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Application of nuclear technology in research on agrifood safety and authenticity in China

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Outline

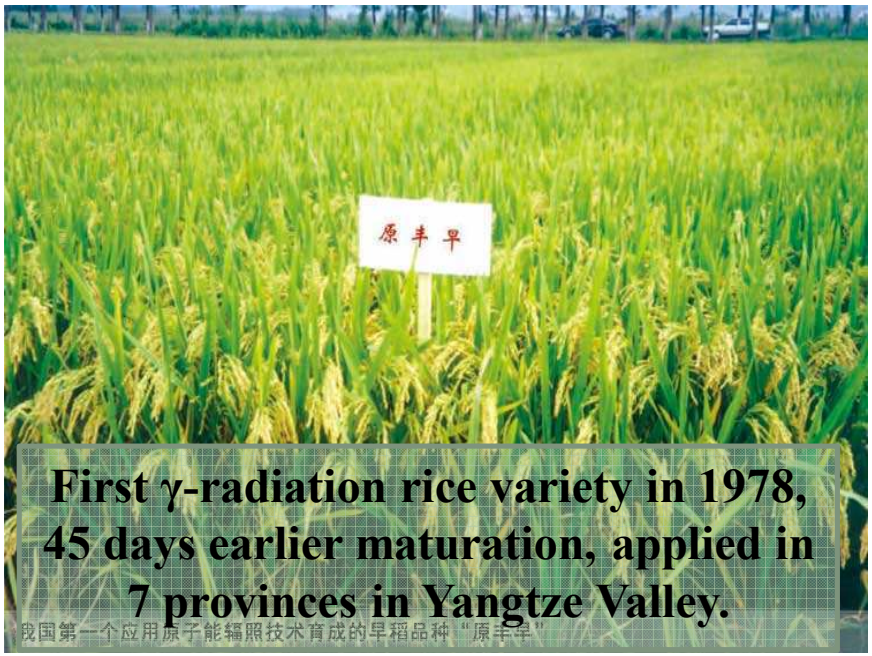
- Introduction
- Research progress
  - Discrimination of fake and adulterated products
  - Verification of product provenance
  - Determination of contaminants
  - Detection of irradiated agrifood
- Perspectives

A brief review: nuclear agricultural research in China

Nuclear techniques have been widely applied in several fields of agricultural research in China since the 1950s. The key areas of the research include:

- Crop breeding
- Insect pest control
- Food irradiation
- Animal health
- Soil and water management

I. Achievements in development of radiation-induced mutant varieties in China



First γ-radiation rice variety in 1978, 45 days earlier maturation, applied in 7 provinces in Yangtze Valley.

我国第一个应用原子能辐照技术育成的早稻品种"原丰早"

A maize variety for cereal and silage use by γ-radiation in 2009 with 12.77% crude protein (0.32% lysine)

By radiation and selection of the induced mutants, 823 mutagenic plant varieties with better quality, higher yield and stronger resistance or tolerance, were released, which accounts for 25.2% globally.

II. Successful control of insect pests by SIT

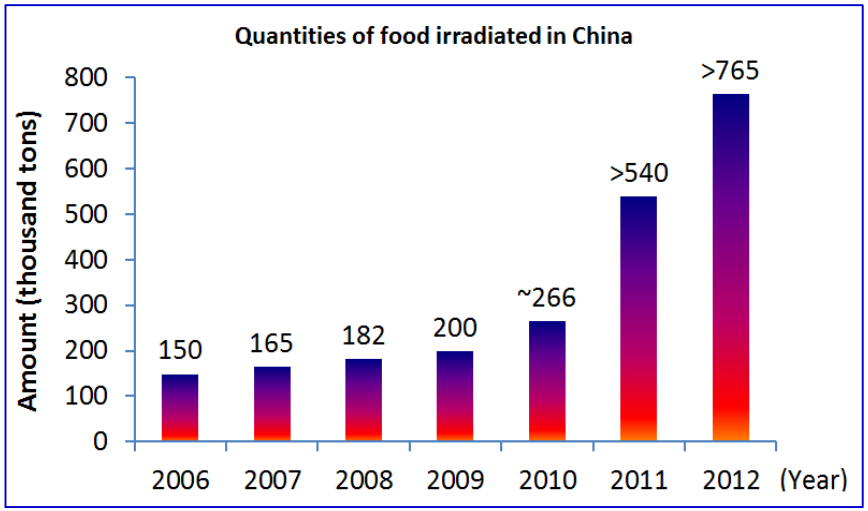
Control of Asian Corn Borer by SIT (1982-1984)

In China, control of insect pests by sterile male technique was started in the early 1960s and covered a wide range of pest species. Successful results were obtained from experiments on Asian corn borer, pine caterpillar, Chinese citrus fly and Oriental fruit fly, etc.

Zhang et al., 1985, *Appl. Atomic ener. Agri.*

III. Commercial application of food irradiation

- 140 ^{60}Co irradiators and 11 EB accelerators have been built for irradiation of agrifood in China
- Applications of the irradiation :
  - Decontamination of spices, fishery products, raw and cooked meat
  - Disinfestations of dry fruits, grains and beans
  - Sprout inhibition (garlic)



Adopted from Li Shurong, 2013

IV. Animal production and health management

表 1 1.5 岁秦川牛 GH、INS 含量GH and INS in year 1.5

激素	GH (ng/ml)					INS(μIU/ml)				
样本	1	2	3	4	X±s	1	2	3	4	X±s
公牛	1.67	3.02	2.92	1.13	2.19±0.93	25.77	20.24	49.56	72.49	42.02±23.97
母牛	1.46	1.88	1.17	1.34	1.46±0.73	46.64	25.13	29.36	33.94	33.77±9.31

表 2 2.0 岁秦川牛 GH、INS 含量GH and INS in year 2.0

激素	GH(ng/ml)				INS (μIU/ml)			
样本	1	2	3	X±s	1	2	3	X±s
公牛	2.42	2.41	2.85	2.56±0.205	135.71	110.50	108.39	118.2±15.2
母牛	1.20	0.64	0.91	0.92±0.280	41.04	46.47	49.22	45.58±4.16

表 3 不同性别不同年龄秦川牛的体尺指数Body composition index (cm)

性别	年龄	肢长指数	髋胸指数	胸宽指数	体躯指数	胸围指数	尻宽指数
公牛	1.5 岁	47.2	99.7	65.5	126.7	137.6	60.0
	2.0 岁	46.3	104.4	66.5	131.0	140.0	59.9
母牛	1.5 岁	48.0	92.1	61.2	134.8	137.2	62.9
	2.0 岁	45.6	111.4	65.0	135.1	141.2	66.9

表 4 秦川牛体重实测表 Weight of cattle (kg)

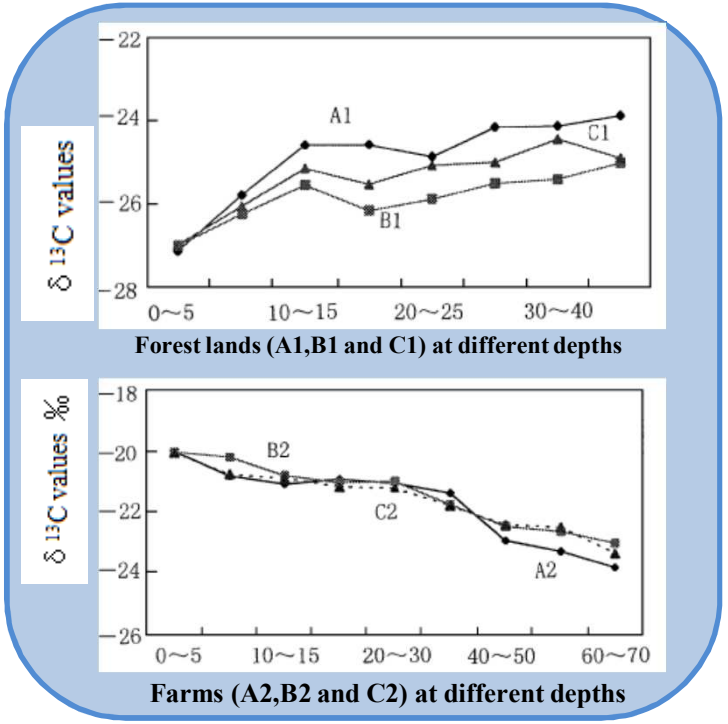
年龄	体重								
	公牛				母牛				X±s
1.5岁	380	450	400	470	340	300	300	320	370±66.117
2.0岁	500	490	490	--	370	360	360	--	428.3±71.39

Men et al., 2004, *Chin. Agri. Sci. bul.*

The immunoassay with radio isotopic markers have provided sensitive and speedy diagnosis. Nuclear-based techniques have also been applied in studies of ecological behavior of parasites and pharmaceutical toxicity of insecticides.

V. Water and soil management

Joint observationt of water and heat fluxes with stable isotope and micro-meteorological technique at Luancheng Agro-Ecosystem Experimental Station, CAS.



^{13}C of organic carbon in soil at different depths

Liu et al., 2002, *Envir. Sci*

More than 40 tracing nuclides were involved in R&D on agricultural resources and eco-environment. Integrated water, plant nutrients and soil management systems were developed in different cropping systems and production regions in China.

Nuclear agricultural research on food safety and traceability: emerging requirements

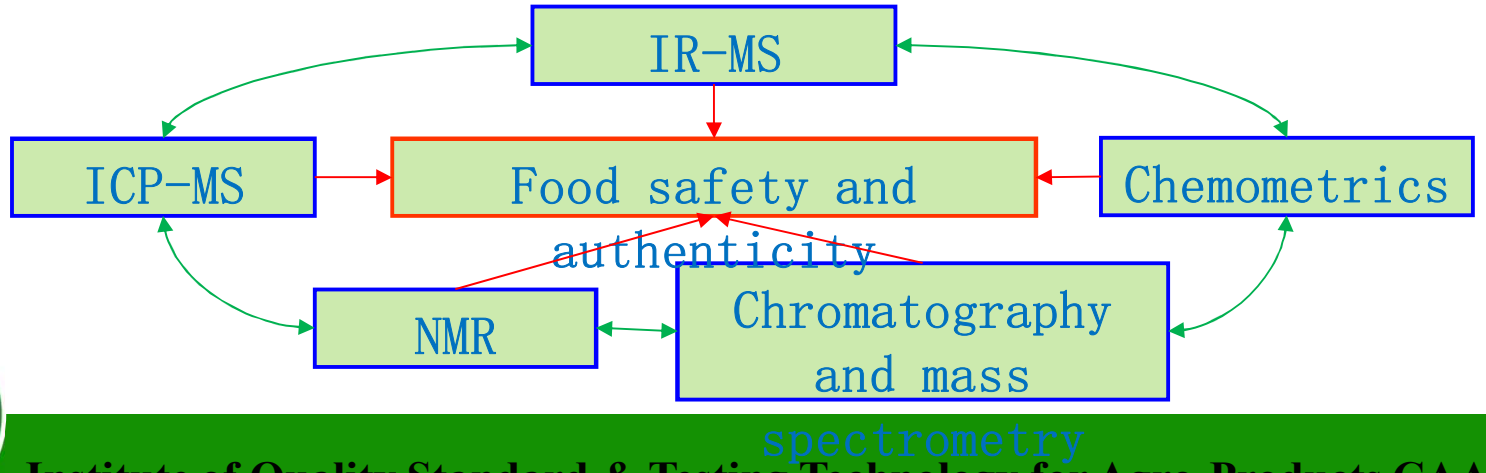
Research on application of nuclear technique in agriculture in China has been shifted to agrifood safety and authenticity in recent years to deal with emerging incidents of food safety and authenticity, such as:

- False labeling of origins
- Fake claims of products
- Adulteration of products
- Added contaminants

Nuclear agricultural research on food safety and traceability: method development

Research on application of nuclear techniques in agrifood quality and safety issues was primarily focused on development of analytical methods and relevant techniques :

- Stable isotope mass spectrometry
- Inductively coupled plasma-mass spectrometry
- Nuclear magnetic resonance
- Chromatography and mass spectrometry
- Chemometrics combined with analytical techniques



Nuclear agricultural research on food safety and traceability: pioneer research works

The pioneer research works on application of nuclear and complimentary techniques in the country have been carried out on:

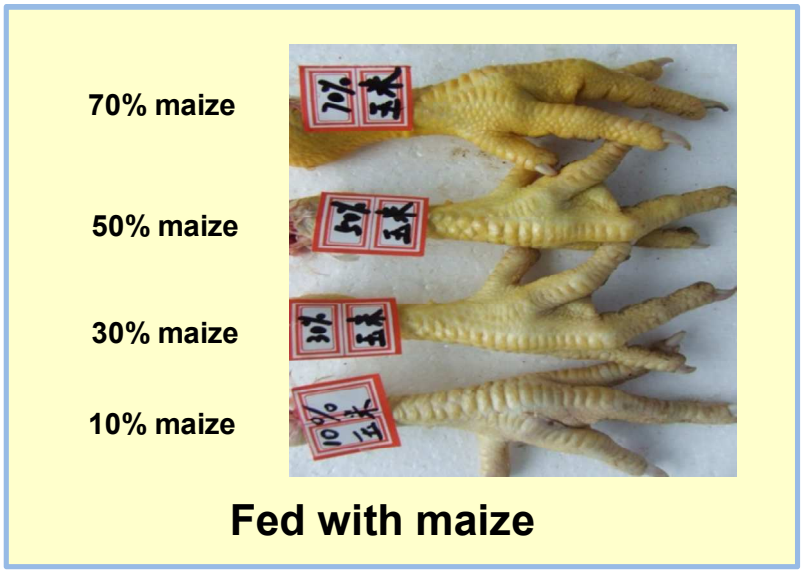
- Discrimination of fake and fraud products
- Verification of product provenance
- Determination of contaminants
- Detection of irradiated agrifood

Discrimination of fake and fraud products

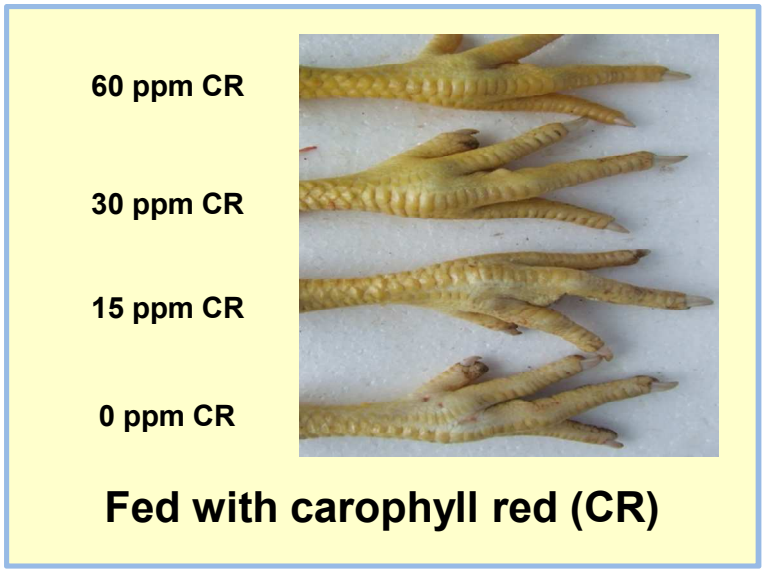
I. Discrimination of honey adulteration by stable isotope techniques

A method with IRMS has been developed to identify honey products adulterated with syrups (i.e. HFCS) from C4 plants, and adopted as a national analytical standard (*GB/T 18932.1-2002*).

II. Tracing the origin of pigment in broilers by nitrogen and carbon isotope analysis



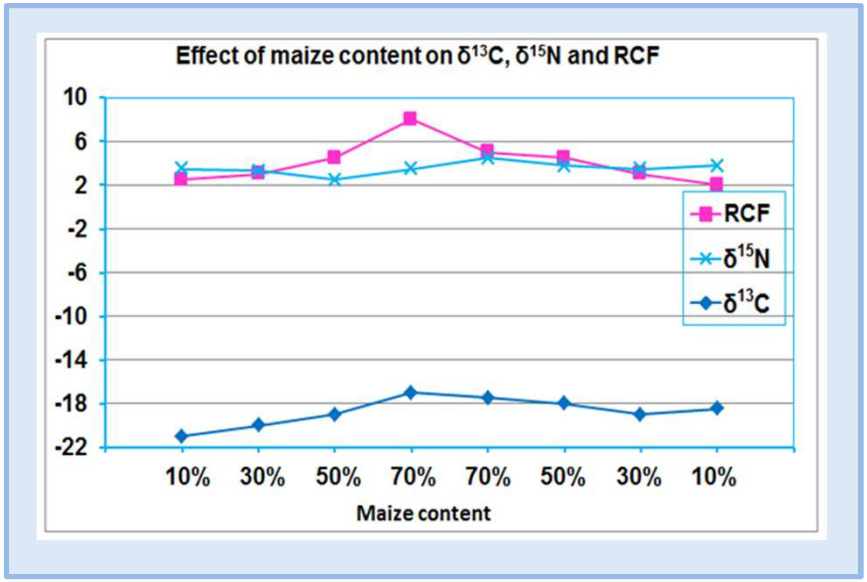
Fed with maize

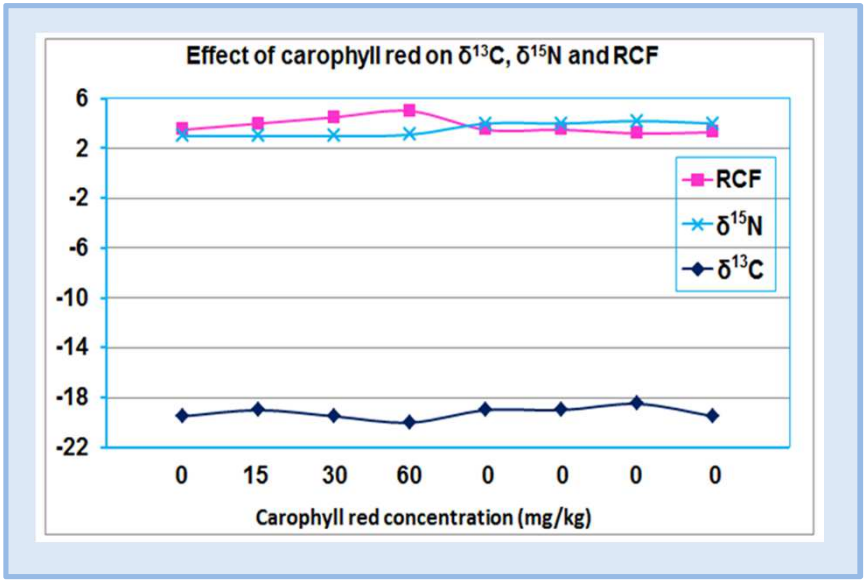


Fed with carophyll red (CR)

- 320 broilers were assigned to 8 groups fed on diets of four levels of maize and four levels of carophyll red for 8 weeks.
- $\delta^{13}C$ and $\delta^{15}N$ of skin samples were measured by IRMS.
- Skin's chrome was tested by Roche Color Fan and scores were recorded.

II. Tracing the origin of pigment in broilers by nitrogen and carbon isotope analysis (cont'd)





- The more carophyll red was added, the deeper yellow skin of broiler was, but the $\delta^{13}C$ values were NOT changed much.
- When maize content increased in diets, the chrome of the broiler skin and the $\delta^{13}C$ values of the fat free dry mass also increased.

Wang et al., 2007, *J. Instru. Anal*

III. Tracing the origin of organic pork

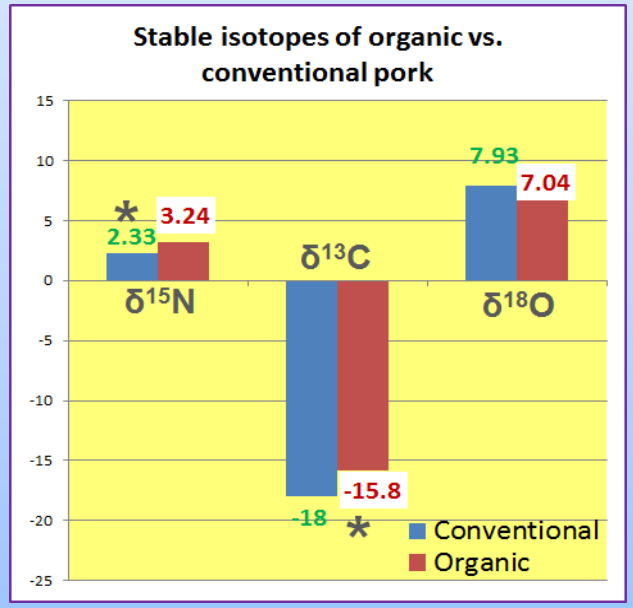
The study aims to establish robust techniques to verify pork from organic origin, in order to prevent the fraudulent labelling

Tracing techniques

Chemometrics

- **Stable isotopes**
- **Multi- elements**
- **Fatty acids**
- **NMR**

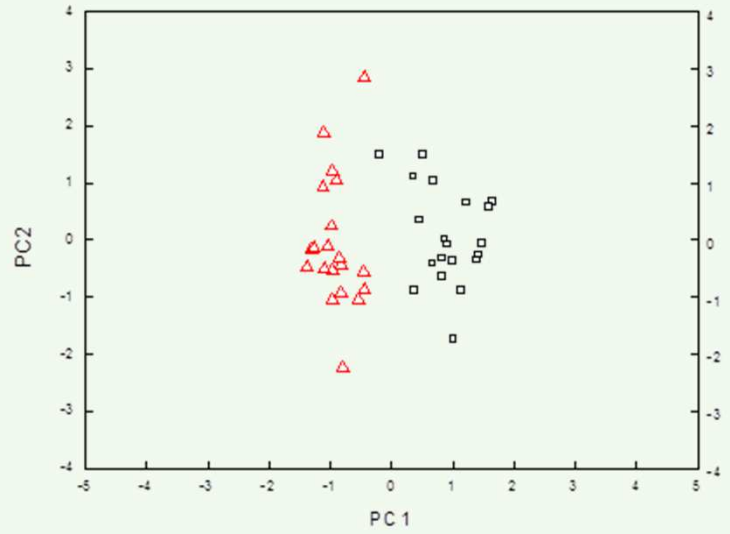
→ **PCA**, **LDA**



$\delta^{15}N$ and $\delta^{13}C$ were significantly higher in organic pork than in conventional pork.

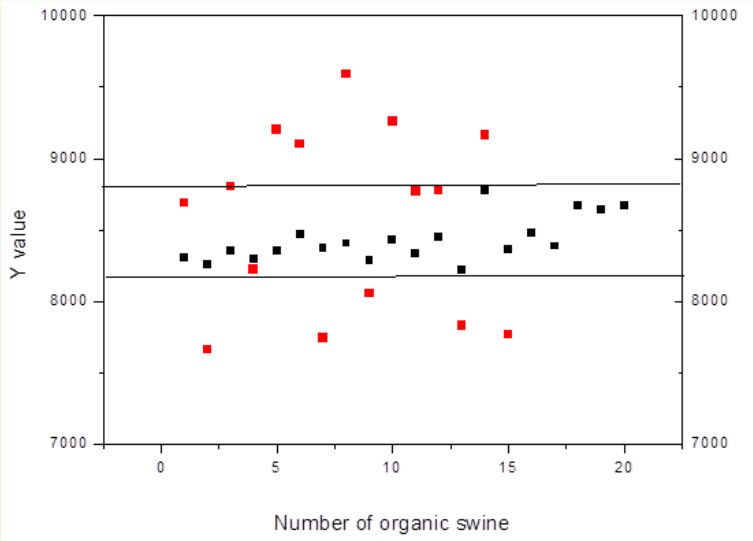
III. Tracing the origin of organic pork (cont'd)

PCA model



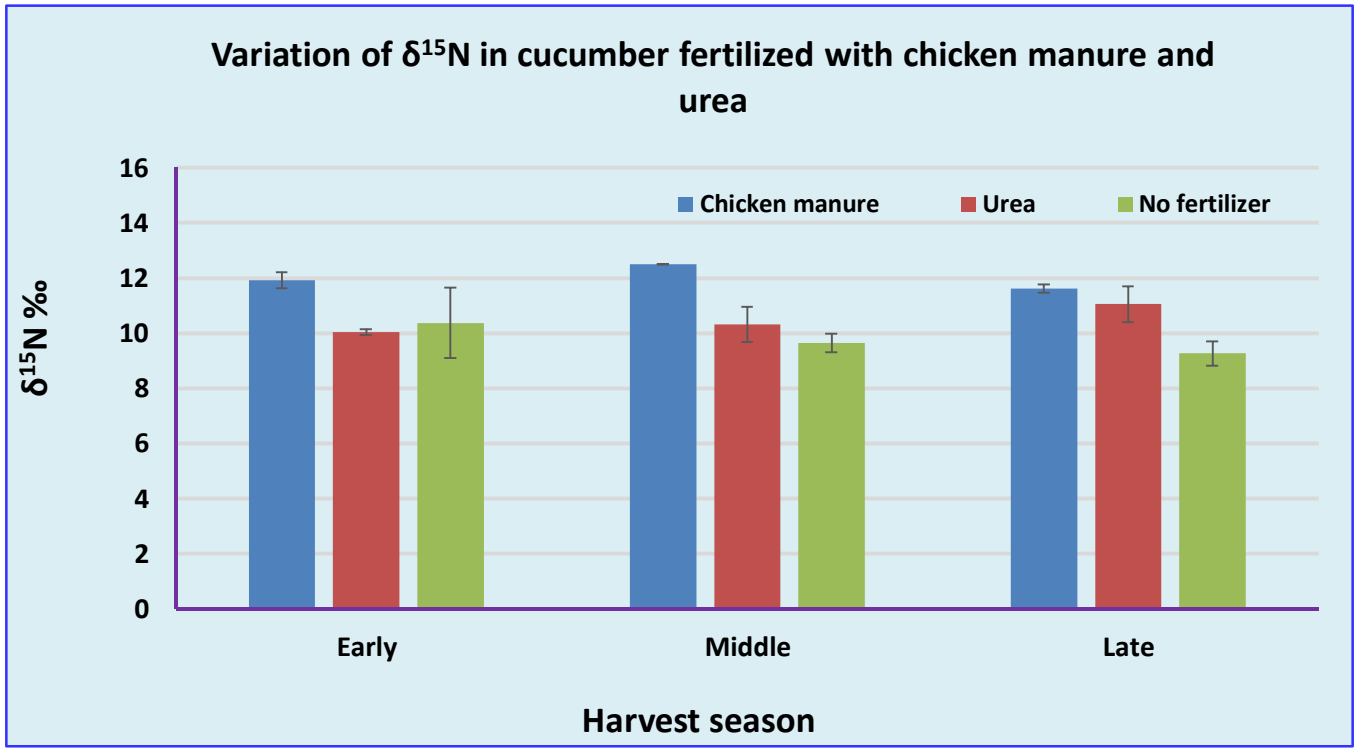
- Differentiation of conventional vs. organic pork using PCA model, based on $\delta^{13}C$, $\delta^{15}N$, multi elements, and fatty acids. The two types of samples were clearly separated along the PC1 axis.

Model validation



- 15 organic pork and 15 conventional pork were taken from another farm, in order to validate the organic pork model.
- Using the established model, 30% of organic pork from QingQuanWan were classified as organic.

IV. Cucumber fertilized with manure and urea

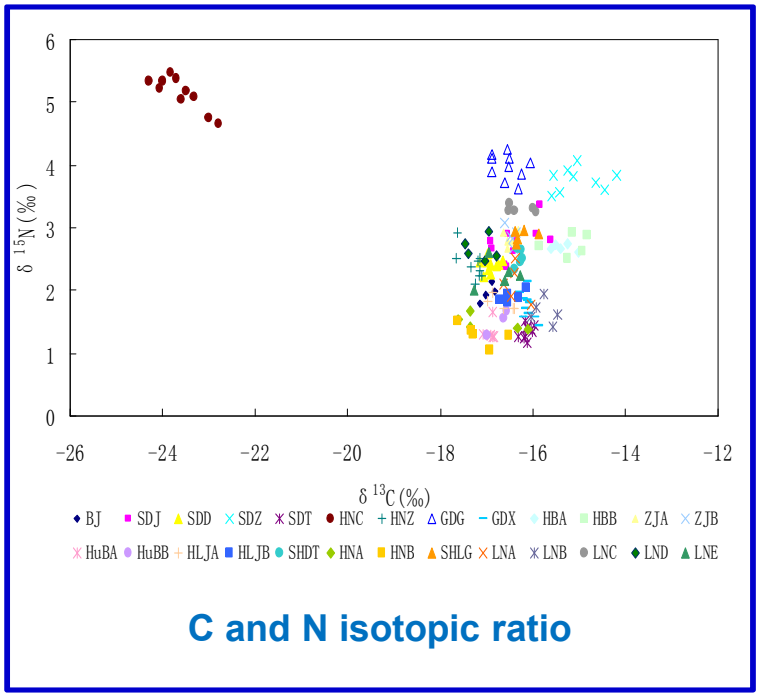


Cucumbers raised with chemical fertilizer and manure can be clearly characterized by testing their stable isotopes. The $\delta^{15}N$ values of cucumber raised with chicken manure were significantly higher than that of cucumber raised with urea.

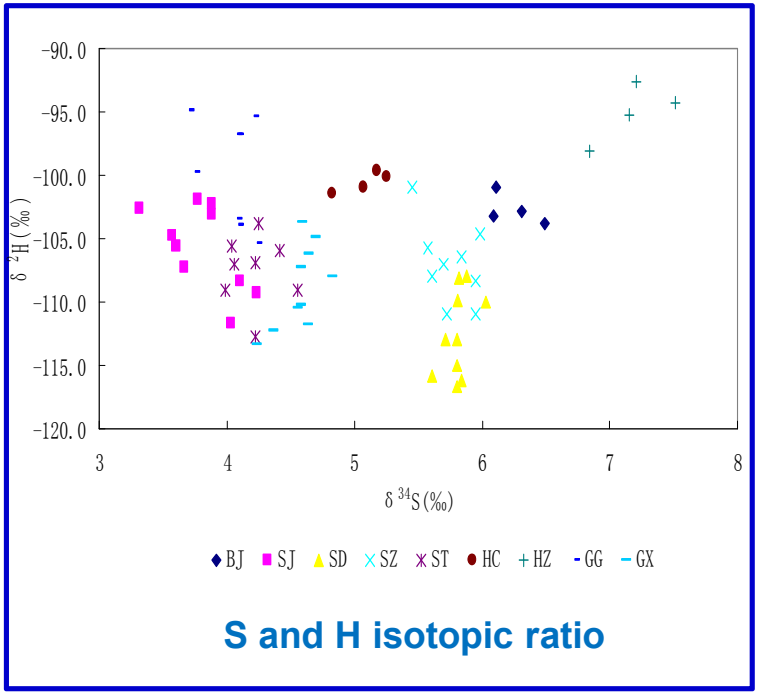
Yuan et al., 2010 J. Nucl. Agri. Sci.

Verification of product provenance-

I. Differentiation of geographical origin of chicken meat



C and N isotopic ratio



S and H isotopic ratio

Stable isotope ratios of C,N,H,O,S and Sr of chicken meat samples were measured. Scatter plotting of C vs N, S vs H could roughly group samples from different regions as shown in the figures above.

I. Geographical origin of chicken meat (cont'd)

LDA in verification of geographical origins of chicken

Classification results 判别结果		Actual region of origin 地区	Classified region of origin 判别地区									
			BJ	SJ	SD	SZ	ST	HC	HZ	GG	GX	Total
Original count 初始判别结果		BJ	4	0	0	0	0	0	0	0	0	4
		SJ	0	10	0	0	0	0	0	0	0	10
		SD	0	0	10	0	0	0	0	0	0	10
		SZ	0	0	0	9	0	0	0	0	0	9
		ST	0	0	0	0	7	0	0	0	1	8
		HC	0	0	0	0	0	4	0	0	0	4
		HZ	0	0	0	0	0	0	4	0	0	4
		GG	0	0	0	0	0	0	0	8	0	8
		GX	0	0	0	0	1	0	0	0	9	10
)bservations	correctly classified (%)正确判别率（%）		100	100	100	100	87.5	100	100	100	90	99.975
	Cross- validated count		BJ	SJ	SD	SZ	ST	HC	HZ	GG	GX	Total
	交叉验证后的判别结果	BJ	4	0	0	0	0	0	0	0	0	4
		SJ	0	9	0	0	0	0	0	1	0	10
		SD	0	0	10	0	0	0	0	0	0	10
		SZ	0	0	0	9	0	0	0	0	0	9
		ST	0	0	0	0	7	0	0	0	1	8
		HC	0	0	0	0	0	4	0	0	0	4
		HZ	0	0	0	0	0	0	4	0	0	4
		GG	0	0	0	0	0	0	0	8	0	8
		GX	0	0	0	0	1	0	0	0	9	10
Observations correctly classified (%)正确判别率（%）			100	90	100	100	87.5	100	100	100	90	99.964

The correct classification in cross validation were 87.5% to 100% for the samples from 9 regions.

Sun et al., 2008, *J. Instru. Anal*

II. Geographical origin of beef using $\delta^{13}C$

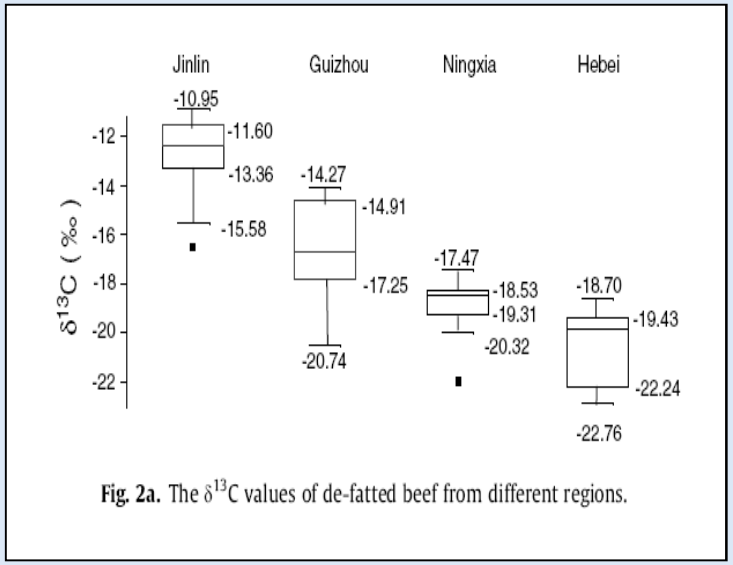


Fig. 2a. The $\delta^{13}C$ values of de-fatted beef from different regions.

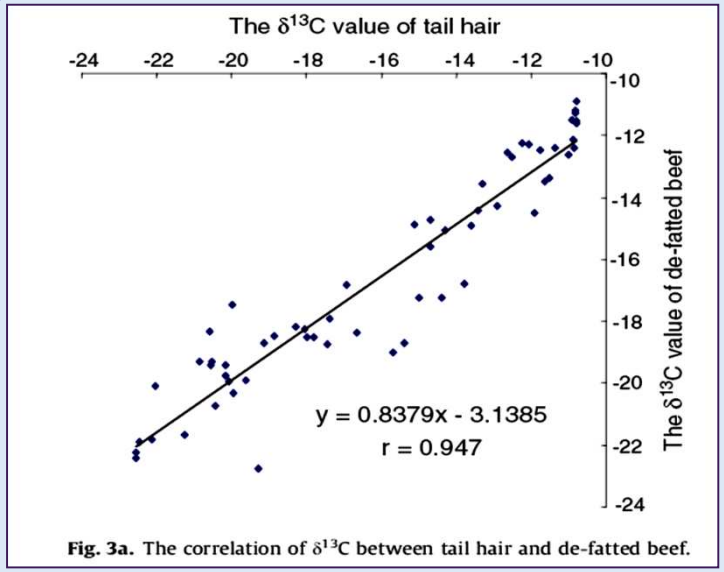


Fig. 3a. The correlation of $\delta^{13}C$ between tail hair and de-fatted beef.

Variation has been reported in $\delta^{13}C$ and $\delta^{15}N$ stable isotope ratios of different cattle tissues from 4 provinces. The results implied the tissues responded to the diet in a similar pattern. $\delta^{13}C$ was a better indicator for tracing the origin of cattle.

Guo et al., 2010, *Food Chem.*

III. Origins of beef using stable isotopes in tail hair

The liner discriminant analysis results of $\delta^{13}C$, $\delta^{15}N$ and $\delta^{2}H$ values in cattle tail hair from different beef production regions.

		Predicted group membership				
		IMP	NCP	LP	NP	Total
Original						
Count	IMP	22	3	0	4	29
	NCP	3	67	6	0	76
	LP	0	8	22	0	30
	NP	5	0	0	27	32
%		75.9	88.2	73.3	84.4	82.6
Cross-validated						
Count	IMP	20	4	0	5	29
	NCP	3	65	7	1	76
	LP	0	9	21	0	30
	NP	5	0	0	27	32
%		69.0	85.5	70.0	84.4	79.6

Note: IMP, Inner Mongolian Plateau; NCP, North China and Central Plains; LP, Loess Plateau; NP, Northeast Plain.

The $\delta^{13}C$, $\delta^{15}N$ and $\delta^{2}H$ values of cattle tail hair were measured. Variance and LDA showed significant differences among 7 sub-regions of 4 production regions. Overall correct classification and cross-validation rates were 82.6% and 79.6%, respectively.

Liu et al., 2013, *Food Chem*.

IV. Tracing beef by stable isotope & multi-element analysis

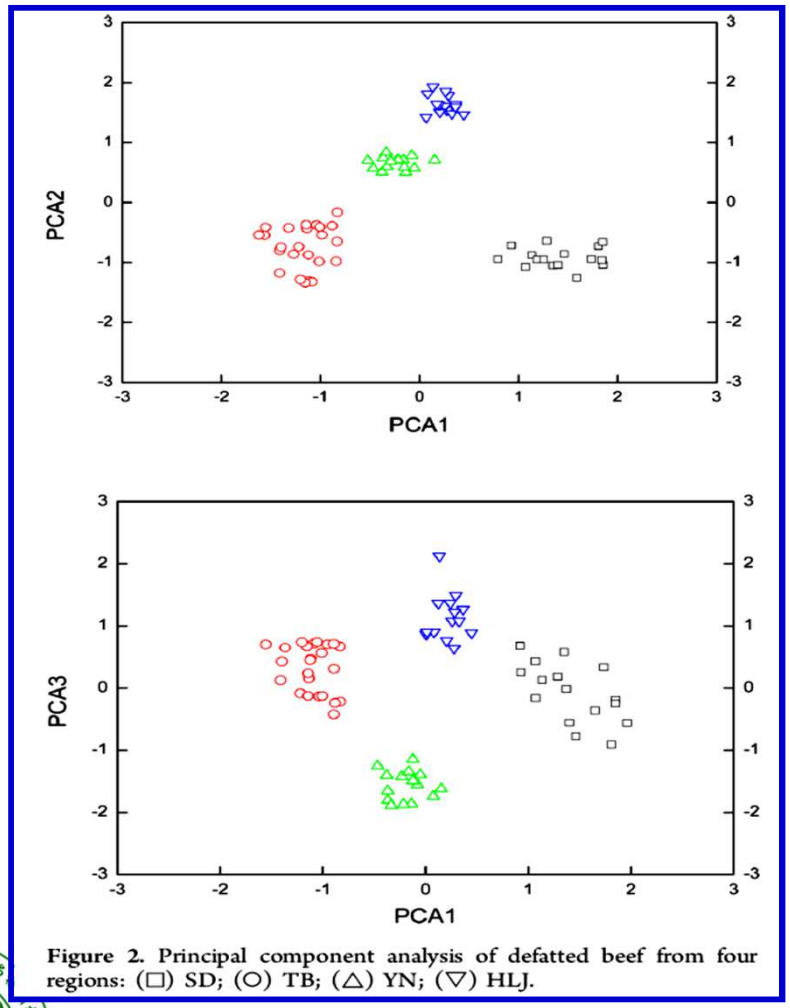
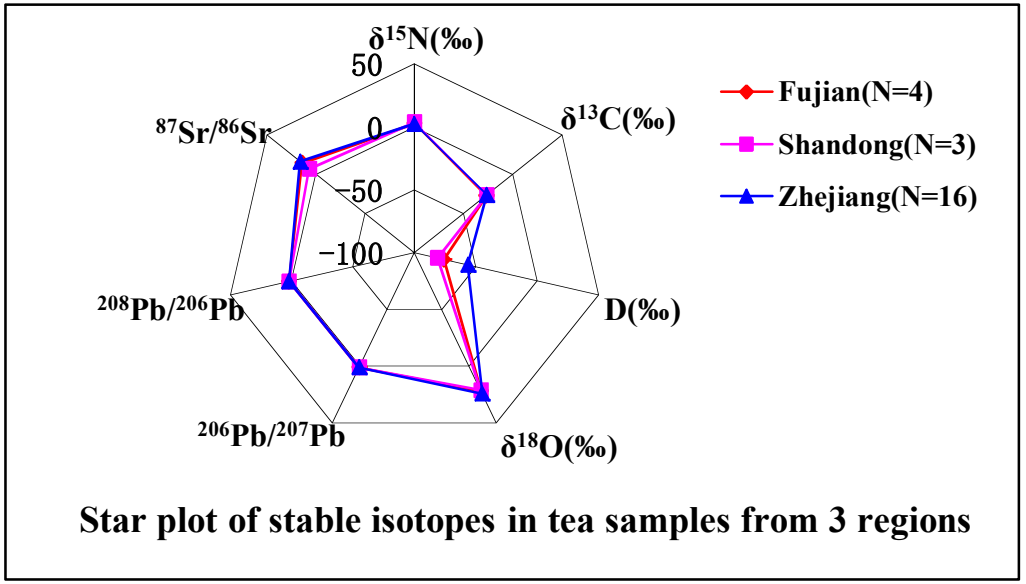


Figure 2. Principal component analysis of defatted beef from four regions: (□) SD; (○) TB; (△) YN; (▽) HLJ.

Combined analysis of stable isotopes and multi-elements was applied to trace the geographical origins of beef from four different provinces. PCA results indicated that eight key variables, including $\delta^{13}C$, $\delta^{15}N$, Mg, K, Mn, Zn, Se and Zr, were identified as maximum discriminants among samples. Both overall correct classification and cross-validation rates were 100%.

Zhao et al., 2013, *J. Agri. Food Chem*

V. Stable isotope and multi-element analysis for tea



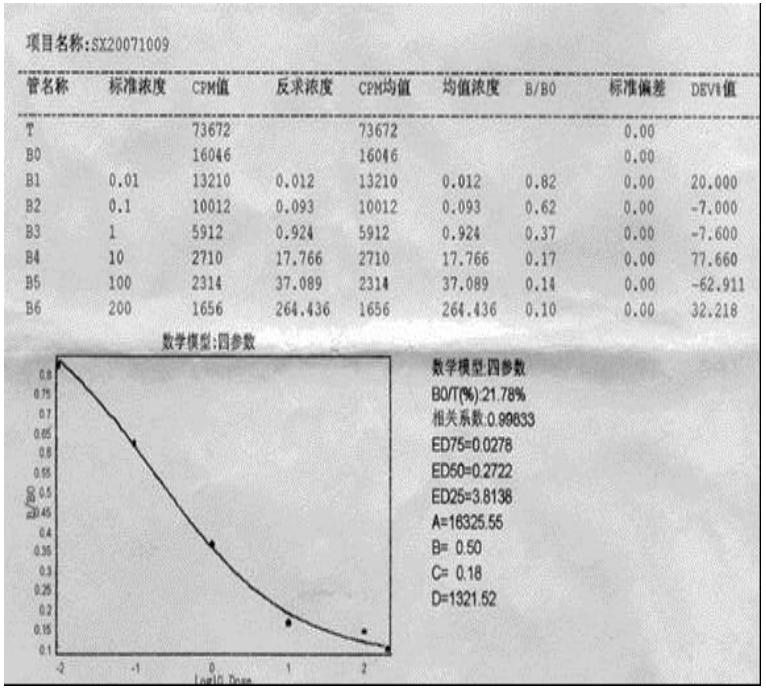
Star plot of stable isotopes in tea samples from 3 regions

Pattern recognition techniques with PCA and LDA were used to classify geographical origins of tea from Fujian, Shandong and Zhejiang province. Results revealed that it is feasible to classify the geographical origin of tea by PCA-LDA based on isotopes and multi-elements.

Yuan et al., 2013, *J. Nucl. Agri. Sci.*

Determination of contaminants

I. A new radio labeled assay for detecting multi-residues of veterinary drugs



项目名称:SX20071009

管名称	标准浓度	CPM值	反求浓度	CPM均值	均值浓度	B/B0	标准偏差	DEVI值
T		73672		73672			0.00	
B0		16046		16046			0.00	
B1	0.01	13210	0.012	13210	0.012	0.82	0.00	20.000
B2	0.1	10012	0.093	10012	0.093	0.62	0.00	-7.000
B3	1	5912	0.924	5912	0.924	0.37	0.00	-7.600
B4	10	2710	17.766	2710	17.766	0.17	0.00	77.660
B5	100	2314	37.089	2314	37.089	0.14	0.00	-62.911
B6	200	1656	264.436	1656	264.436	0.10	0.00	32.218

数学模型:四参数

数学模型:四参数
B0/T(%):21.78%
相关系数:0.99833
ED75=0.0278
ED50=0.2722
ED25=3.8138
A=16325.55
B= 0.50
C= 0.18
D=1321.52

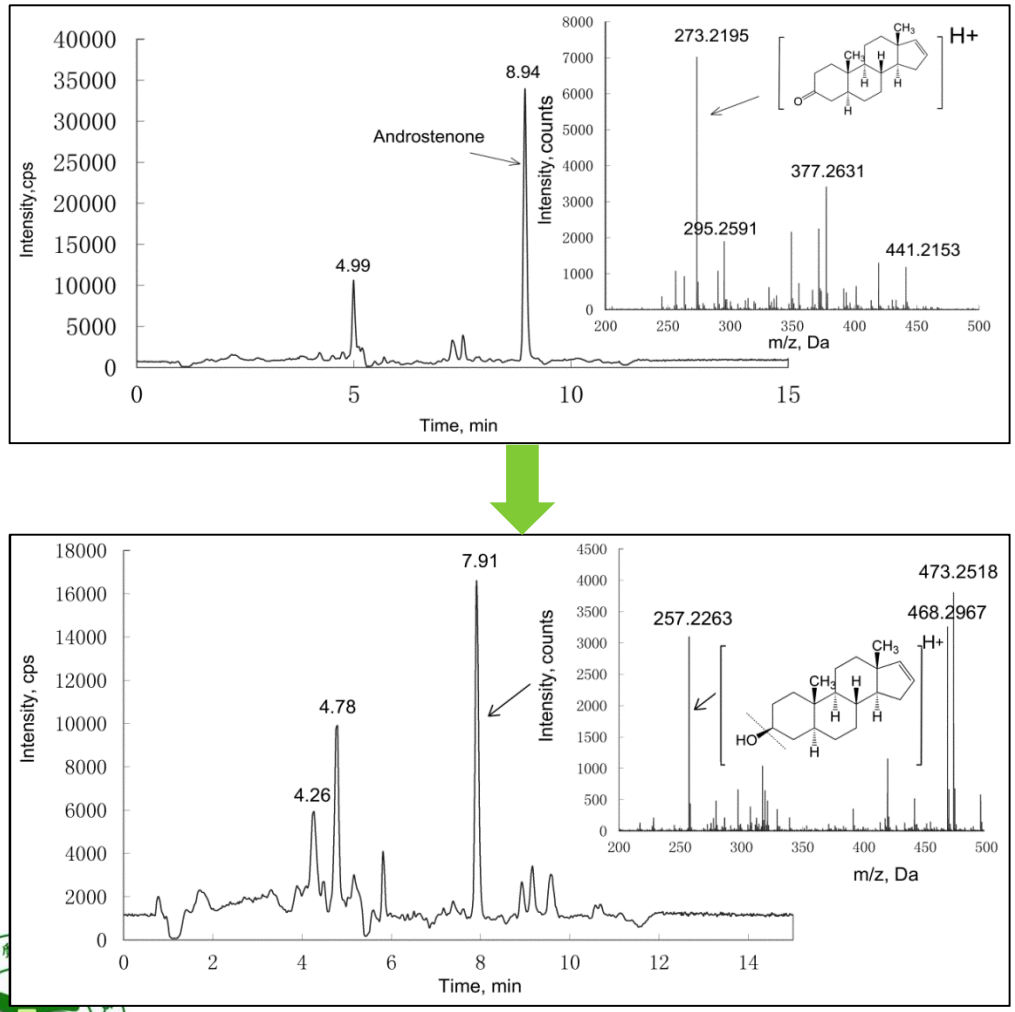
表2: 利用受体检测β2激动剂多残留

Table 2: Detection of the multiresidue of β 2-agonist by β 2AR

包被蛋白种类 \ 竞争物 吸光值		Clenbuterol(ppb)				ractopamine(ppb)			Salbutamol(ppb)		
		0	10	100	1000	10	100	1000	10	100	1000
大肠杆菌细胞对照（仅表达MBP）	粗膜蛋白	0.397	0.264	0.263	0.379	0.434	0.269	0.234	0.241	0.215	0.257
	纯化MBP	0.214	0.301	0.312	0.335	0.421	0.363	0.391	0.236	0.136	0.198
	未结合蛋白	0.26	0.242	0.297	0.258	0.212	0.234	0.206	0.206	0.115	0.211
诱导表达大肠杆菌细胞	粗膜蛋白	0.892	0.599	0.413	0.192	0.582	0.379	0.165	0.615	0.37	0.132
	纯化β_2AR	0.808	0.457	0.308	0.152	0.65	0.367	0.11	0.608	0.375	0.113
	未结合蛋白	0.852	0.605	0.447	0.167	0.561	0.357	0.098	0.468	0.276	0.128
转染表达哺乳动物细胞	细胞破碎后	0.879	0.574	0.395	0.168	0.568	0.39	0.192	0.493	0.282	0.154
	粗膜蛋白	0.907	0.622	0.435	0.178	0.563	0.3415	0.123	0.528	0.364	0.149
	纯化β_2AR	0.842	0.612	0.314	0.195	0.638	0.441	0.137	0.578	0.437	0.145
	未结合蛋白	0.719	0.476	0.219	0.094	0.46	0.299	0.132	0.569	0.351	0.142

A multi-screening tool was established with β_2-androenergic receptor and iodocyanopindolol ([3H]ICYP) to simultaneously detect the total drug residues of β-agonists. Wang et al., 2008, *Life Sci. Res.*

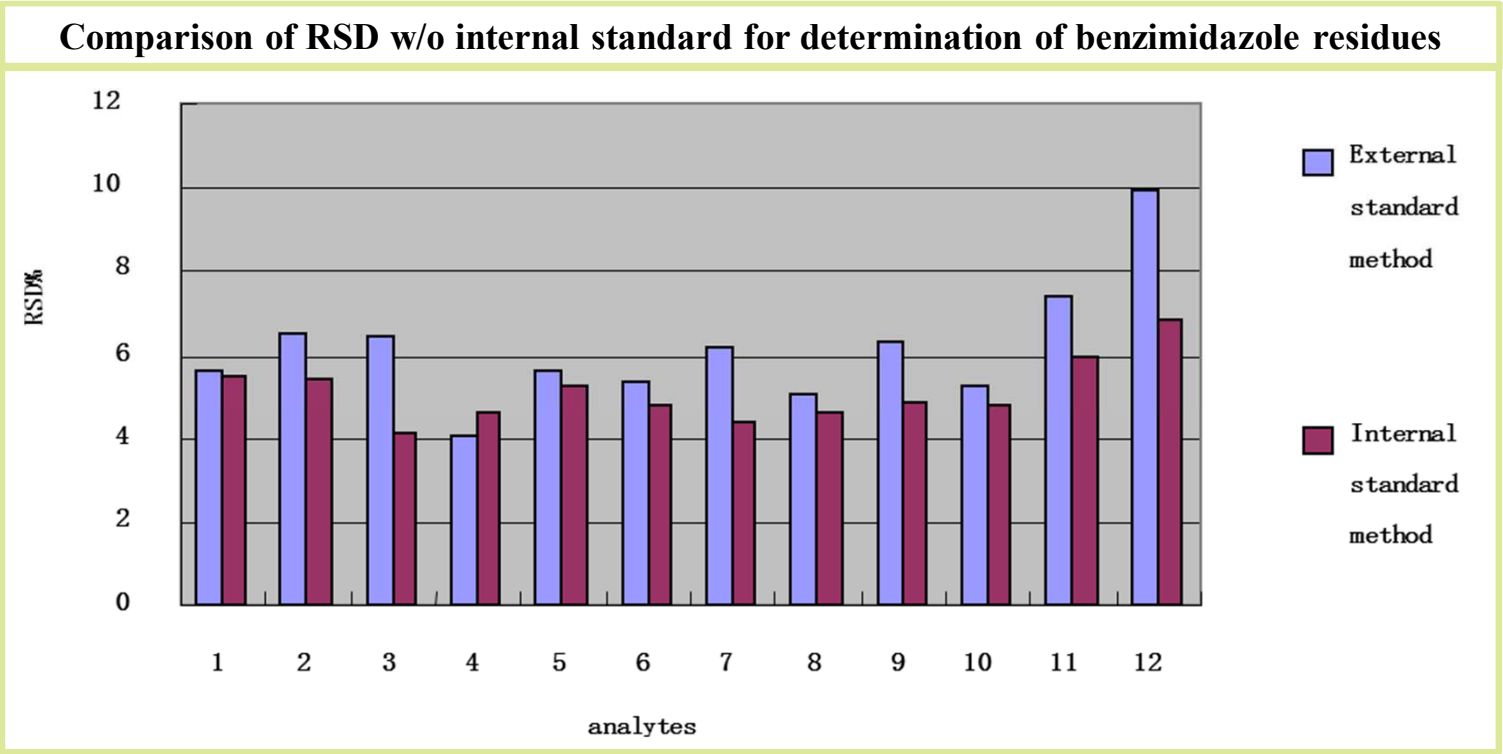
II. Metabolomics of steroids labelled by ^{2}H in hepatocytes



Steroid (androstenone) was labelled with ^{2}H to investigate its metabolism in pig hepatocytes. The metabolites were analyzed by LC-TOF-MS. Metabolites from ^{2}H labelled steroid could be differentiated clearly from the samples without labeling. Therefore, the steroid metabolites can be clearly characterized.

Chen et al.,2012, *PLoS ONE*

III. Stable Isotope dilution in analysis of residues



Internal standards labelled with stable isotopes have been adopted in more than 30 Chinese national standards. The figure above demonstrates the comparison of mean RSD with or without 6-^{13}C-thiabendazole as internal standard for LC-MS/MS analysis of 12 benzimidazole residues in milk.

Liu et al, 2012, *Chin. J. Ana.Lab*

Detection of irradiated agrifood

I. Discrimination of irradiated/untreated food

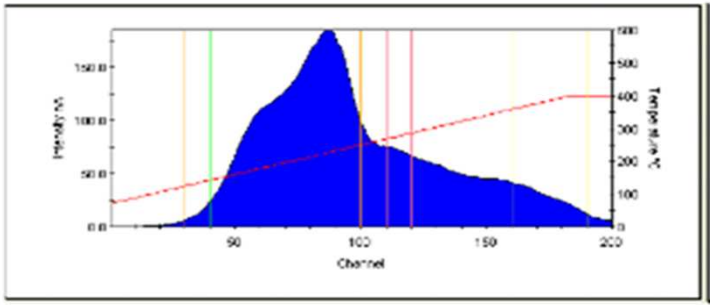


图 1：黑胡椒的一次发光曲线 $Glow_1$

Fig.1 TL curve $Glow_1$ of black pepper

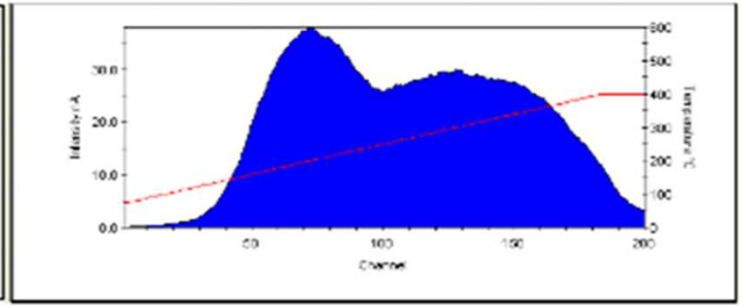


图 2：黑胡椒的二次发光曲线 $Glow_2$

Fig.2 TL curve $Glow_2$ of black pepper

TL 发光比=G_1/G_2

$G_1=(Glow_1-B_1)/(m_1-m_0)$ (nC/mg)　　$G_2=(Glow_2-B_2)/(m_2-m_0)$ (nC/mg)

$G_1/G_2 \geqslant 0.1$ 则判定样品经过辐照处理

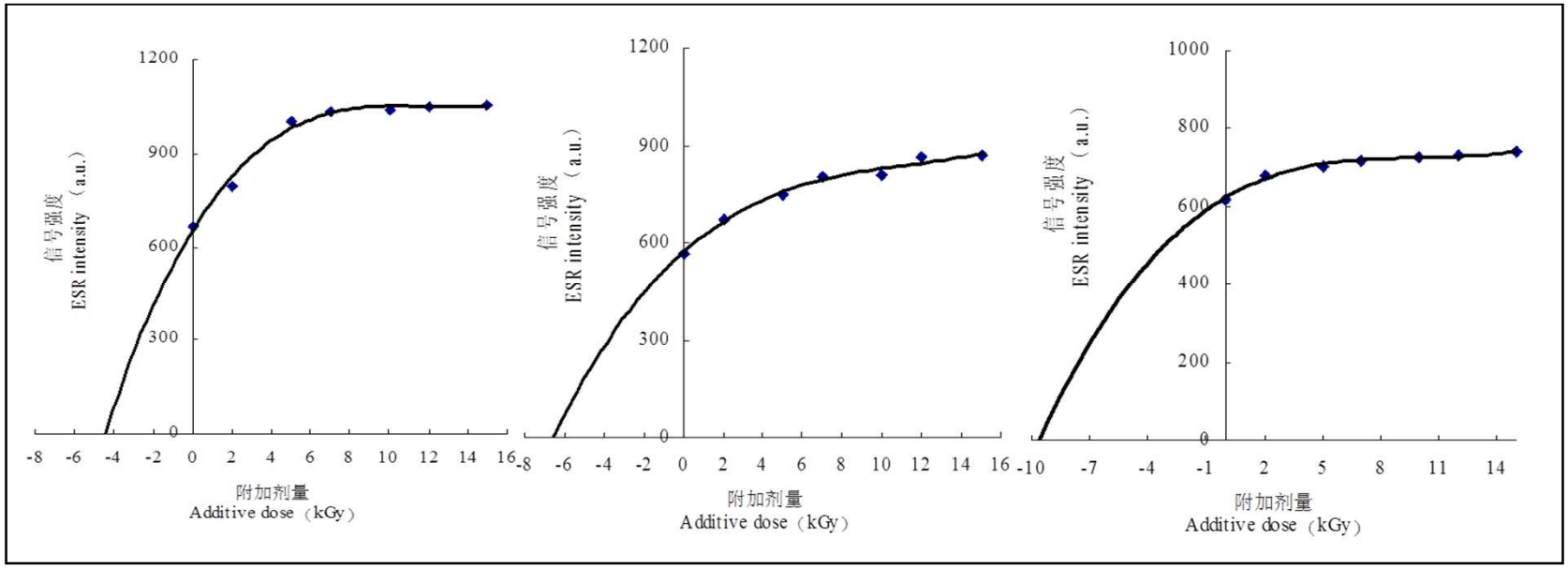
$G_1/G_2 < 0.1$ 则判定样品未经过辐照处理

It is found that thermoluminescence intensity of inorganic minerals, such as silicate, silica & clay, treated by ionizing radiation is positively correlated with the irradiation dose. A detective method was developed to discriminate the irradiated and untreated agrifoods.

Ha et al., 2009, *Food Machinery*

Detection of irradiated agrifood

II. Estimation of irradiation dose

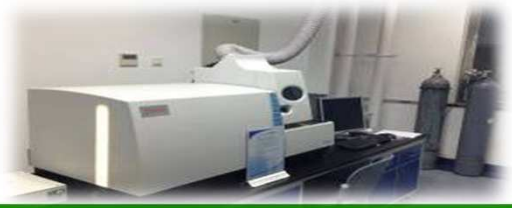


Irradiation dose in cumin samples was estimated by analyzing ESR intensity with a dose-additive method. Figures showed that irradiation dose was positively correlated with ESR intensity. The deviation between the initial dose and the dose estimated was less than 1 kGy.

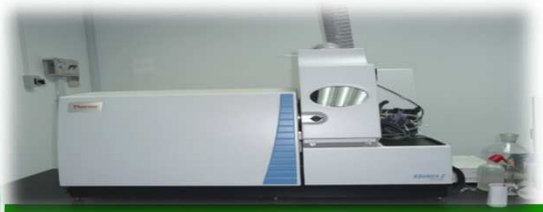
Li et al., 2011, *Sci. Agri. Sinica*

Future perspectives: research priorities

- To develop chromatography-IRMS combined techniques for identifying authenticity, combating fraud and tracing origins of specific agrifood commodities.
- To exploit integrated discrimination and verification methods and models based on analysis of multi-isotopes and related geographical and meteorological data.
- To apply tracer techniques in proteomics, metabolomics and foodomics studies.


- To carry out risk assessment of radioactive contamination in agrifood.
- To develop standard operating procedures (SOP), harmonized protocols and standards for determination of the provenance and authenticity of agrifood commodities.



Future perspectives: application

Established integrated analytic approaches together with standards, SOPs and protocols from researches on food safety and traceability with nuclear and related techniques will help to build and strengthen capacities in supporting:

- Food monitoring and regulation of governmental agencies
- Quality control and quality assurance of food industries
- Fair trade of food in domestic and international markets
- Protection of consumers' health and benefits

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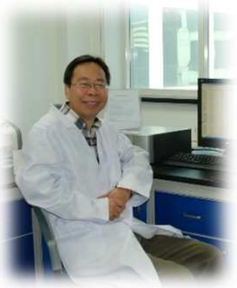
Co-authors



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