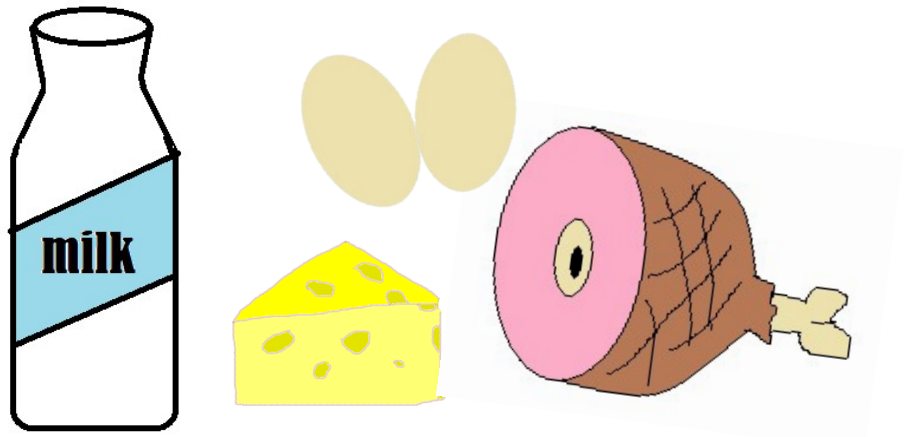
#42301
Session: 86

Welcome to the EAAP + WAAP + Interbull Congress 2023
Lyon, France - August 26th / September 1st, 2023

Technological tools for authentication assessment of animal products



Carmen L. Manuelian

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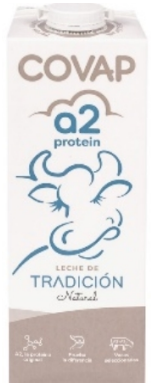
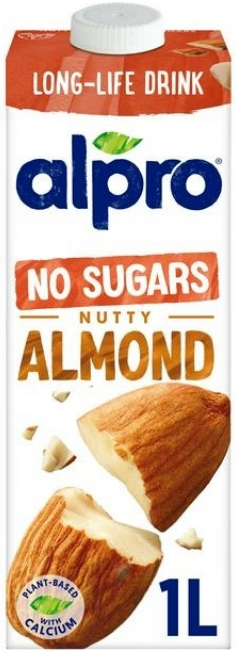
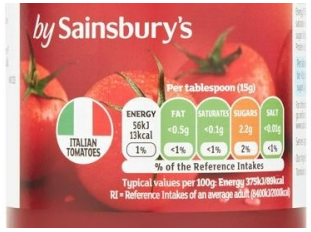
Introduction

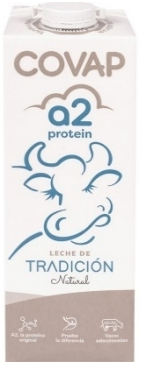






(https://www.sainsburys.co.uk/; https://groceries.morrisons.com/; Regulation (EC) No 1924/2006)

1,06 €/L - 1,24 €/L

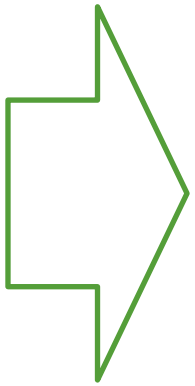


1,29 €/L - 1,47 €/L

(Prieto et al., 2017)

Traditional methods

- Expensive
- Time consuming
- Skilled personal
- Toxic solvents and reagents



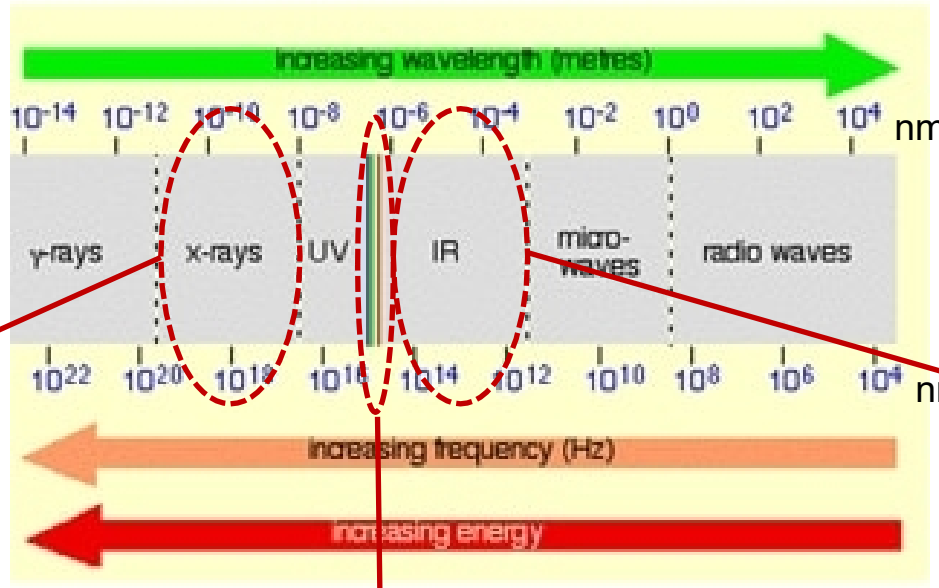
Search alternative analytical methods

- Cost-effective
- Rapid
- Easy-to-use
- Environmentally friendly

(Prieto et al., 2017)

Infrared (IR) spectroscopy

Energy-dispersive X-ray fluorescence (EDXRF) spectroscopy



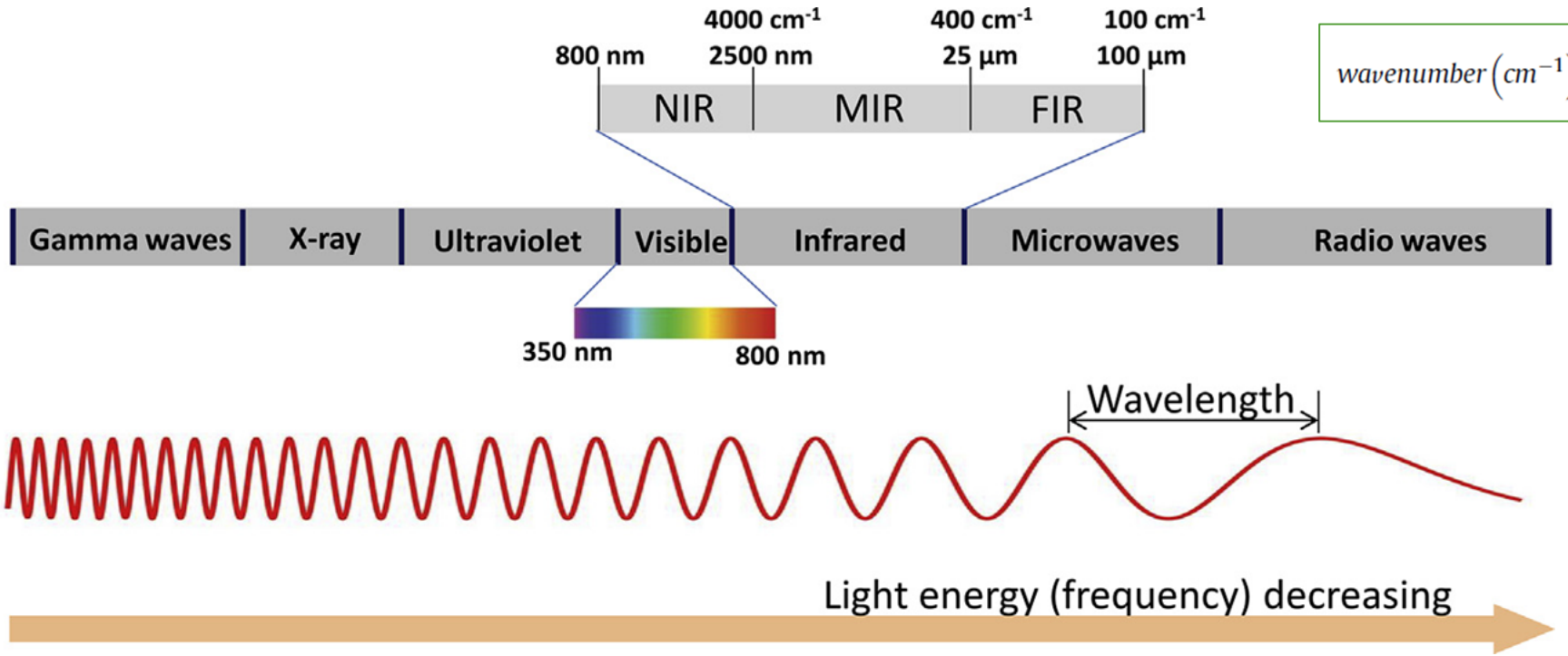
EDXRF spectroscopy

IR spectroscopy

(https://www.ssi.shimadzu.com/service-support/faq/uv-vis/light-and-theory/4/index.html)

Infrared (IR) Spectroscopy

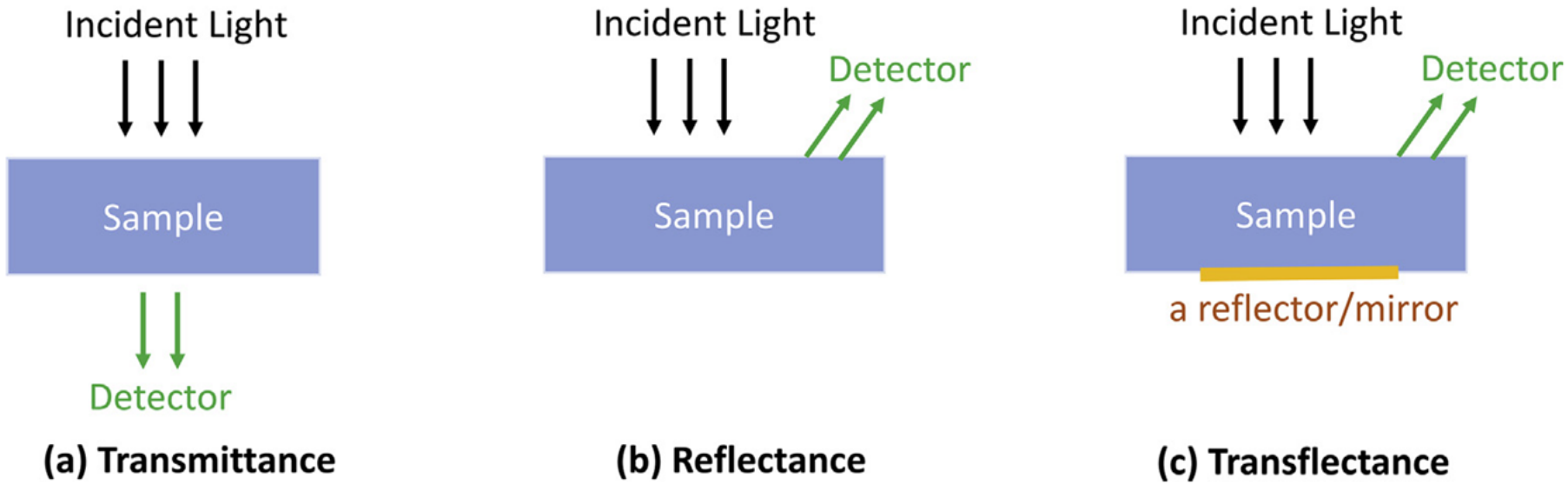
Principles



$$wavenumber\left(cm^{-1}\right) = \frac{10^7}{wavelength\ (nm)}$$

(Pu et al., 2020)

Principles

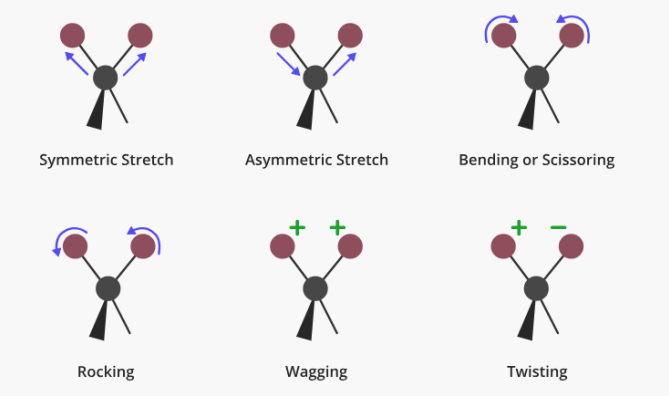


(Pu et al., 2020)

(https://www.inno-spectra.com/about.php?lang=cn)

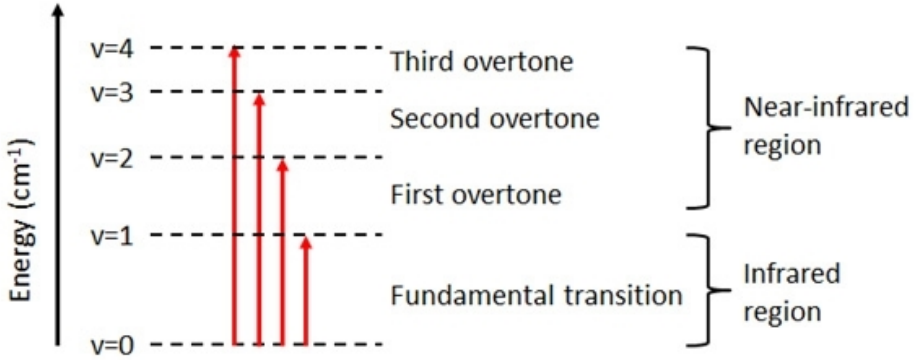
Principles

Vibrational mode



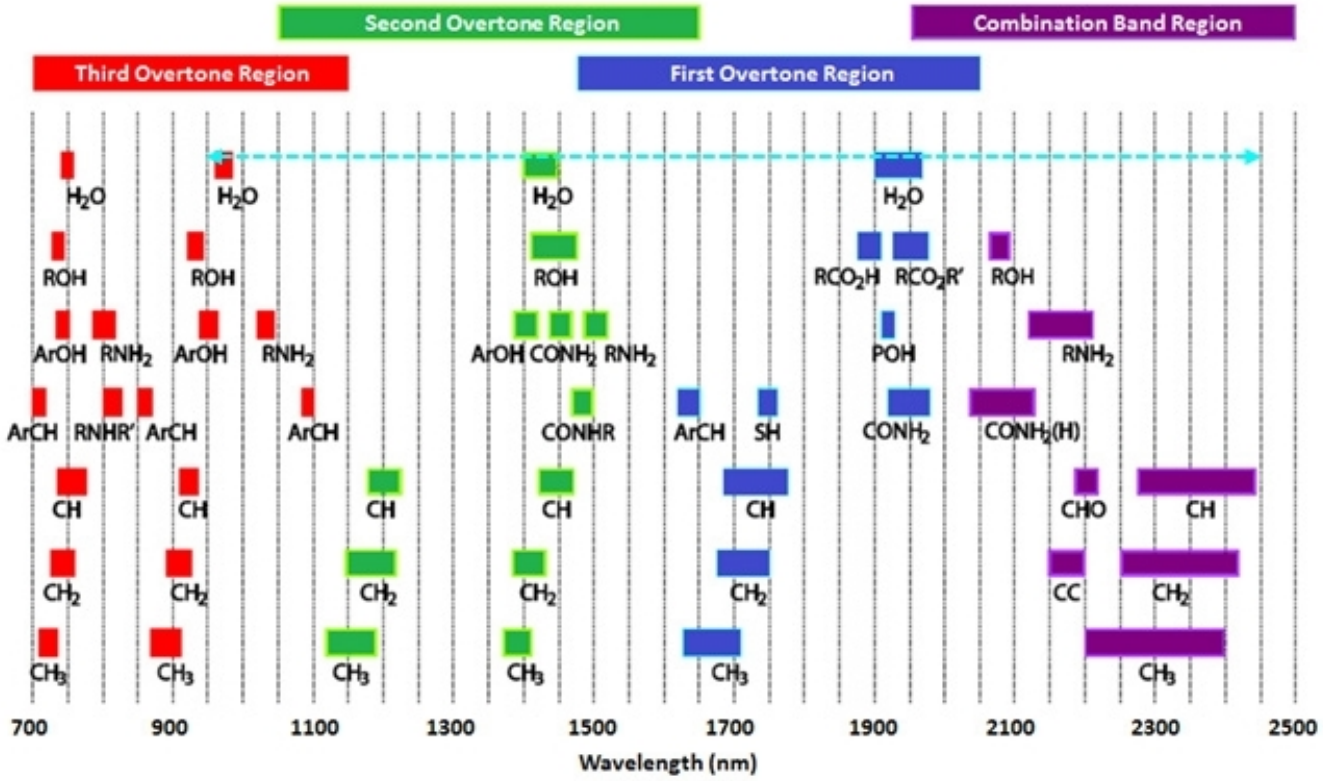
(https://psiberg.com/infrared-spectroscopy/?utm_content=cmp-true#)

Vibrational Frequencies



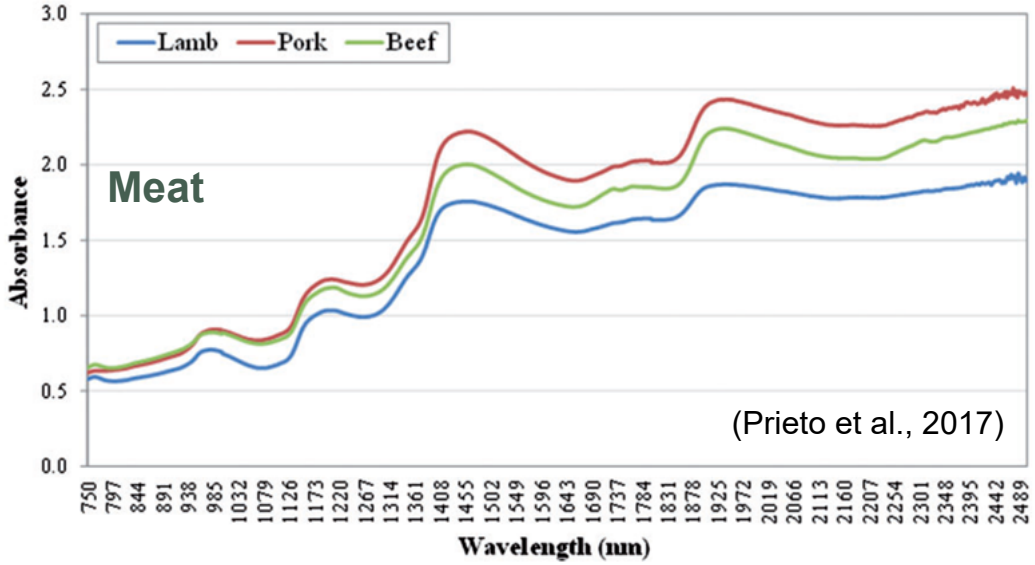
(https://www.inno-spectra.com/about.php?lang=cn)

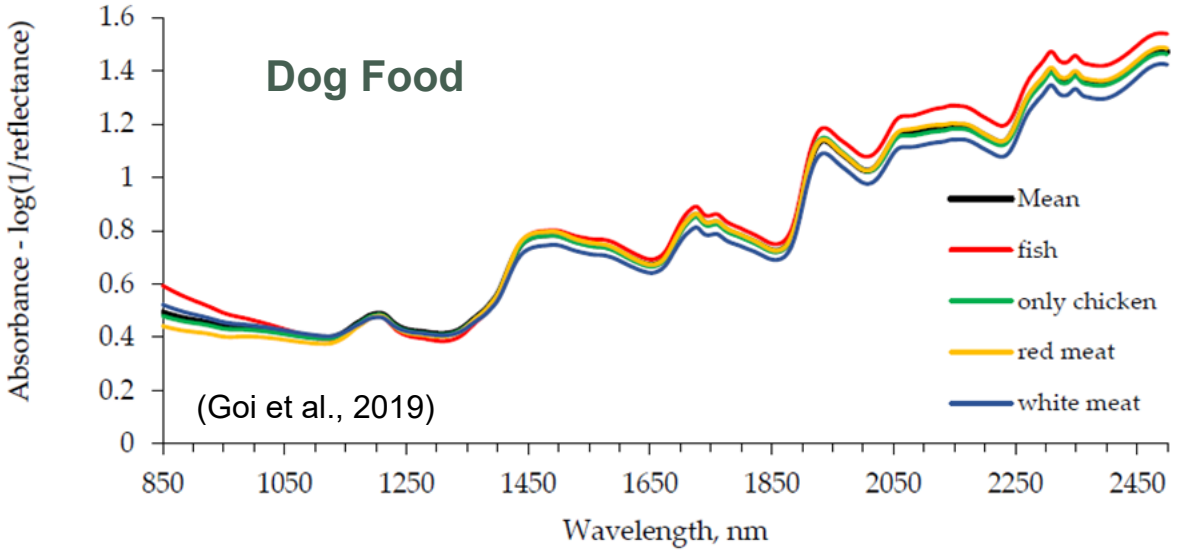
NIR Absorption Bands

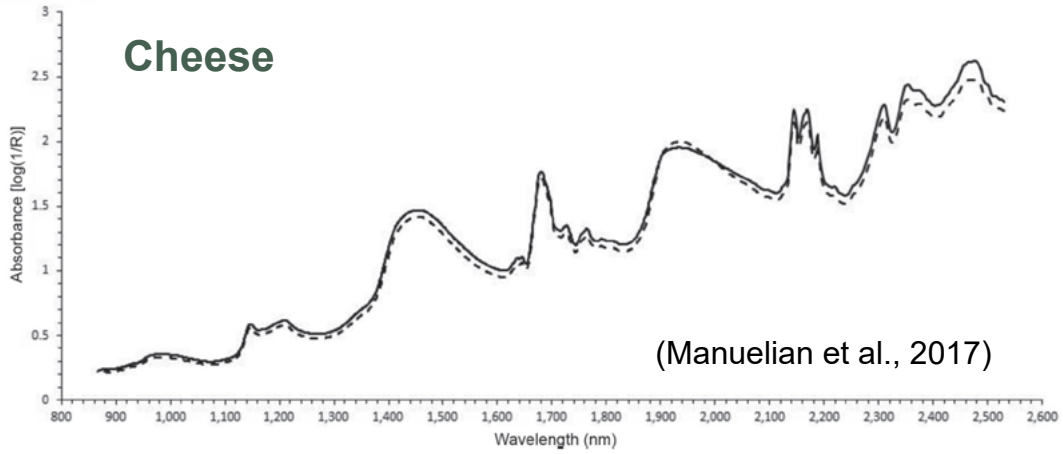


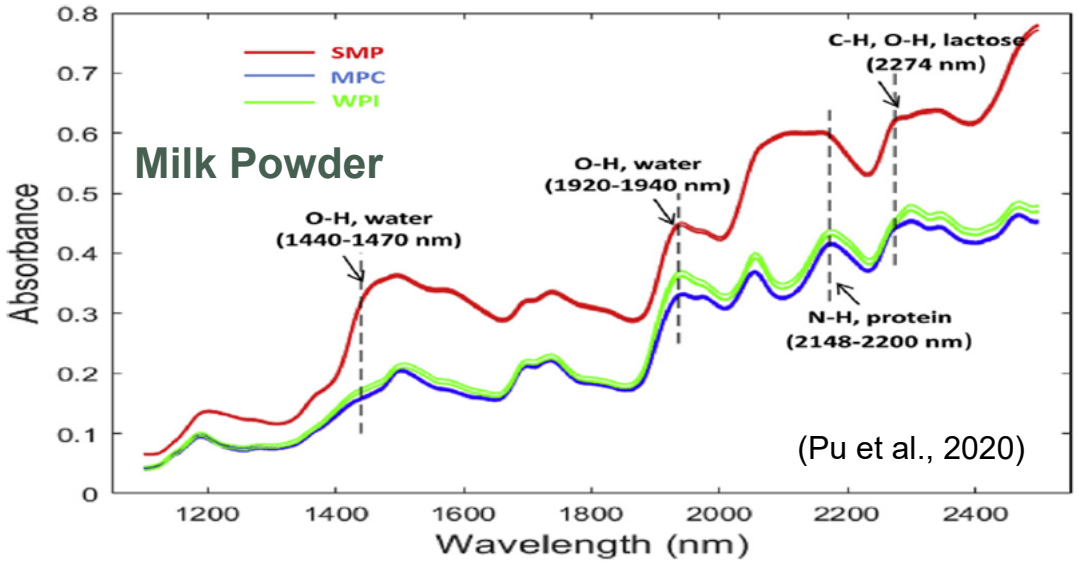
(https://www.inno-spectra.com/about.php?lang=cn)

(Pu et al., 2020)

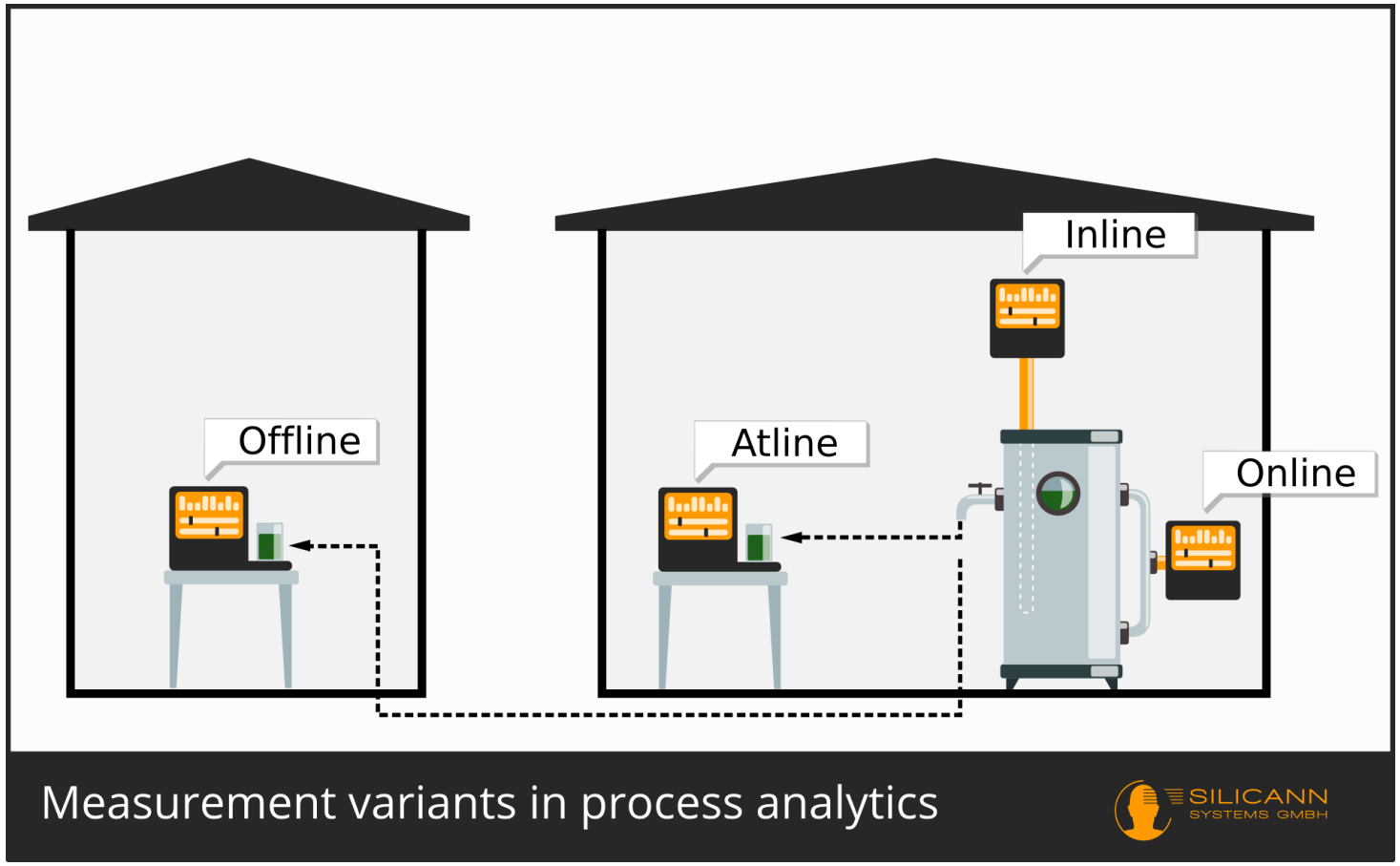

Lamb
Pork
Beef
Meat
Absorbance
Wavelength (nm)
(Prieto et al., 2017)


Dog Food
Absorbance - log(1/reflectance)
Wavelength, nm
Mean
fish
only chicken
red meat
white meat
(Goi et al., 2019)


Cheese
Absorbance [log(1/R)]
Wavelength (nm)
(Manuelian et al., 2017)


SMP
MPC
WPI
Milk Powder
O-H, water
(1440-1470 nm)
O-H, water
(1920-1940 nm)
C-H, O-H, lactose
(2274 nm)
N-H, protein
(2148-2200 nm)
Absorbance
Wavelength (nm)
(Pu et al., 2020)

Location



(https://www.inno-spectra.com/about.php?lang=cn)

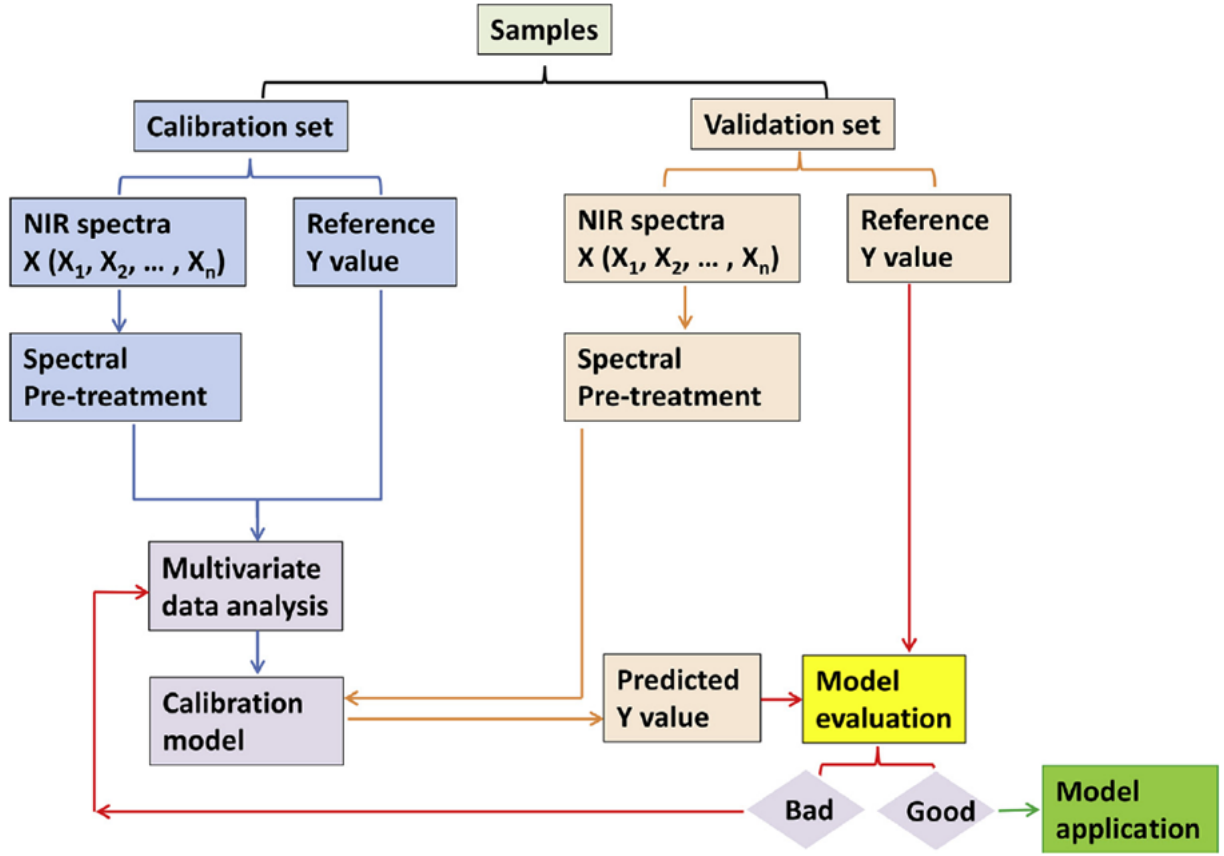
NIR vs MIR

Parameter	NIR	MIR
Spectral Region	800 – 2500 nm (shorter wavelength)	4000 – 400 cm^{-1} (longer wavelength)
Light Energy	Higher	Lower
Vibrational Frequencies	Overtones and combinations	Fundamental
Spectral Peaks	Broad and overlapped, weak intensity	Sharp, strong intensity
Peak Assignment	Not straightforward	Can be assigned to specific functional groups, providing 'fingerprint' information of the sample
Cost	Lower	Higher
Sample preparation	None	Liquid

(adapted from Pu et al., 2011)

Models development

- At least 120 samples for robust calibration model
- At least 25 samples for validation
- Regularly validated and updated
- Standardisation needed for calibration transfer



(Pu et al., 2020)

Prediction models assessment

- **R2**: coefficient of determination
- **RMSECV** or **RMSEP**: root mean square error of cross-validation or prediction
- **RPD**: Ratio of performance deviation SD/SEP or SECV
- **Bias**

Guidelines for model prediction performance indices.[a]

R^2	RPD	Interpretation of R^2
<0.66	0.75	Not recommended: further research needed
0.66–0.81	<1.7	Screening and some other 'approximate' applications
0.83–0.90	2.3	Usable with caution for many applications
0.92–0.96	3.6	Usable for most applications including quality control
>0.98	>5.0	Usable in any application

(Pu et al., 2020)

Table 1. Degrees of merit for the ratio of performance deviation (RPD) to the application of NIR spectroscopy.[27]

RPD value	Classification	Application
0.0–1.9	Very poor	Not recommended
2.0–2.4	Poor	Rough screening
2.5–2.9	Fair	Screening
3.0–3.4	Good	Quality control
3.5–4.0	Very good	Process control
4.1+	Excellent	Any application

(Prieto et al., 2017)

(De Marchi et al., 2018; Prieto et al., 2017)

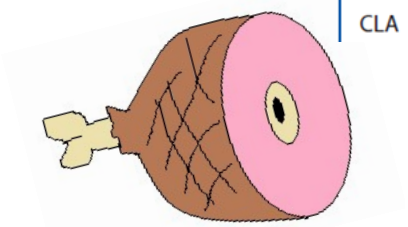
In Meat Products

Table 2. Cross-validation statistics for modified partial least squares regression models developed to predict FA in the overall dataset of ground samples of commercial processed meat

	Percentage of total FA				g FA kg^{-1} of edible product			
Trait	Math	SECV	R^2_{CV}	RPD	Math	SECV	R^2_{CV}	RPD
Fatty acid groups								
SFA	D(2,5,5,1)	2.42	0.75	1.99	NONE(2,5,5,1)	1.03	0.94	3.92
UFA	SNV(2,5,5,1)	2.45	0.75	2.00	D(0,0,1,1)	1.57	0.95	4.54
MUFA	MSC(2,5,5,1)	2.03	0.51	1.43	NONE(0,0,1,1)	1.26	0.95	4.64
							0.94	4.18
							0.76	2.02
							0.93	3.72
							0.64	1.66

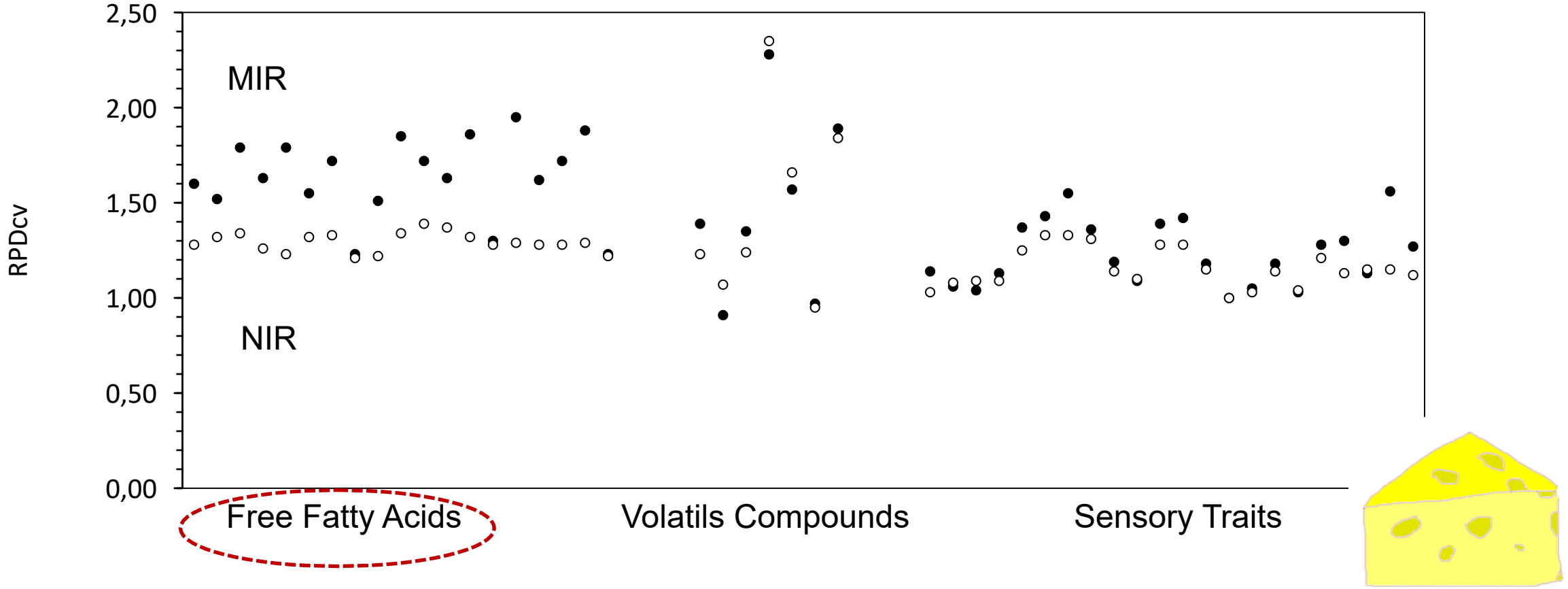
Table 4. Cross-validation statistics for modified partial least squares regression models developed to predict main FA groups in intact and ground samples of each processed meat category

	Intact						Ground					
	Percentage of total FA			g FA kg^{-1} of edible product			Percentage of total FA			g FA kg^{-1} of edible product		
Trait	SECV	R^2_{CV}	RPD	SECV	R^2_{CV}	RPD	SECV	R^2_{CV}	RPD	SECV	R^2_{CV}	RPD
Fresh meat												
SFA	3.09	0.79	2.17	1.29	0.91	3.29	2.36	0.86	2.68	0.39	0.97	5.72
UFA	3.05	0.77	2.09	0.54	0.97	5.53	2.44	0.85	2.58	2.44	0.85	2.56
MUFA	2.68	0.35	1.25	1.56	0.93	3.69	2.52	0.49	1.40	0.52	0.96	4.92
PUFA	3.23	0.62	1.63	0.46	0.91	3.29	1.60	0.89	3.03	0.19	0.95	4.35
ω-3	0.46	0.11	1.06	0.46	0.11	1.06	0.44	0.18	1.10	0.04	0.54	1.45
ω-6	3.20	0.66	1.72	3.20	0.66	1.72	2.54	0.78	2.12	0.30	0.89	2.99
CLA	0.10	0.68	1.79	0.02	0.76	2.04	0.09	0.76	2.03	0.02	0.77	2.08
C18:1*n*7	MSC(2,5,5,1)			0.53	0.34	1.23	NONE(0,0,1,1)			0.14	0.91	3.27
C18:3*n*3	MSC(0,0,1,1)			0.28	0.33	1.22	D(1,4,4,1)			0.04	0.81	2.31
C20:0	MSC(2,10,10,1)			0.04	0.07	1.04	NONE(0,0,1,1)			0.01	0.80	2.26
C20:3*n*3	MSC(1,4,4,1)			0.17	0.14	1.07	SNV + D(2,5,5,1)			0.03	0.28	1.17
C20:4*n*6	NONE(2,10,10,1)			0.13	0.11	1.06	NONE(0,0,1,1)			0.03	0.55	1.49



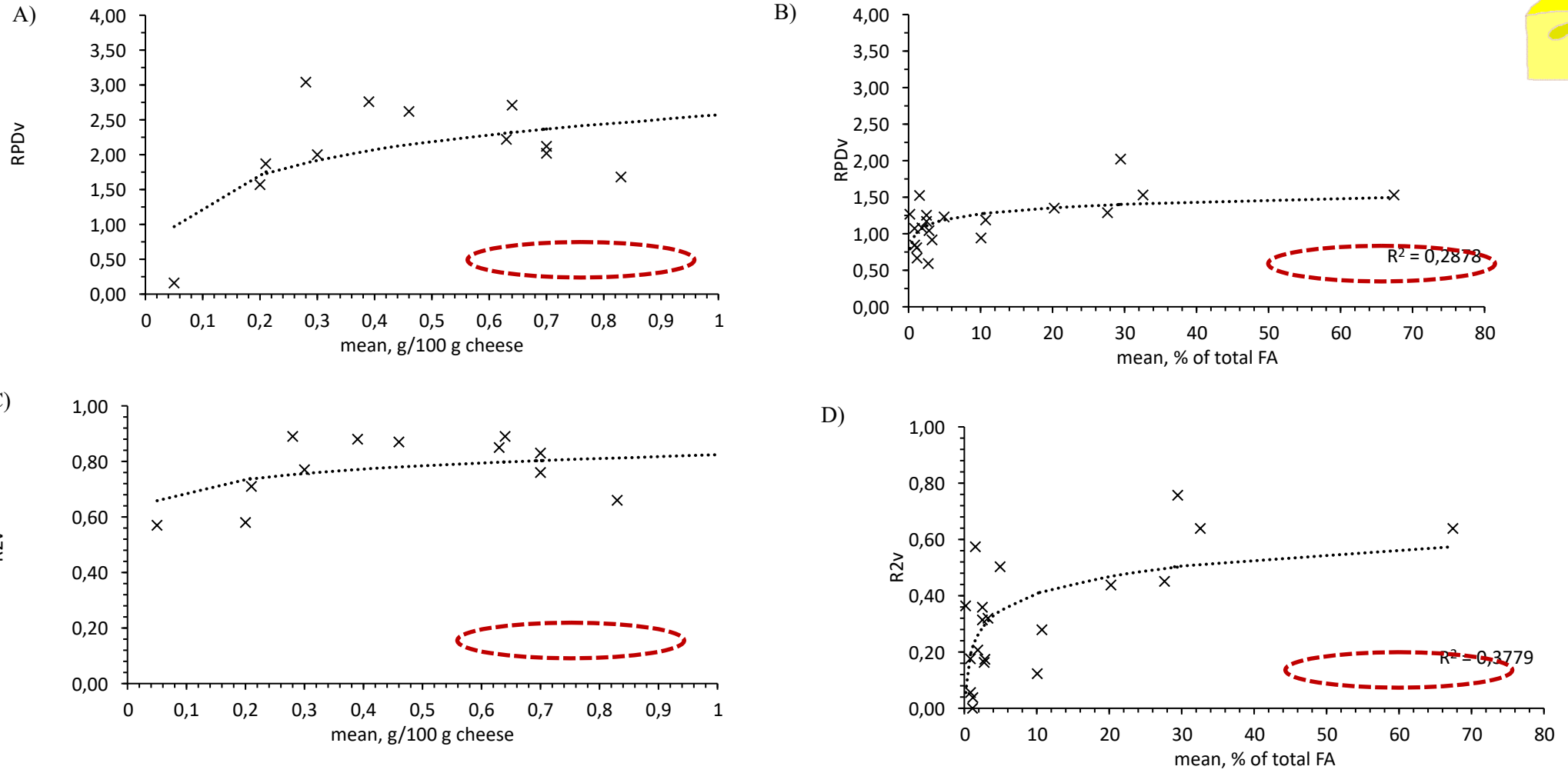
(De Marchi et al., 2017)

In Cheese



(adapted from Kraggerud et al. (2014) by De Marchi et al. (2018))

In Cheese



(De Marchi et al., 2018)

Authentication Organic Milk

Table 2
Summarized results of PLS-DA classification models based on Micro-NIRS, FT-NIRS and FAs by GC data.

		Micro-NIRS	
		Training %	Validat
			Interna
OM vs NOM	Sensitivity	61	78
	Specificity	85	83
	Accuracy	75	81
OM vs CM	Sensitivity	89	56
	Specificity	100	100
	Accuracy	95	77

(Liu et al., 2018)

Table 3. Confusion matrix and statistical measures of performance of the partial least squares-discriminant analysis model for two components for the classification of organic and conventional bulk milk in the Vis/NIR region.

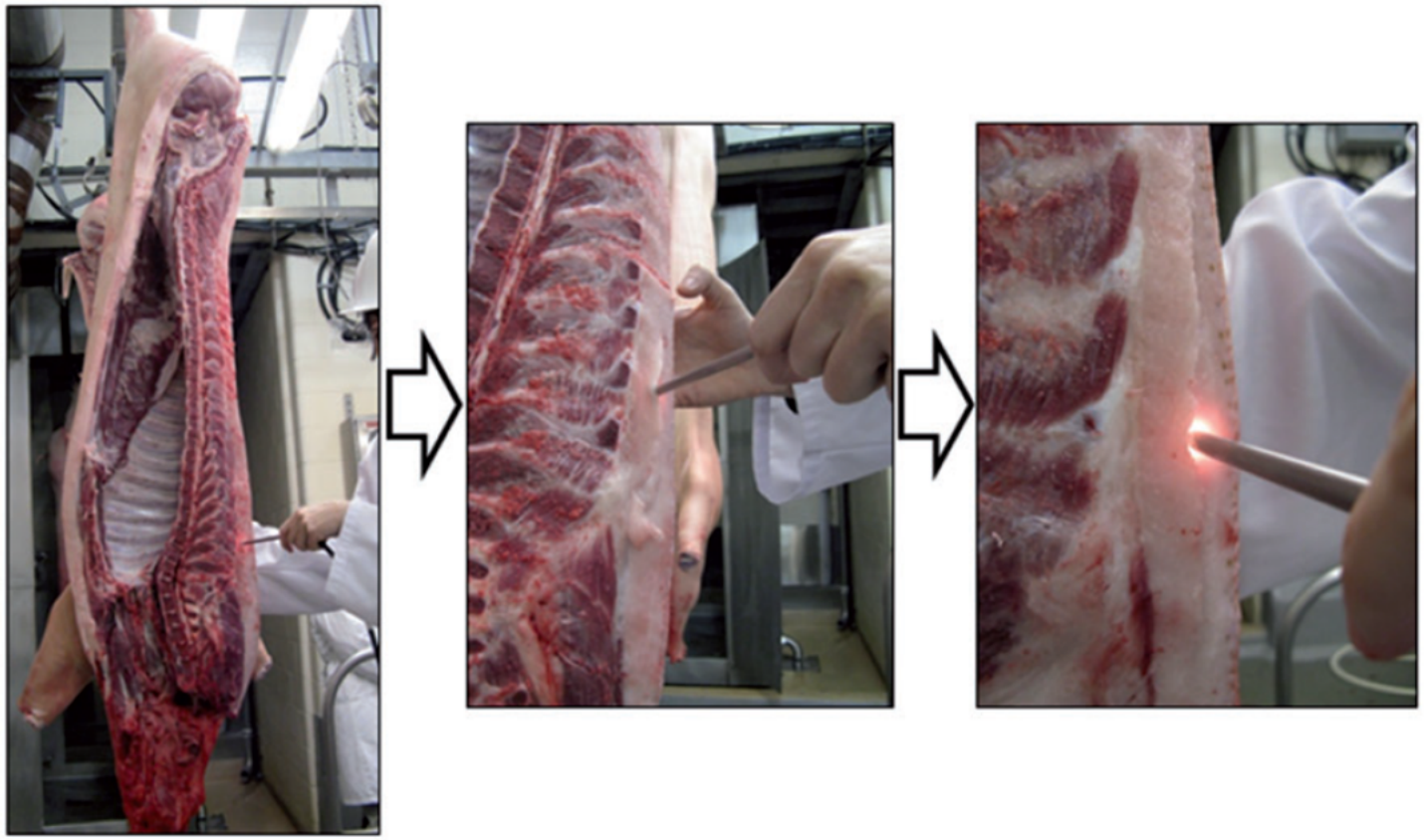
	Predicted		Predicted	
	Train set ($n = 121$)		Test set ($n = 62$)	
Original class	Organic	Conventional	Organic	Conventional
Organic	32	26	14	17
Conventional	23	40	6	25
Model performance, %				
Error rate	0.40		0.37	
Accuracy	0.60		0.63	
Sensitivity	0.55		0.45	
Specificity	0.63		0.81	
False positive rate	0.37		0.19	
Precision	0.58		0.70	

(Manuelian et al., 2021)

Portable devices

(Prieto et al., 2017)

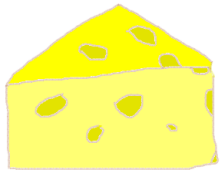
Portable devices



(Prieto et al., 2017)

Portable devices

Pocket-sized NIR:
67.7 × 40.2 × 18.8 mm; SCiO™
Every 1 nm from 740 to 1070 nm



Trait (g/100 g)[b]	N[a]	Math	LF	SE_C	R^2	SE_{cv}	R^2_{cv}	SE_{nv}	R^2_{nv}	RPD_{nv}
Fatty acids	513	SND1441	8	0.84	0.98	0.95	0.98	0.95	0.98	6.61
SFA	494	SNV0011	6	1.44	0.89	1.52	0.88	1.51	0.88	2.92
C4:0	472	SND2551	2	0.07	0.86	0.07	0.84	0.07	0.84	2.51
C6:0	474	DET2551	2	0.07	0.78	0.07	0.76	0.07	0.76	2.06
C8:0	477	NO0011	4	0.04	0.76	0.05	0.74	0.05	0.74	1.98
C10:0	470	DET210101	2	0.10	0.77	0.11	0.76	0.11	0.76	2.04
C12:0	472	NO0011	5	0.14	0.76	0.14	0.73	0.23	0.41	1.30
C14:0	476	DET210101	2	0.55	0.69	0.57	0.67	0.57	0.67	1.74
C15:0	473	NO2551	4	0.03	0.83	0.04	0.80	0.04	0.79	2.17
C16:0	463	SND0011	7	0.71	0.90	0.79	0.87	0.78	0.87	2.83
C18:0	451	MSC2551	1	0.62	0.43	0.64	0.40	0.70	0.39	1.28
MUFA	459	MSC2551	1	1.28	0.60	1.32	0.58	1.33	0.58	1.54
C14:1	466	SND1441	4	0.04	0.73	0.05	0.69	0.05	0.69	1.79
C16:1	482	DET2551	4	0.07	0.79	0.07	0.75	0.08	0.73	1.93
C18:1 c	459	SND2551	1	0.12	0.52	0.12	0.49	0.12	0.49	1.40
C18:1	483	DET1441	2	1.18	0.64	1.21	0.62	1.20	0.62	1.63
PUFA	499	NO210101	2	0.20	0.60	0.20	0.57	0.20	0.57	1.53
□ -3	492	DET210101	2	0.04	0.54	0.04	0.50	0.04	0.50	1.41
C18:3	433	SND210101	1	0.02	0.68	0.02	0.67	0.02	0.66	1.71
C18:4	421	NO2551	6	0.002	0.71	0.003	0.62	0.003	0.56	1.52
C20:3	475	MSC1881	3	0.01	0.71	0.01	0.67	0.01	0.66	1.71
C20:5	435	SND1441	7	0.004	0.57	0.004	0.44	0.004	0.33	1.23
C22:6	462	SND1441	4	0.004	0.63	0.004	0.55	0.005	0.51	1.42
□ -6	487	DET1881	2	0.17	0.52	0.17	0.50	0.17	0.49	1.40
C18:2	489	MSC0011	2	0.16	0.52	0.16	0.49	0.16	0.49	1.40

Trait	N	Math	LF	SE_c	R^2	SE_{cv}	R^2_{cv}	SE_{nv}	R^2_{nv}	RPD_{nv}
Nitrogen related traits (%)										
Total nitrogen	500	SNV1881	9	0.80	0.98	0.89	0.97	0.91	0.97	5.95
Soluble nitrogen	504	MSC1441	7	0.40	0.83	0.46	0.78	0.46	0.78	2.15
Ripening index	509	NO1441	8	2.86	0.59	3.21	0.48	3.40	0.44	1.34
Minerals (g/100 g)										
Ca	506	MSC0011	12	0.05	0.89	0.06	0.87	0.06	0.87	2.77
P	509	SND1881	7	0.03	0.95	0.03	0.94	0.03	0.94	4.04
K	512	NO1881	9	0.02	0.82	0.02	0.78	0.02	0.77	2.07
Mg	509	NO1441	7	0.009	0.82	0.010	0.78	0.01	0.78	2.13
Na	511	MSC0011	11	0.05	0.92	0.06	0.90	0.05	0.90	3.12

(Manuelian et al., 2022)

Limitations

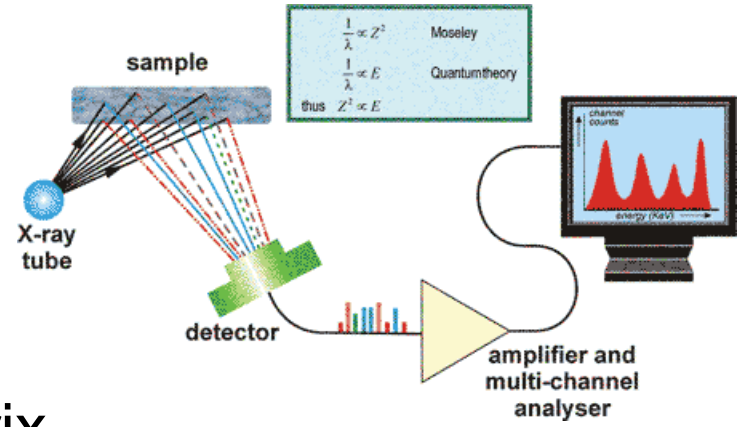
- Complex data analysis (chemometrics)
- Sometimes only for screening or classification
- Compounds not related to organic molecules (minerals)
- Limit detection of constituents/contaminants (< 0.1%)
- Unit of measurement (fatty acids)

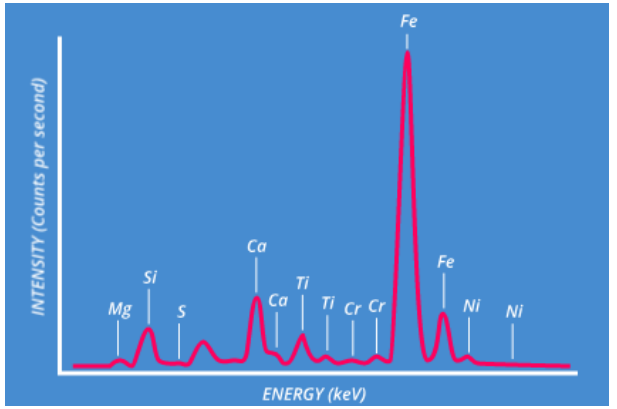
(De Marchi et al., 2018; Pu et al., 2011)

Energy-dispersive X-ray fluorescence (EDXRF) spectroscopy

Principles

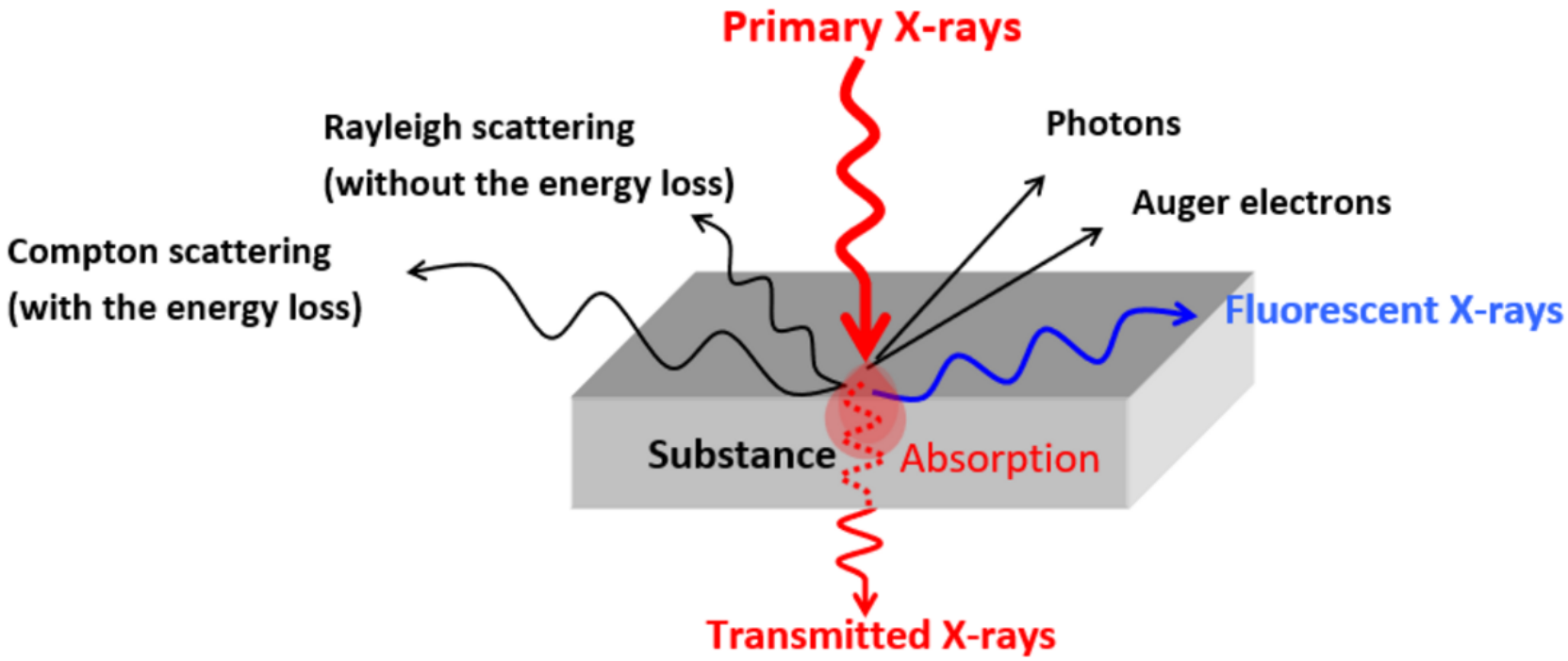
- Covers all elements from Na → Uranium
- From 100% to ppm
- Limit of detection depends on the element and matrix
- Heavier elements better detected
- Qualitative and quantitative analysis





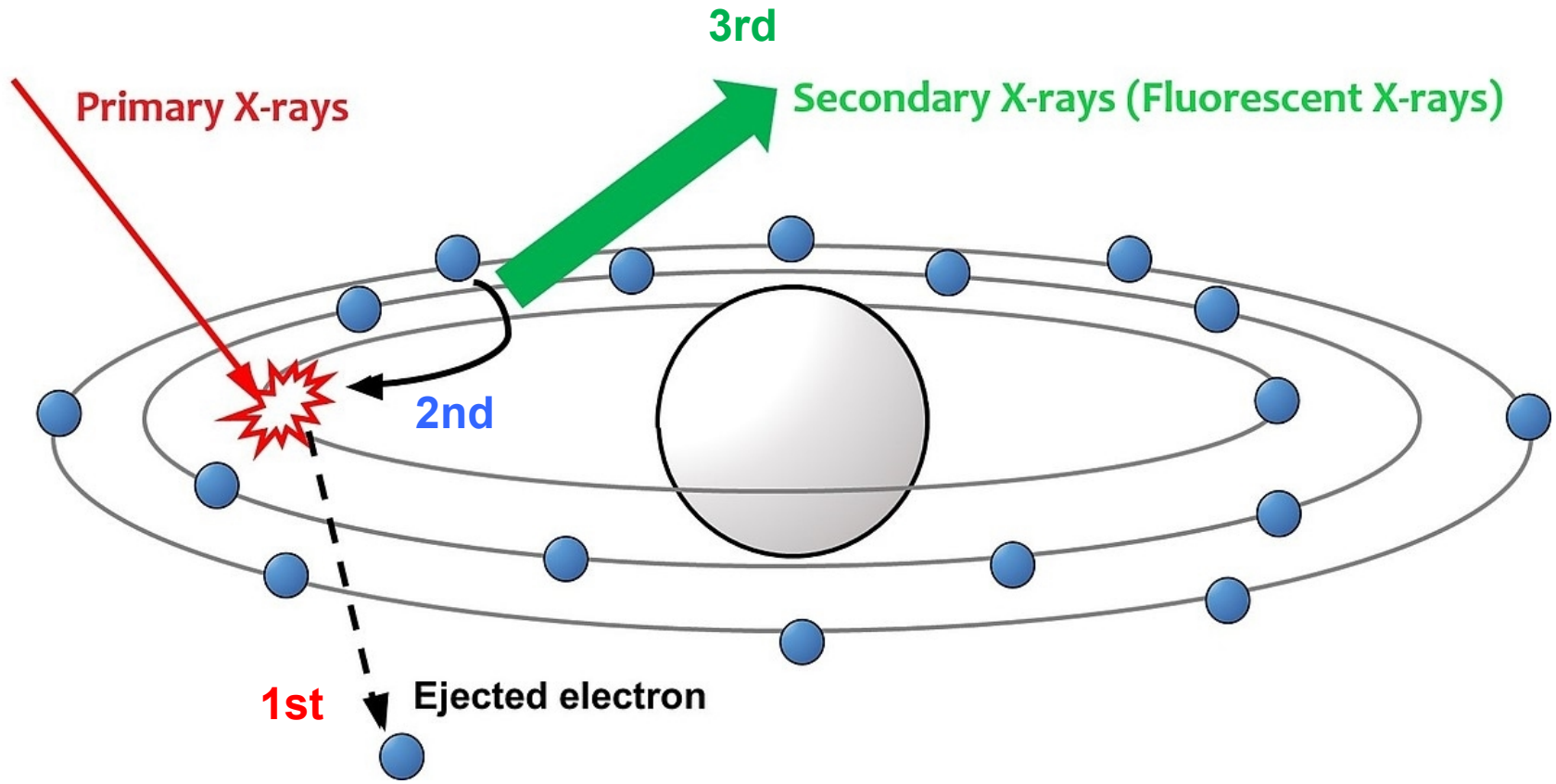
(https://www.horiba.com/int/scientific/technologies/energy-dispersive-x-ray-fluorescence-ed-xrf/what-is-x-ray-fluorescence-xrf/)

Principles



(https://www.horiba.com/int/scientific/technologies/energy-dispersive-x-ray-fluorescence-ed-xrf/what-is-x-ray-fluorescence-xrf/)

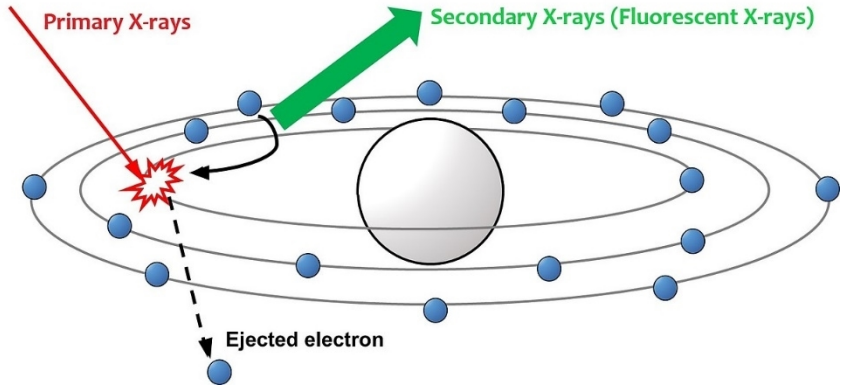
Principles



(https://www.horiba.com/int/scientific/technologies/energy-dispersive-x-ray-fluorescence-ed-xrf/what-is-x-ray-fluorescence-xrf/)

Principles

- Energy of the emitted fluorescent X-ray directly link specific element
- Calcium peak same position for CaCO3, CaO and CaCl2



(https://www.horiba.com/int/scientific/technologies/energy-dispersive-x-ray-fluorescence-ed-xrf/what-is-x-ray-fluorescence-xrf/)

Models development

- Calibration curves using standard references (<10 samples)
- Own calibration curves (<50 samples)
- Regression model between reference (concentration) and intensity produced by ED-XRF
- **R2**: coefficient of determination
- About 20 min per sample read

(Visentin et al., 2020)

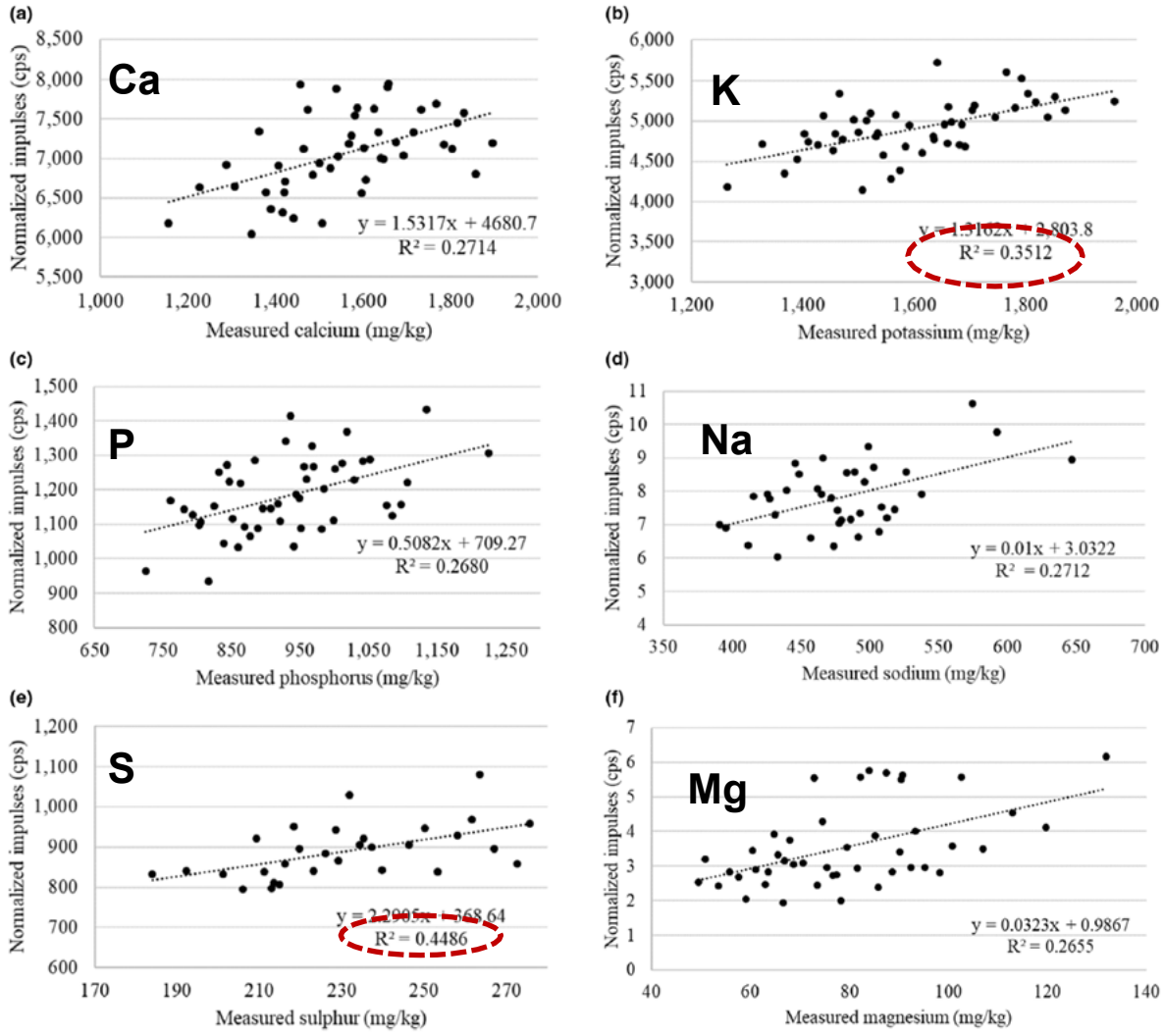
Application in milk and dairy products

Table 1
Analyses of milk and dairy products by XRF.

Sample	XRF/source of excitation	Analytes	Sample preparation
Cow milk	EDXRF/^{109}Cd	I	Freeze drying, pressing tablets ($m = 3$ g, $d = 3$ cm, $p = 5$ t)
Milk powder	EDXRF/X-ray tube (W anode), secondary targets (Cu, Mo, Sn)	Fe, Cu, Zn, Br, Rb, Sr	Filling a sample holder with powder ($m = 4$ g)
Non-fat milk powder SRM 1549	EDXRF/SR	K, Ca, Cr, Mn, Fe, Ni, Cu, Zn, As, Se, Br, Rb, Sr, Hg, Pb	Pressing tablets ($m = 0.5$ g, $d = 2.54$ cm, $p = 700$ kg/cm^2)
Non-fat milk powder SRM 1549, sour cream	EDXRF/Gd, Ag and Ge secondary excitation	Mn, Fe, Cu, Zn	Freeze drying, pressing tablets ($m = 0.5$ g, $d = 3.2$ cm, $p = 2300$ kg/cm^2)
Human and cow milk, formula milk	EDXRF/^{109}Cd	K, Ca, Mn, Fe, Ni, Cu, Zn, Br, Rb, Se, Pb	Freeze drying, pressing tablets ($m = 0.1$ g, $d = 1.0$ cm)
Milk powder	EDXRF/X-ray tube (Mo anode), secondary target (Zr)	K, Ca	Drying and pressing tablets ($m = 1$ g, $d = 2.5$ cm, $p = 3000$ kg/cm^2)
Milk powder, SRM A-11, IAEA-153	EDXRF/X-ray tube	Fe, Zn, As, Br, Rb	Pressing tablets of intermediate thickness
Milkshake, Non-fat milk powder SRM 1549	EDXRF/Gd, Ag and Ge secondary excitation	P, S, Cl, K, Ca, Mn, Fe, Zn, Br, Rb, Sr	Freeze drying, pressing tablets ($m = 0.5$ g, $d = 3.2$ cm, $p = 2300$ kg/cm^2)
Human milk	EDXRF/^{109}Cd,^{55}Fe	Cl, K, Ca, Mn, Fe, Cu, Zn, Cr, Co, Mo	Freeze drying, pressing tablets ($m = 0.8$–1 g, $d = 2.5$ cm)
Colostrum milk	TXRF/SR	Fe, Cu, Zn	Treating 0.5 mL of sample with mixture of HNO_3/H_2O_2, adding internal standard Ga
Melted cheese, cottage cheese	WDXRF/X-ray tube	P	Drying samples at 200°C for 2 h
Milk-based products	EDXRF/X-ray tube (Pd anode)	P, S, Cl, K, Ca, Fe, Zn	Pressing tablets ($m = 4$ g, $d = 3.2$ cm, $p = 2$ t)

(Pashkova et al., 2018)

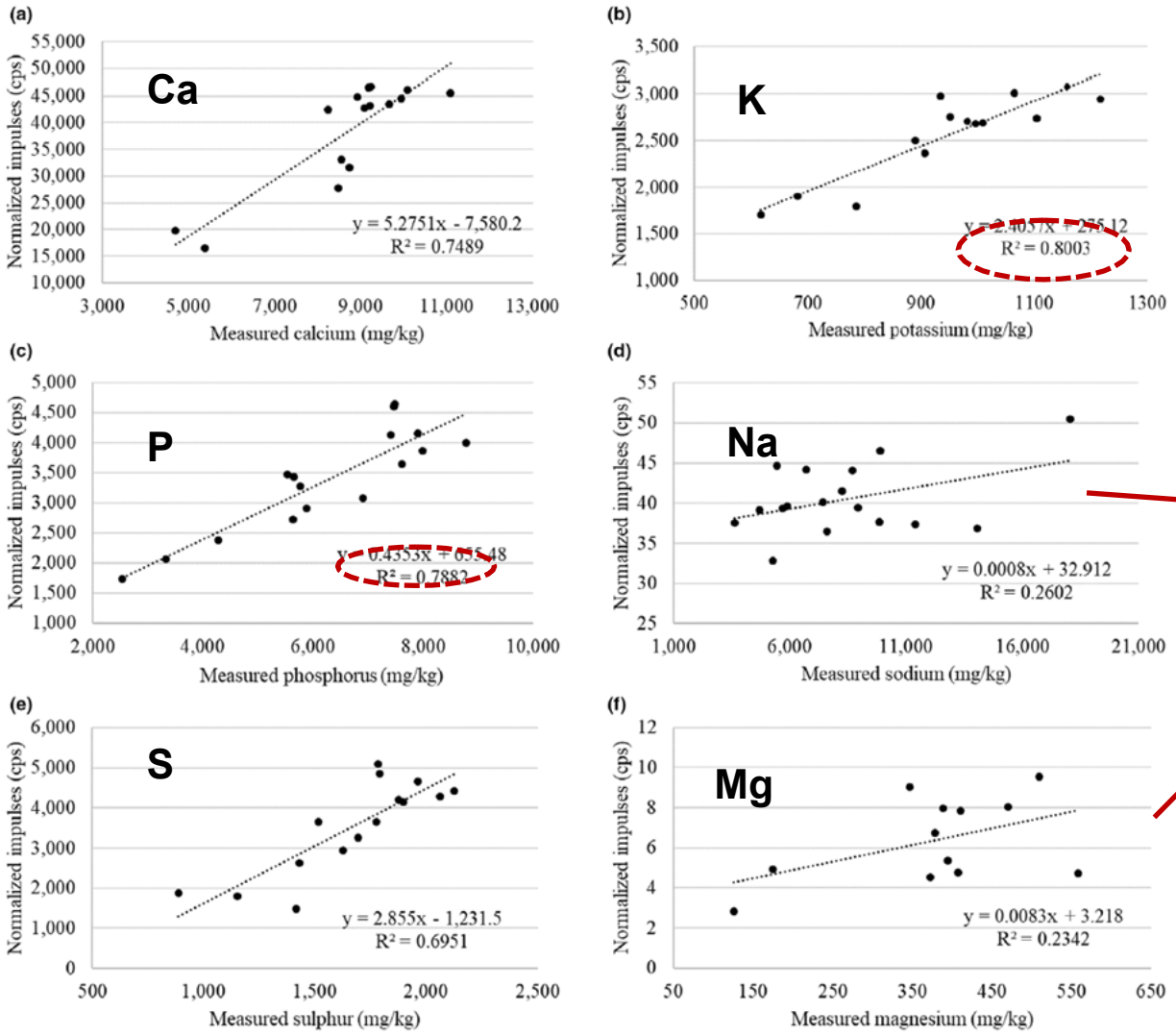
Fluid milk



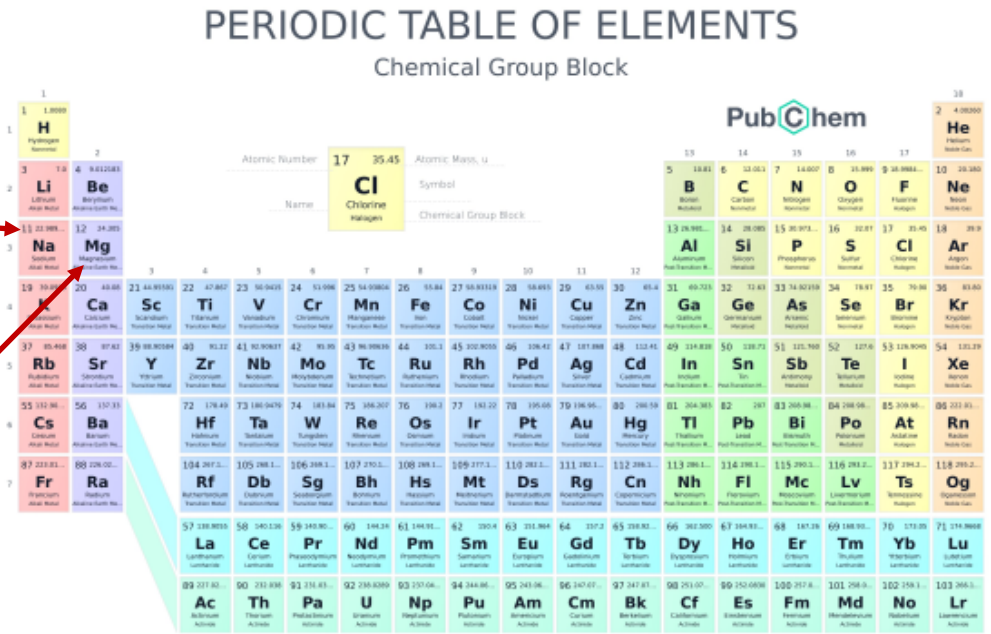
- Need sample freeze-drying to reduce the moisture content

(Visentin et al., 2020)

Cheese



- Affected low amount Na and Mg, and low atomic weight



(Visentin et al., 2020)

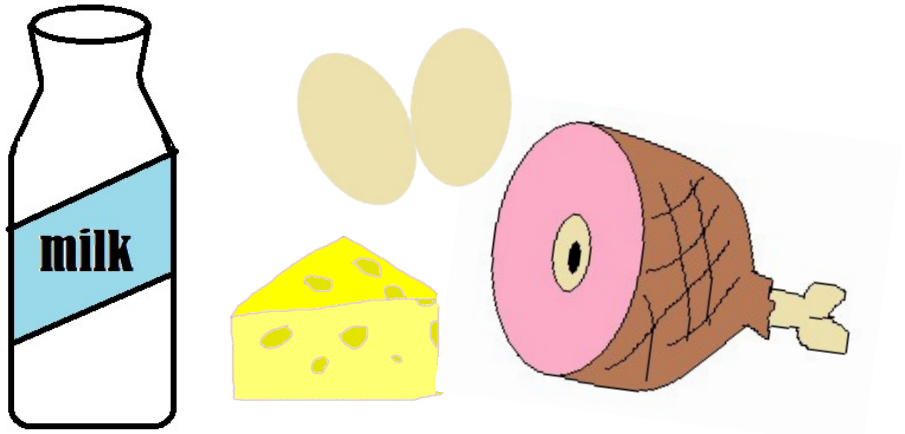
Conclusions

Infrared Spectroscopy

- Benchtop → portable devices
- Gross → detailed composition (fatty acids, amino acids, minerals…)
- Quantification → authentication (organic, free-range…)
- Ground → intact samples

Energy-dispersive XRF

- Introduced to predict mineral composition
- More research is needed in animal-derived products



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... Thank you for your attention

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