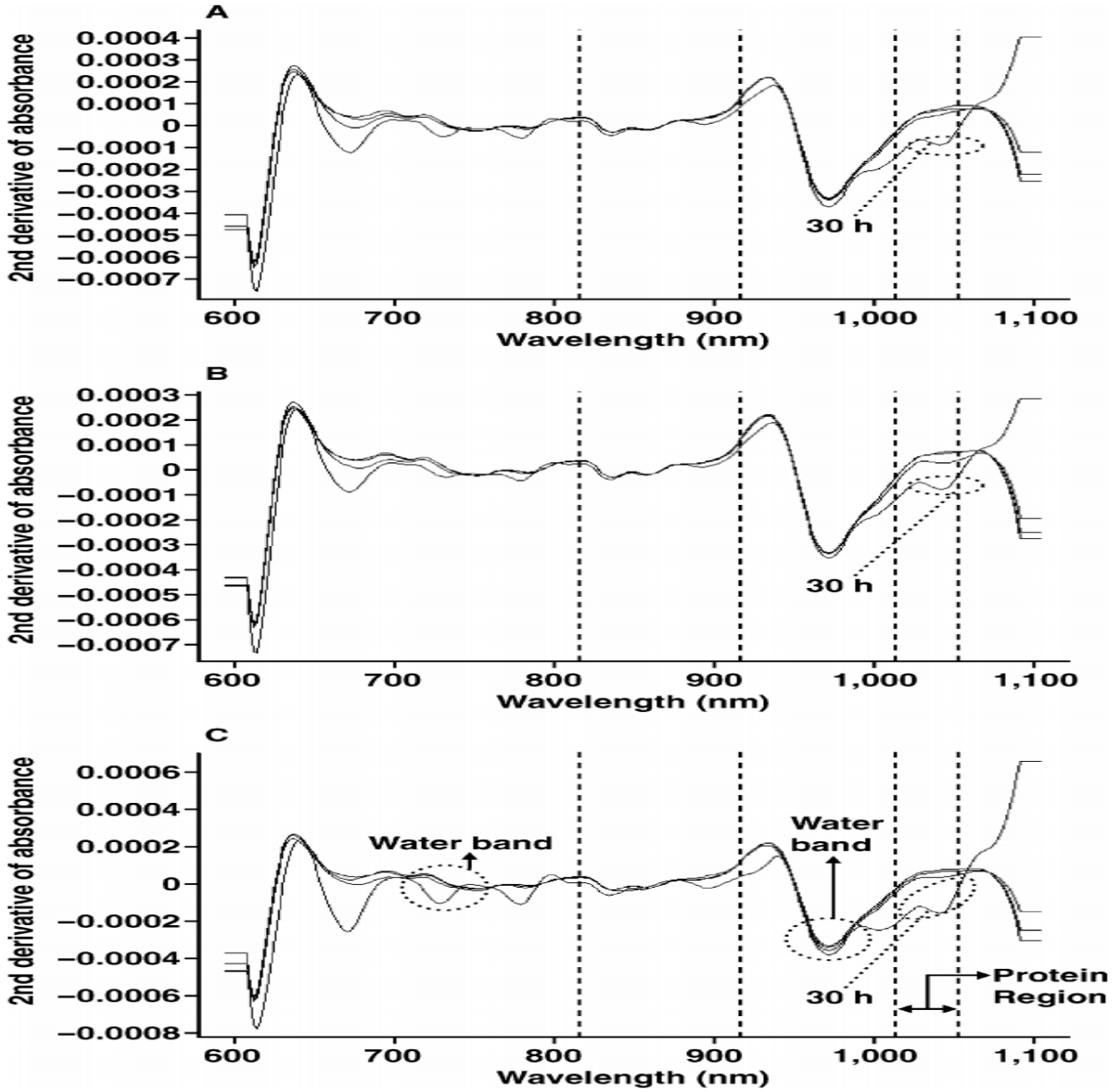
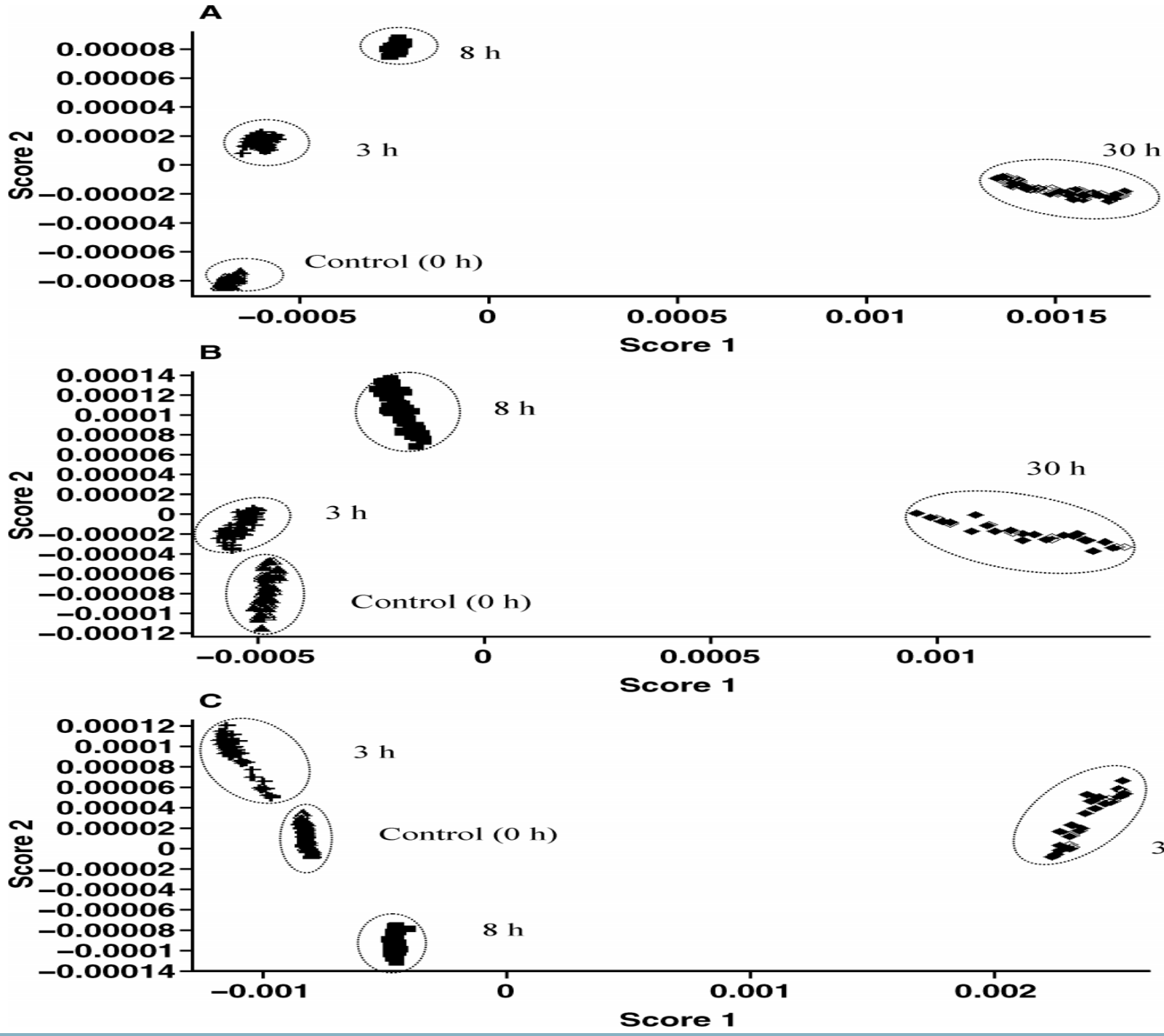


Representative second-derivative transformation of spectral patterns for control (t = 0 h) and milk samples stored at 6 (A), 21 (B), and 37°C (C) for t = 3, 8, and 30 h.


A
8 h
3 h
30 h
Control (0 h)
Score 2
Score 1
0.00008
0.00006
0.00004
0.00002
0
−0.00002
−0.00004
−0.00006
−0.00008
−0.0005
0
0.0005
0.001
0.0015
B
8 h
3 h
30 h
Control (0 h)
Score 2
Score 1
0.00014
0.00012
0.0001
0.00008
0.00006
0.00004
0.00002
0
−0.00002
−0.00004
−0.00006
−0.00008
−0.0001
−0.00012
−0.0005
0
0.0005
0.001
C
3 h
Control (0 h)
8 h
30 h
Score 2
Score 1
0.00012
0.0001
0.00008
0.00006
0.00004
0.00002
0
−0.00002
−0.00004
−0.00006
−0.00008
−0.0001
−0.00014
−0.001
0
0.001
0.002

6 °C

21 °C

37 °C

Soft independent modeling of class analogy classification results of each storage treatment compared with the other test treatments regarding storage temperature at t = 3, 8, and 30 h

	Correctly classified spectra					
	3 h		8 h		30 h	
Storage treatment	n	%	n	%	n	%
Control (0 h)	49	82	54	90	55	92
6°C	50	83	53	88	56	93
21°C	51	85	52	87	53	88
37°C	54	90	53	88	55	92

Rapid and quantitative detection of the microbial spoilage in chicken meat by diffuse reflectance spectroscopy (600–1100 nm)

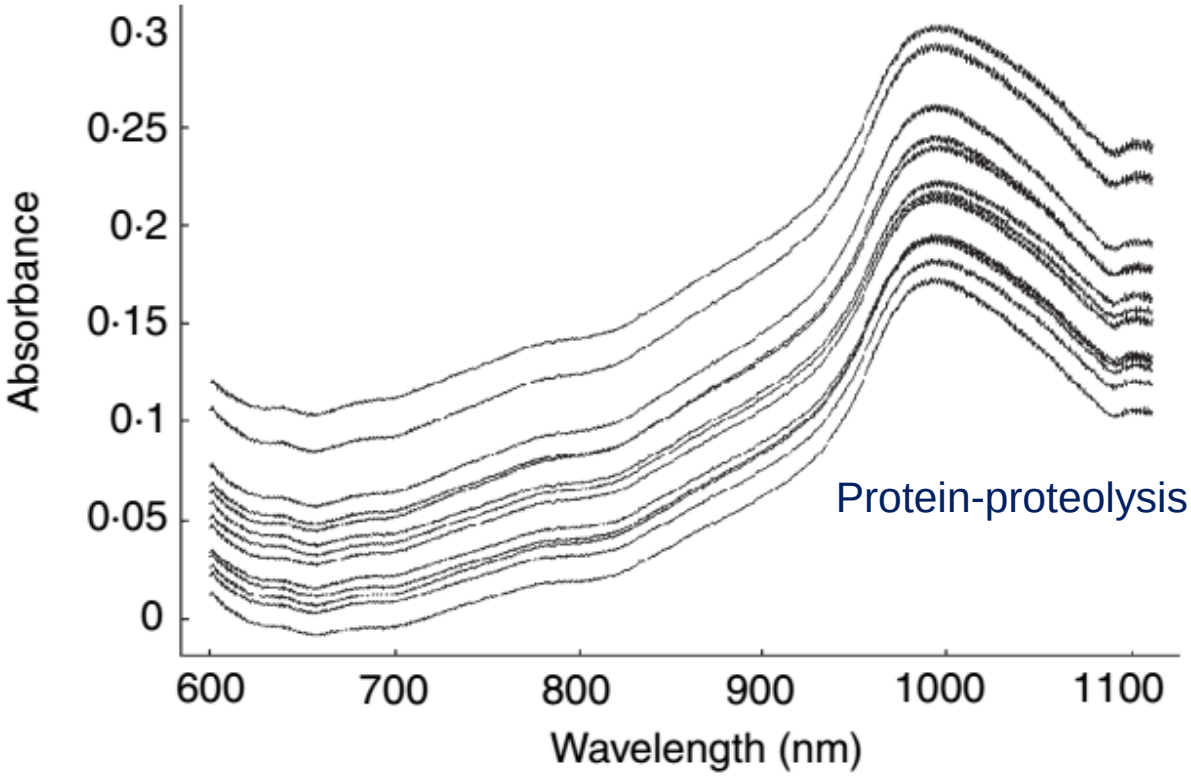


Fig. 1 Representative visible and SW-NIR diffuse reflectance spectra for chicken muscle

Rapid and quantitative detection of the microbial spoilage in chicken meat by diffuse reflectance spectroscopy (600–1100 nm)

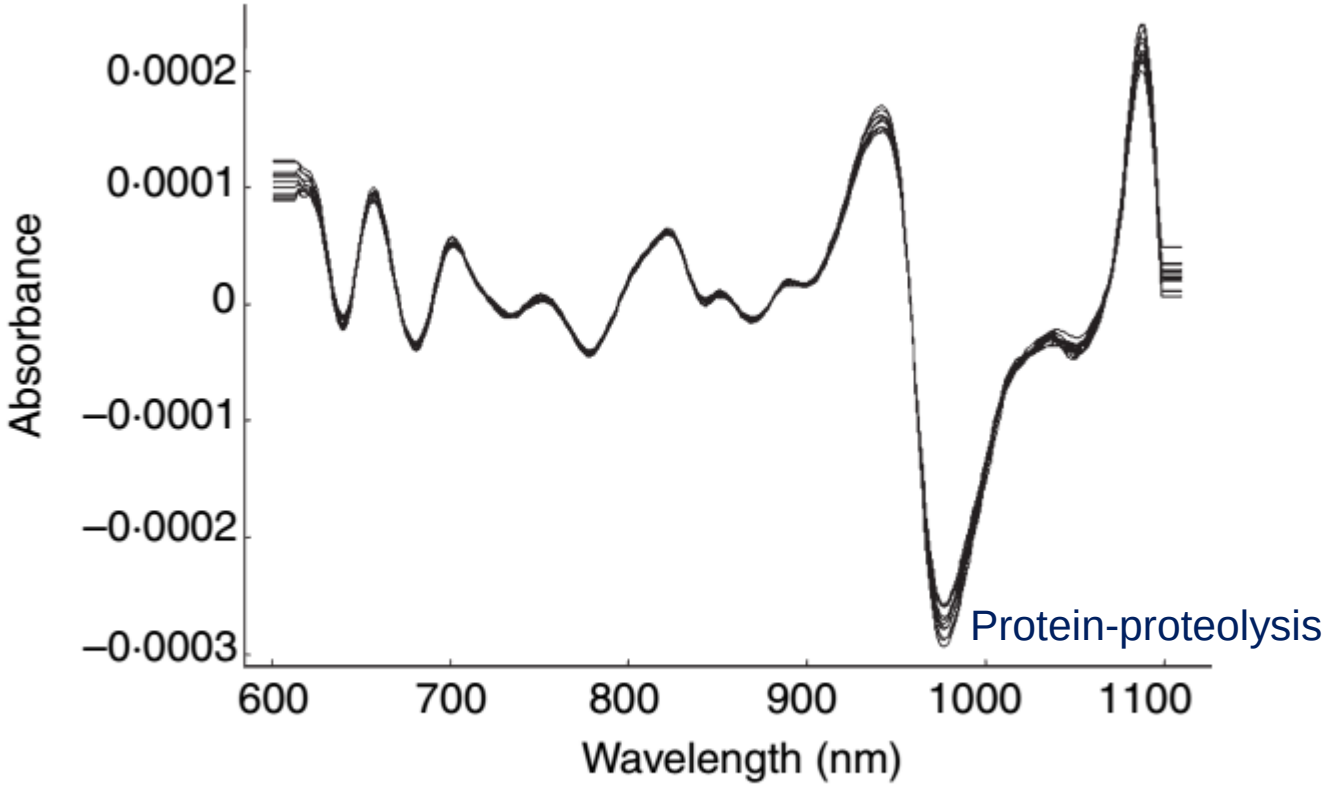


Fig. 2 Second derivative transformation of spectra for chicken muscle

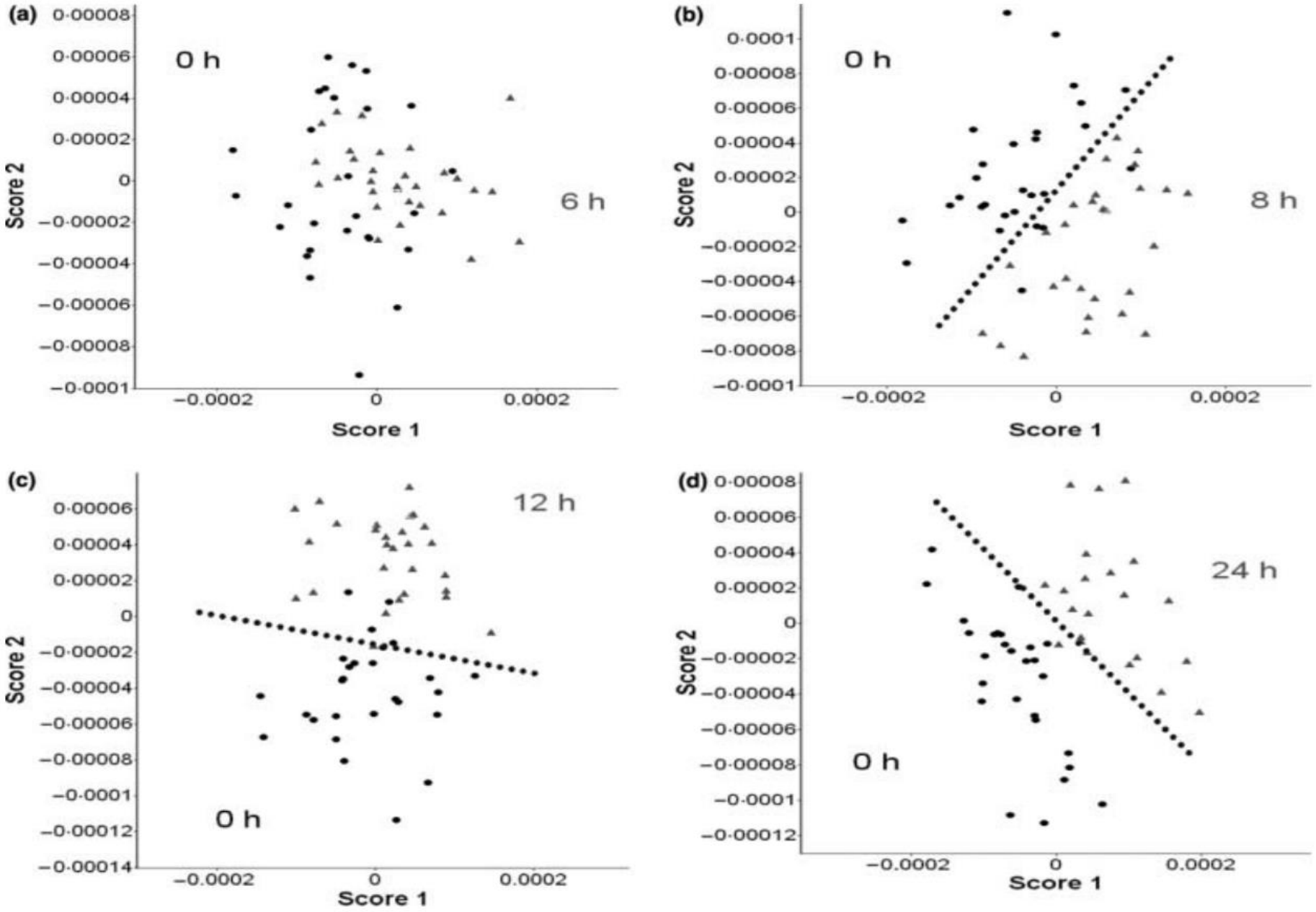


Fig. 3 PCA results for chicken muscle samples held 0 and 6 h (a), 0 and 8 h (b), 0 and 12 h (c) and 0 and 24 h (d) at 21°C. ●, 0-h control; ▲, 6, 8, 12 and 24-h samples

Rapid and quantitative detection of the microbial spoilage in chicken meat by diffuse reflectance spectroscopy (600–1100 nm)

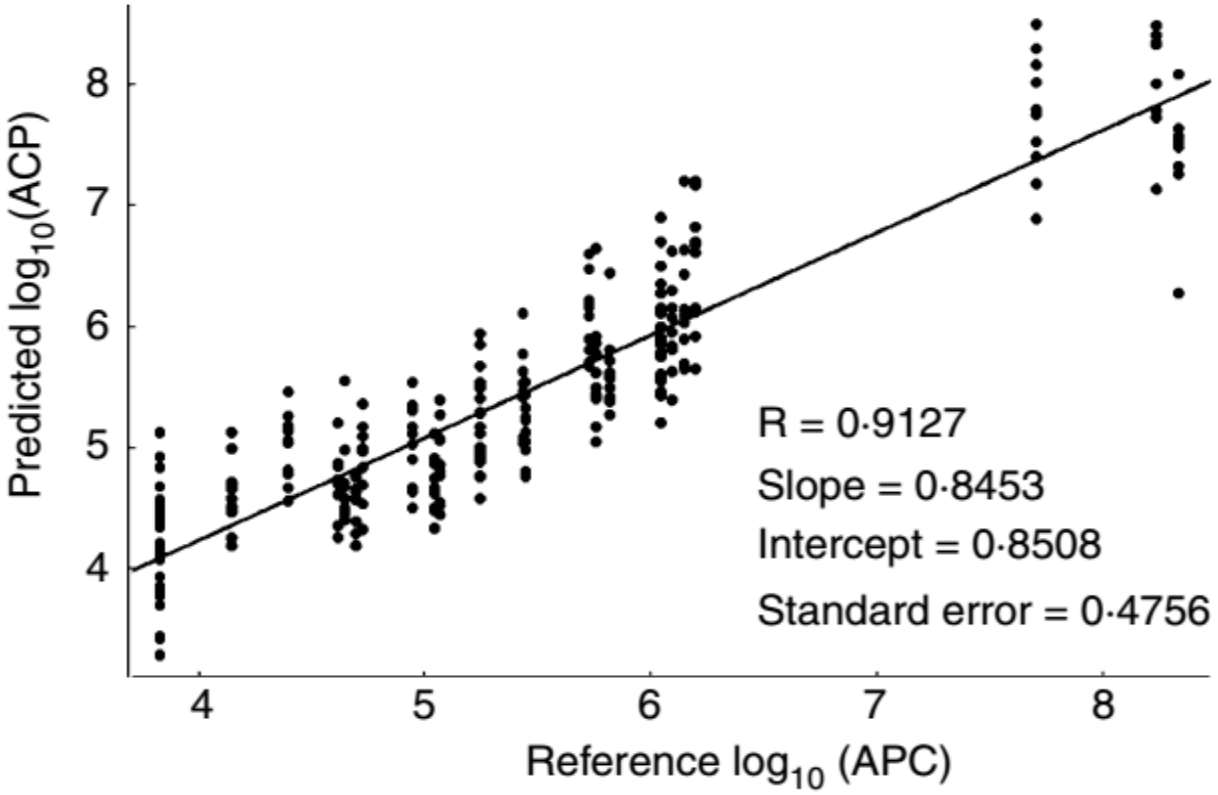


Fig. 5 Comparison between the actual and predicted aerobic plate counts (APC) for chicken muscle

Rapid and quantitative detection of the microbial spoilage in chicken meat by diffuse reflectance spectroscopy (600–1100 nm)

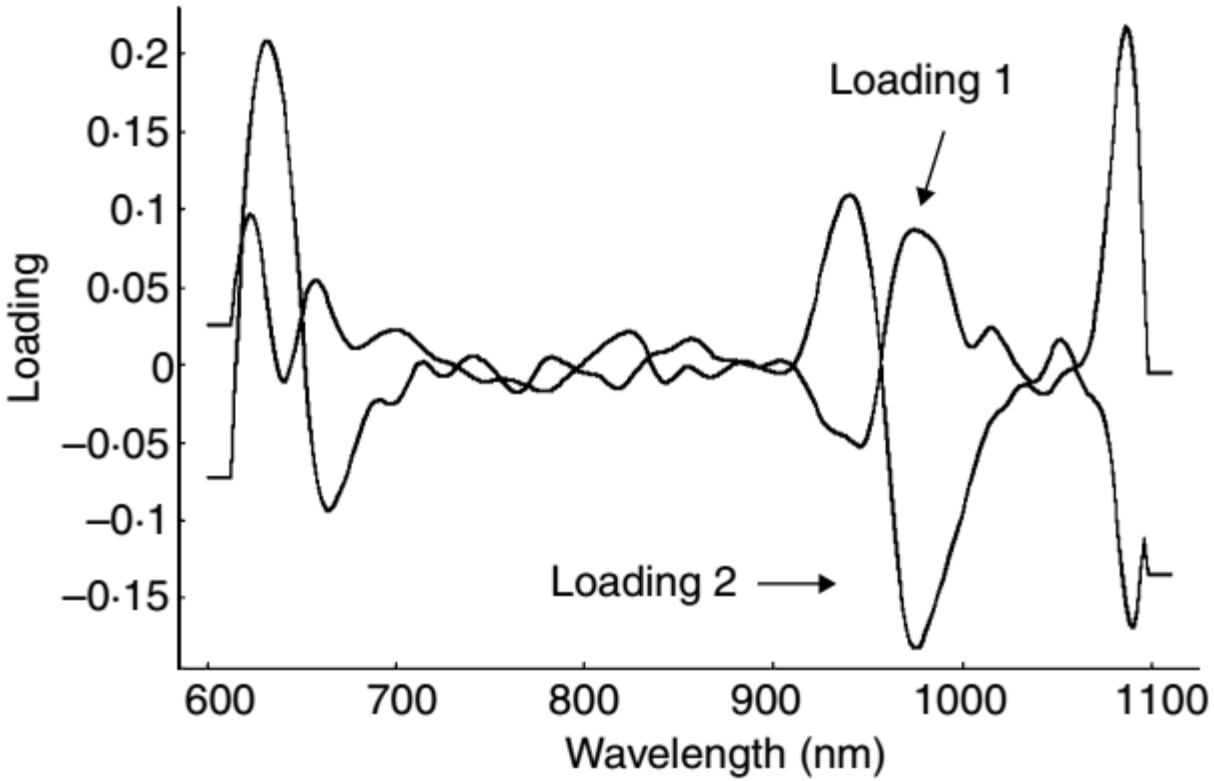
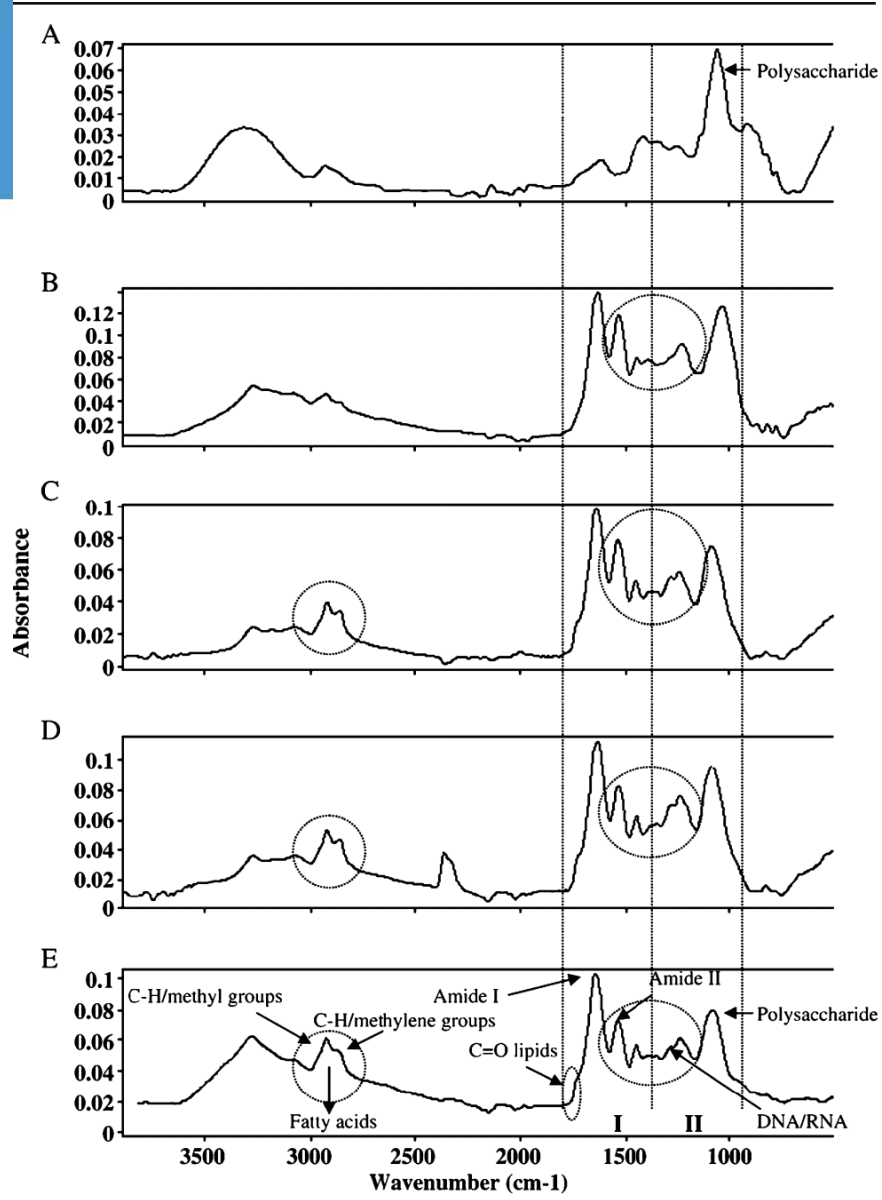


Fig. 6 Loading 1 and 2 from the PLS modelling of chicken muscle samples

Loading 1 (1000-1060 nm): Protein functional group, such as R-CO-NH2, R-NH2, R-CO-NH-R and R-NH-R, with absorption band in the region of 1000–1060 nm. The most significant metabolic process that occurs at the early phases of spoilage may be the start of proteolysis.

FT-IR



Detection of *Salmonella enterica* Serotype Typhimurium, *Escherichia coli* O157:H7 and and *Listeria monocytogenes* Using Fourier Transform Infrared (FT-IR) Spectroscopy (4000 to 600 cm−1)

HAMZAH ALQADIRI, JFS, 2008

Table 1 Assignment of some bands frequently found in FT-IR and Raman spectra respective of biological specimens (a revision of Maquelin et al. 2002)

FT-IR frequency (cm^{-1})	Assignment [a]	Raman frequency (cm^{-1})	Assignment
3,500	O—H str of hydroxyl groups	3,059	$(C{=}C{-}H)_{(aromatic)}$ str
3,200	N—H str (amide A) of proteins	2,975	CH_3 str
2,955	C—H str (asym) of —CH_3 in fatty acids	2,935	CH_3 and CH_2 str
2,930	C—H str (asym) of>CH_2	2,870–2,890	CH_2 str
2,918	C—H str (asym) of>CH_2 in fatty acids	1,735	>C=O ester str
2,898	C—str of C—H in methine groups	1,650–1,680	Amide I
2,870	C—H str (sym) of—CH_3	1,614	Tyrosine
2,850	C—H str (sym) of>CH_2 in fatty acids	1,606	Phenylalanine
1,740	>C=O str of esters	1,575	Guanine, adenine
1,715	>C=O str of carbonic acid	1,440–1,460	C—H_2 def
1,680–1,715	>C=O in nucleic acids	1,295	CH_2 def
1,695, 1,685, 1,675	Amide I from β-turns of proteins	1,230–1,295	Amide III
1,655	Amide I of α-helical structures	1,129	C—N and C—C str
1,637	Amide I of β-sheet structures	1,102	>PO_2^- str (sym)
1,550–1,520	Amide II	1,098	CC skeletal and COC str from glycosidic link
1,515	Tyrosine band	1,085	C—O str
1,468	C—H def of>H_2	1,061	C—N and C—C str
1,400	C=O str (sym) of COO—	1,004	Phenylalanine
1,310–1,240	Amide III	897	COC str
1,250–1,220	P=O str (sym) of >PO_2^- phosphodiesters	858	CC str, COC 1,4 glycosidic link
1,200–900	C—O, C—C str, C—O—H, C—O—C def of carbohydrates	852	"Buried" tyrosine
1,090–1,085	P=str (sym) of >PO_2^-	829	"Exposed" tyrosine
		785	Cytosine, uracil
		720	Adenine
		665	Guanine
		640	Tyrosine (skeletal)
		620	Phenylalanine (skeletal)
		540	COC glycosidic ring ref
		520–540	S—S str

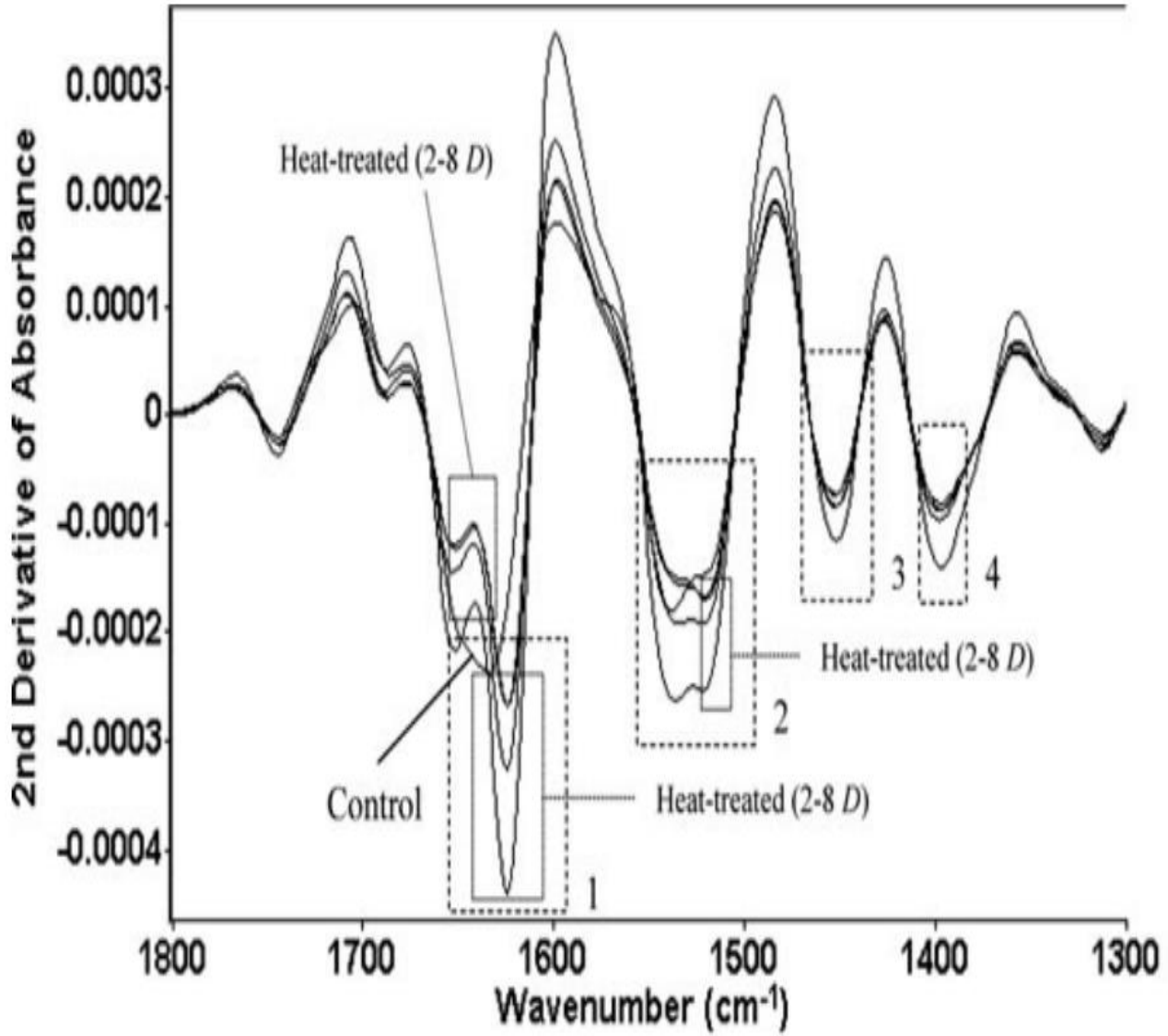


Figure 3 – Representative 2nd derivative spectra (1800 to 1300 cm^{-1}) of *L. monocytogenes* ATCC 19113 at different heat treatments. See caption of Figure 2 for details.

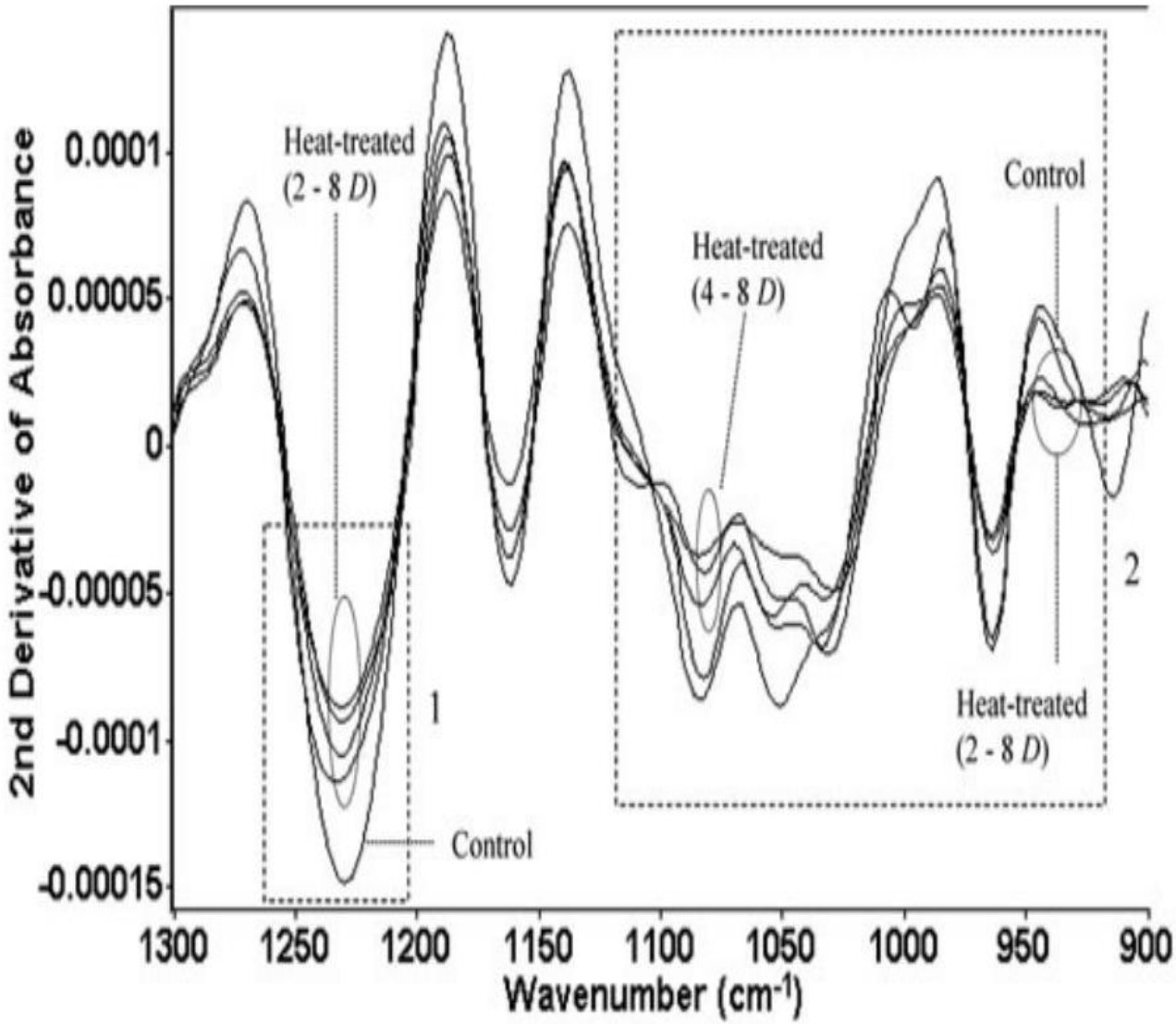


Figure 5—Representative 2nd derivative spectra (1300 to 900 cm^{-1}) of *L. monocytogenes* ATCC 19113 at different heat treatments. See caption of Figure 4 for details.

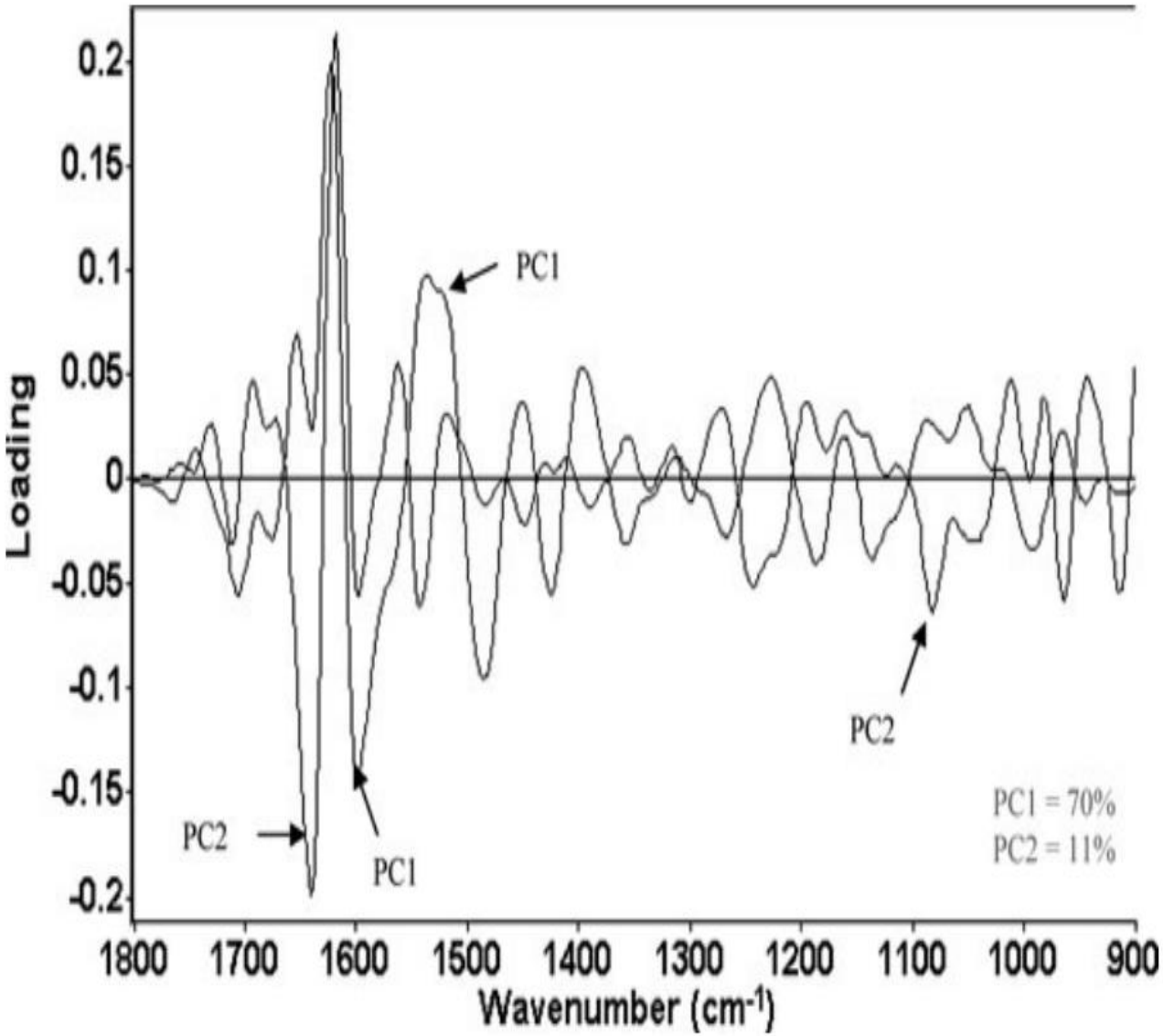
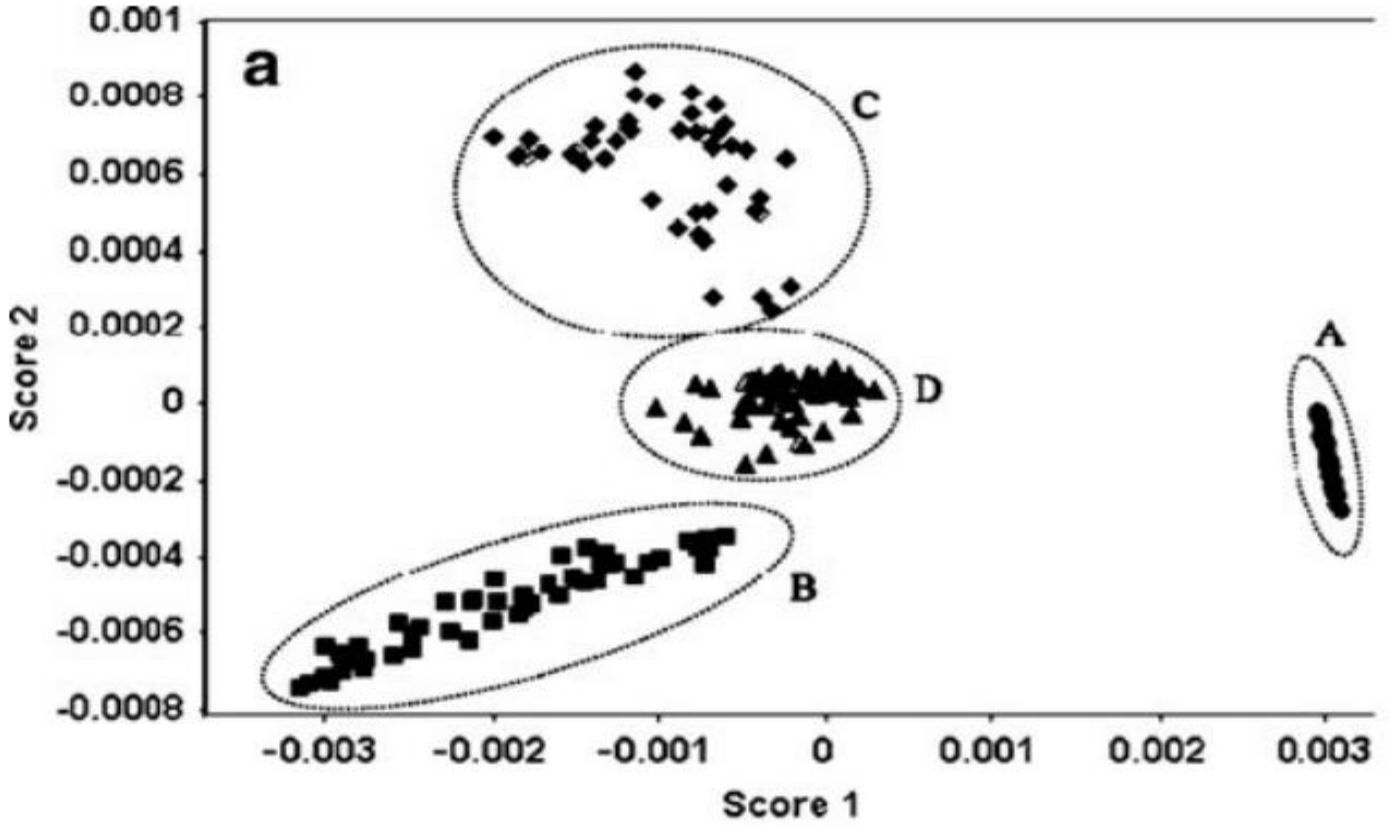
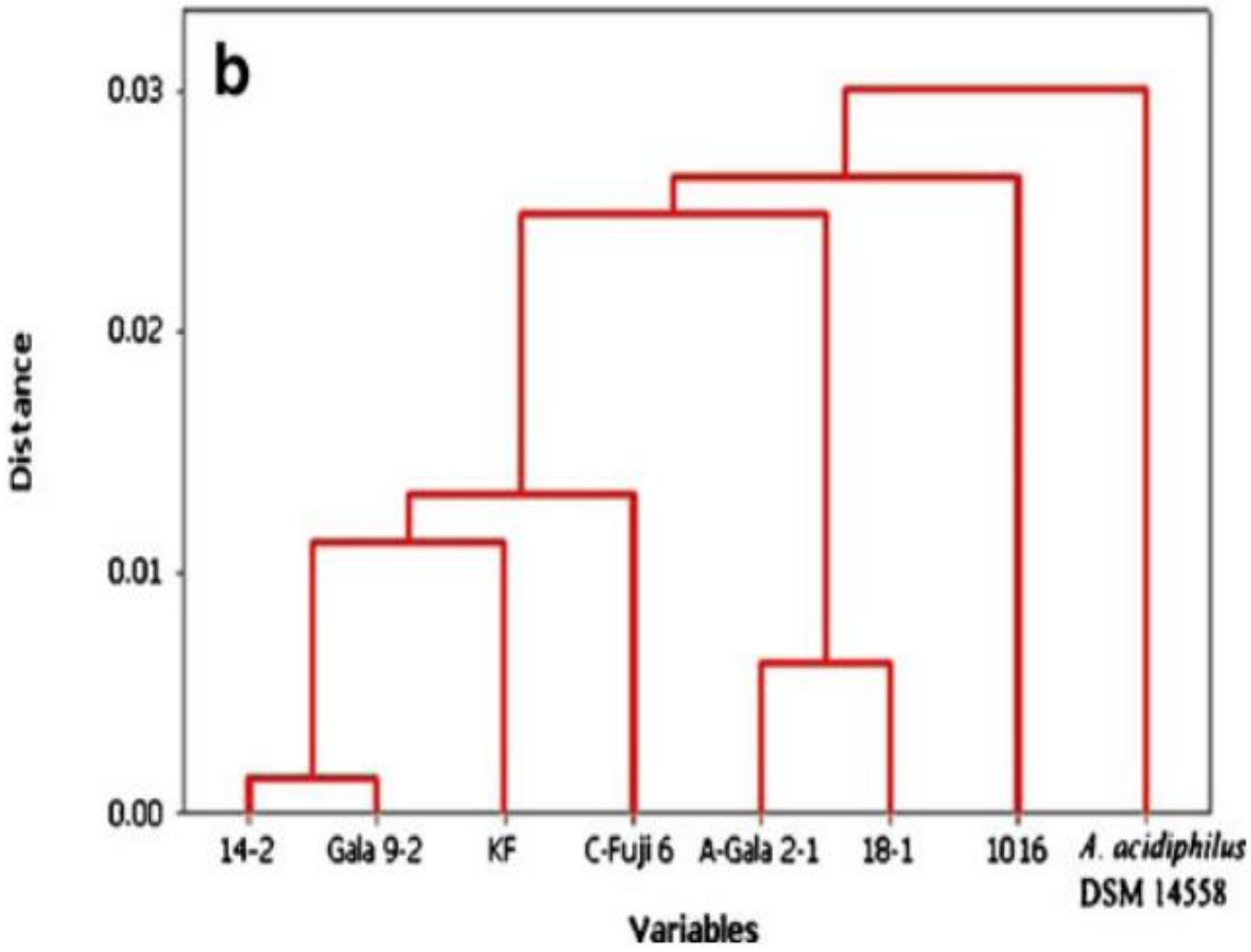


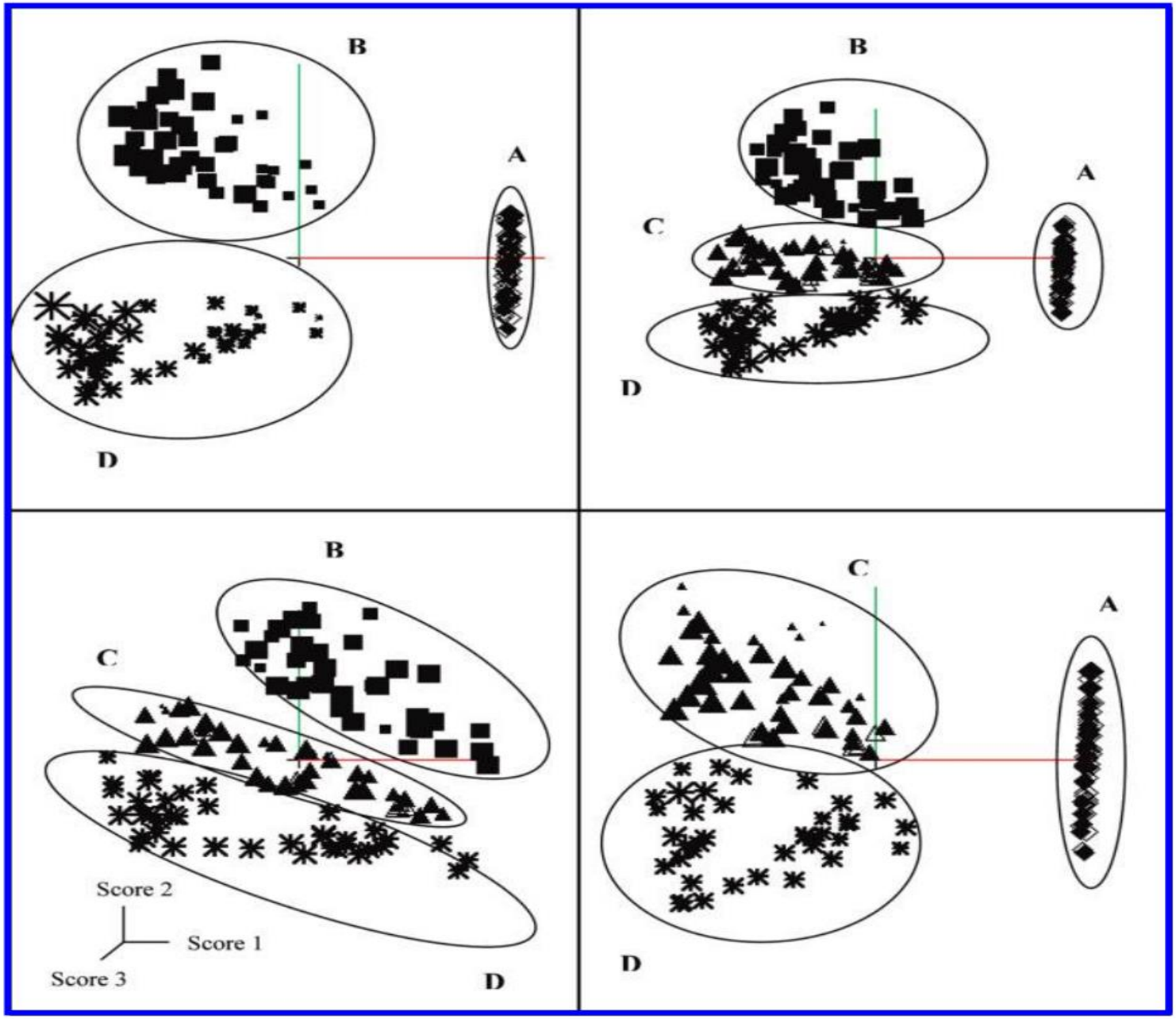
Figure 7 – Loadings of the 1st and the 2nd principal components obtained from PCA analysis of the ATR spectra of heat-injured *L. monocytogenes* ATCC 19113.



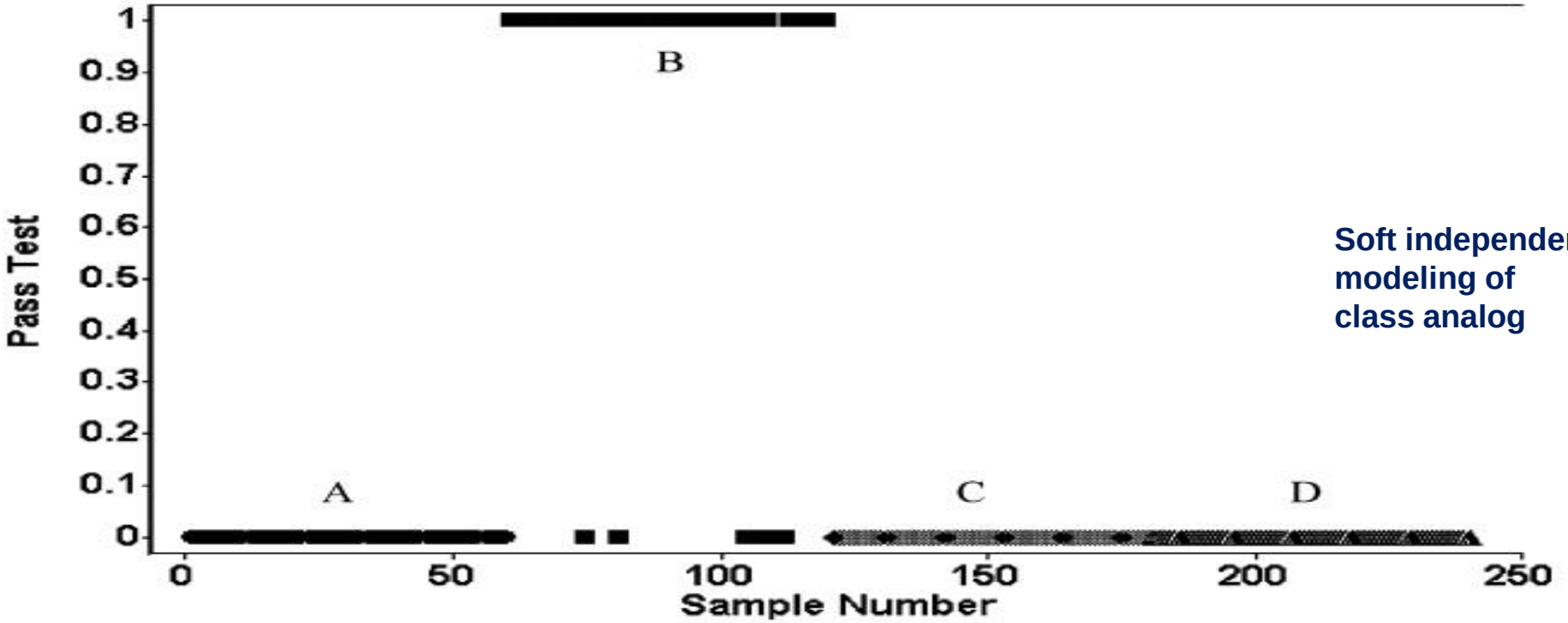
Principal component analysis (PCA) for control (blank without bacteria) (A), E. coli ATCC 25922 (B), P. aeruginosa (C), and mixed (1:1, v/v) culture of E. coli and P. aeruginosa (D). Groups were tightly segregated and significantly different with each other (P<0.05)



Dendrogram of hierarchical cluster analysis (HCA) performed on FT-IR spectral data of eight Alicyclobacillus isolates (citing Lin et al. 2007)



Principal component analysis (PCA) combinations of control (A), 0 ppm (B), 0.3 ppm (C), and 1.0 ppm (D) sodium hypochlorite treatments for mixed bacterial cultures of E. coli ATCC 25922 and P. aeruginosa ATCC 15442.



Soft independent modeling of class analog

Figure 6. SIMCA classification of *E. coli* ATCC 25922 (**B**) as compared to control (**A**), *P. aeruginosa* (**C**), and mixed culture (**D**).

Table 2. SIMCA Classification Results of Each Sample Treatment as Compared to the Other Test Treatments

	correctly classified spectra	
sample treatment	no.	%
control	56	93.3
E. coli ATCC 25922	53	88.3
P. aeruginosa	50	83.3
mixed culture	50	83.3

Conclusion

FT-IR vibrational spectroscopy is a technique used for both the detection and identification of foodborne microbes including bacterial spores and biofilms and for studies involving cell injury and inactivation of microbes by different food preservation methods.

FT-IR spectroscopy provides a basis for studies of food microorganisms but is substantially enhanced when complementary techniques are used that can provide greater insight regarding complicated interactions of a living organism with its environment.

The formation of metabolic by-products, proteolysis, and lipolysis caused by bacterial cell growth leads to a reduction in pH and undesirable biochemical changes.

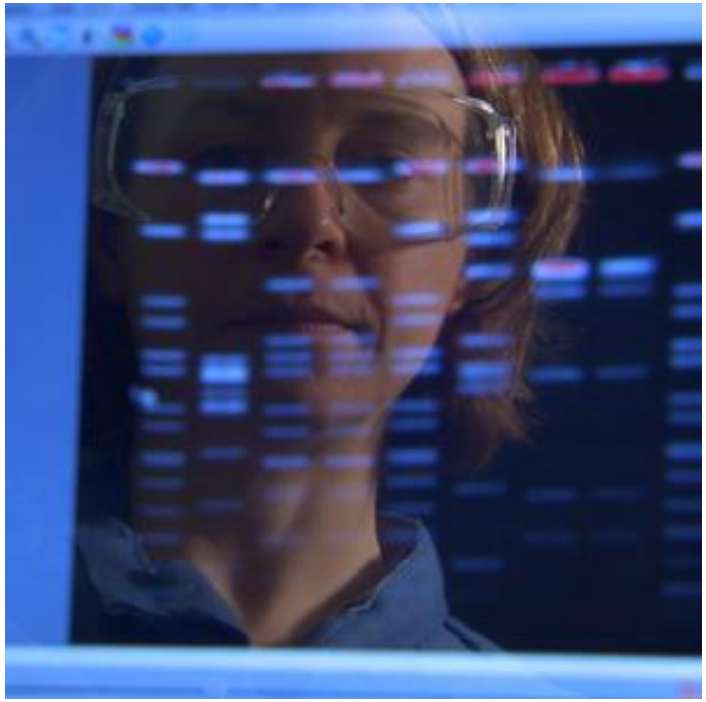
These changes can be detected by visible and SW-NIR diffuse spectroscopy (600 to 1,100 nm) to differentiate wholesome and spoiled food samples without the necessity of enumerating bacteria- applicable for predicting the shelf life and microbial loads.

Multivariate data analytical techniques such as PCA could segregate storage treatments with approximately 90% accuracy.

PulseNet System-DNA fingerprints of bacteria

PulseNet compares the DNA fingerprints of bacteria from patients to find clusters of disease that represent unrecognized outbreaks.

Identifying outbreaks and their sources helps health officials stop outbreaks and helps industry and food regulatory agencies make changes to improve food and water safety and delivery systems. That's where PulseNet comes in.



PulseNet scientist reviews DNA fingerprints

PulseNet System-DNA fingerprints of bacteria

PulseNet is a national US laboratory network that connects foodborne, waterborne, and One Health–related illness cases to detect outbreaks.

PulseNet uses the DNA fingerprints of bacteria making people sick to detect thousands of local and multistate outbreaks. Since the network began in 1996, PulseNet has improved food safety systems through identifying outbreaks early.

This allows investigators to find the source, alert the public sooner, and identify gaps in food safety systems that would not otherwise be recognized. PulseNet International performs a similar role for foodborne illnesses globally.

PulseNet System-DNA fingerprints of bacteria

PulseNet is a national laboratory network consisting of 83 public health and food regulatory laboratories.

PulseNet groups together people who most likely ate the same contaminated food, or who were exposed to illness-causing microorganisms in some other way.

The network does this by analyzing DNA fingerprinting on the bacteria making people sick, and on the bacteria found in food and the environment. People and foods infected or contaminated with bacteria with the same fingerprint are likely to be part of an outbreak.

Public health and regulatory investigators use this information to identify the source of illness, such as an unrecognized problem in the food supply chain.

Microbiologists generate these fingerprints using molecular subtyping tools such as pulsed-field gel electrophoresis (PFGE), multiple-locus variable number tandem repeat analysis (MLVA), and whole genome sequencing (WGS).

PulseNet System Advantages

PulseNet tracks what is being reported to CDC in real time, compares it to what was reported in the past, and looks for any increases that could signal an outbreak.

The PulseNet databases keep growing for this reason. They contain over half a million fingerprints from human illness, food, and the environment.

PulseNet has revolutionized how we detect and investigate foodborne disease outbreaks since it started in 1996. Before PulseNet, these outbreaks often went undetected or were discovered only after they grew very large.

PulseNet is undergoing a major technological transformation from 20 years of PFGE data to a more powerful and precise method, WGS.

This will improve the network's ability to find and investigate outbreaks. Implementing whole genome sequencing will provide the most accurate bacterial fingerprinting data possible today.

PulseNet System Advantages

PulseNet helpes health agencies to create new or improve guidance, policies, and regulations that may prevent thousands of foodborne illnesses making our food supply safer.

PulseNet helps public health scientists determine where our food safety systems have failed so that solutions can be developed to prevent future illnesses.

PulseNet also helps scientists determine whether an outbreak is occurring, even if only a few people are ill or if they are geographically far apart.

The network allows us to identify outbreaks and their causes in a matter of hours rather than days or weeks.

PulseNet System Advantages

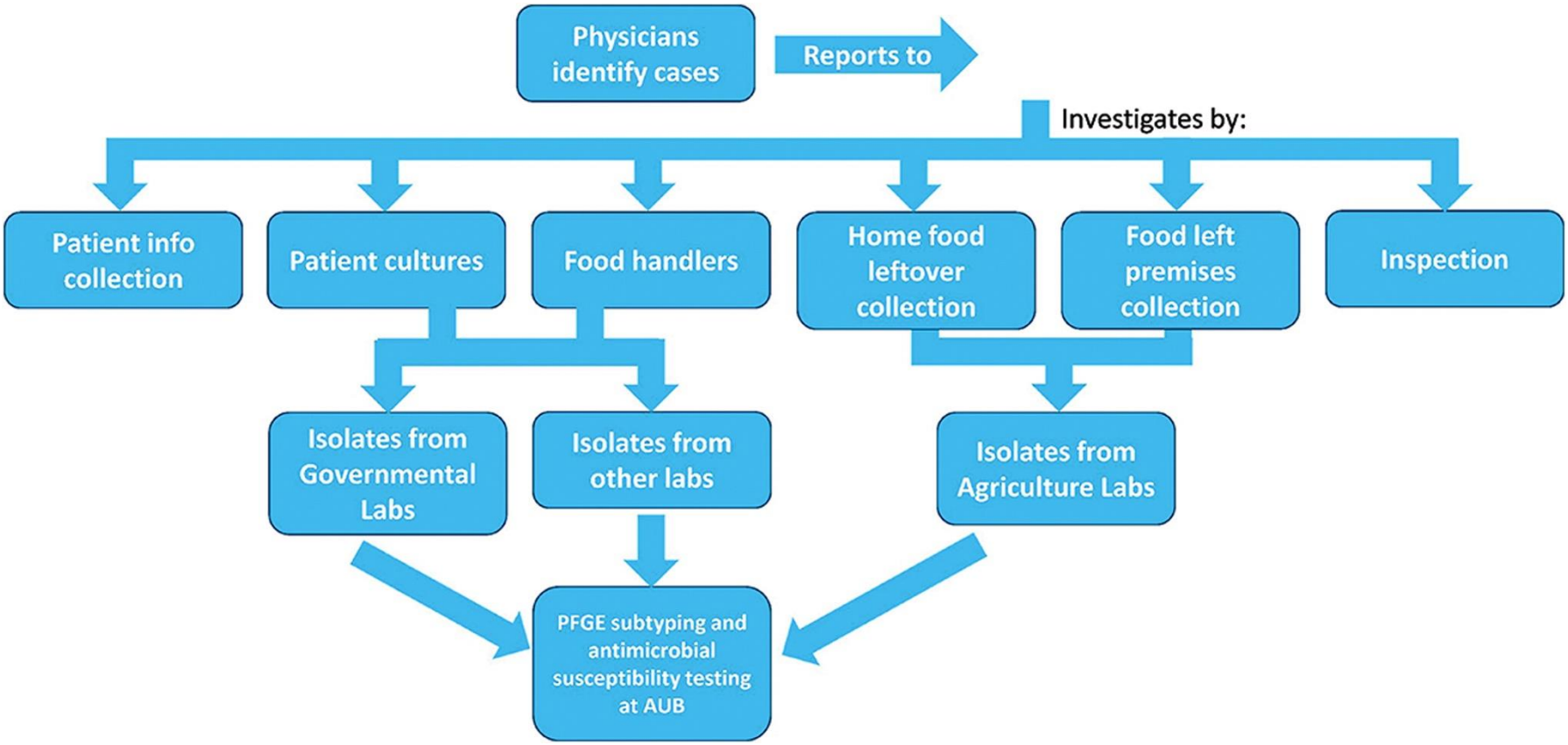
Epidemiologists across the country use PulseNet data to help determine what specifically is making people sick. They also work with food safety and Inspection services, to find solutions to end outbreaks and prevent them from ever happening.

These solutions include immediate measures, such as food recalls, and long-term measures, such as enhanced guidance, policies, and regulations that lead to new production practice.

PulseNet System Advantages

PulseNet is effective because all of the laboratories in its network subtype all foodborne bacteria in real time and follow the same procedures using the same standards. As a result, we can compare fingerprints generated by different laboratories. In PulseNet, the quality and uniformity of the data are ensured by a quality assurance and quality control program.

PulseNet System
Protocol of Investigation



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Conclusion

Programs are being developed and implemented to reduce these the risk of food poisoning, food spoilage and food fraud.

Screening tools to analytically determine the risk of an adulterant being present are being used more widely, with targeted tests being rapidly developed to respond to identified threats.

As these programs and analytical tools continue to evolve, the safety and reliability of our foods will only be strengthened.

Thank you