

第15届年度中国国际食品安全与质量 (CIFSQ) 会议

肉类掺假风险因子高效检测技术研究

**Research on High-efficiency Detection Technologies of Meat
Adulteration Risk Factors**

**中国肉类食品综合研究中心
China Meat Research Center (CMRC)**

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报告提纲 Outline

一 技术背景 technology background •

二 技术研究 technology research •

三 技术应用 technology application •

四 技术展望 technolgy outlook •

一、技术背景 Background

- 肉类食品掺假现象在国内外**普遍存在**
- Meat adulteration is still a common problem in the world



国家地区	公开报道的典型案列 Typical reported cases
中国 CHN	<ul style="list-style-type: none">◆2011年，河北省张家口市桥东区，查获16吨疑似“掺假”羊肉卷。◆2012年，上海市刘某因用鸭肉制作羊肉串，依法判处有期徒刑1年。◆2013年，无锡、上海两地统一行动，捣毁黑窝点50余处，狐狸、水貂、老鼠等未经检验检疫的动物肉制品，冒充羊肉，案值1000余万元。◆2013年，内蒙古包头，现场查获冷冻假牛肉6.4吨、成品假牛肉干14.6吨，以及“一滴香”“牛膏1号”等调味料。◆2014年，台湾，在猪、牛、羊肉中超量使用磷酸盐作为保水剂。◆2017年，北京市食药监局发现3家门店疑似肉品掺假，被责令停业整顿。◆2017年，河间市，马肉冒充驴肉，被河北省食药监局通报。◆2018年，河间通报8起案件 其中涉驴肉掺假的案件2起。◆2020，调研数据，北京市场肉串总体掺假比例为21%，其中羊肉串掺假比例为28%，牛肉串掺假比例为14%。

- ◆ 针对掺假问题，**国务院常务会（2013.5.8）**做出“**严厉打击肉类产品掺假售假等违法违规行为**”的指示。

一、技术背景 Background

- 肉类食品掺假现象在国内外**普遍存在**
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国家地区	公开报道的典型案列 Typical reported cases
欧盟 EU	◆ “马肉风波” ：2013年1月，爱尔兰食品安全局（FSAI）在27份牛肉饼的10份中检出马源性成分（37%），23份中检出猪肉源性成分（85%），FSAI 2013。展开全面调查。
美国 USA	◆ 2016，查普曼大学，市场抽样检测，48个样品中10个存在掺假情况（ 38% ），Dawn E. Kane, Rosalee S. Hellberg, 2016.
加拿大 CAN	◆ 2017，市场上抽检100件香肠产品， 20% 存在掺假情况。Amanda M. Naaum, Hanan R. Shehata, 2018.
南非 RSA	◆ 2013，139件肉制品中，95件存在掺假情况（ 68% ），Cawthorn and others, 2013.
埃及 EGY	◆ 2015年7月，在埃及法尤姆地区发生大规模肉类掺假事件，当地一些餐厅违反伊斯兰宗教规定，为消费者提供了驴肉食品，当地监管机构在一家农场扣留了1500头活驴和30头死驴。



- ◆ 针对马肉风波，欧盟开展了**大规模、系统的市场检测工作**，保障肉类食品的真实性。

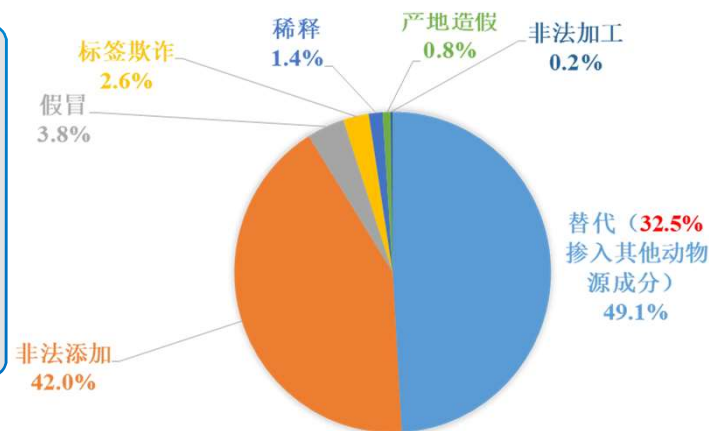
一、技术背景 Background

- | | |
|-------------|---|
| 风险1: 肉种成分掺假 | RISK 1: Meat species adulteration |
| 风险2: 注水药物滥用 | RISK 2: Illegal usage of veterinary drugs |
| 风险3: 超期贮藏肉 | RISK 3: Mislabeling of expire date |
| 风险4: 产地造假 | RISK 4: Origin counterfeiting |



肉类掺假严重打击消费者信心
和政府公信力

- | | |
|---------|------------------------------------|
| ■ 掺假类型多 | ■ Adulteration types are diverse |
| ■ 隐蔽性强 | ■ Strong concealment |
| ■ 危害面广 | ■ Wide range of hazards |
| ■ 防控难度大 | ■ Difficult to prevent and control |



掺杂形式多样

技术需求
technology
needs

- 建立准确、高效、便捷、快速、易用的检测技术和判别方法。
- Establish accurate, efficient, convenient, fast and easy-to-use detection technologies and methods.
- 高效获取样品信息, 样品是否掺假? 掺的什么? 掺假严重程度如何?
- Efficiently obtain sample information, whether the sample is adulterated? Adulterated with what? How serious is the adulteration?

二、技术研究 Technology Research

- 针对需求，构建出肉制品掺假风险的高效检测技术体系和研发平台。
- The efficient detection technology system and R&D platform for the risk of adulteration of meat products were constructed.

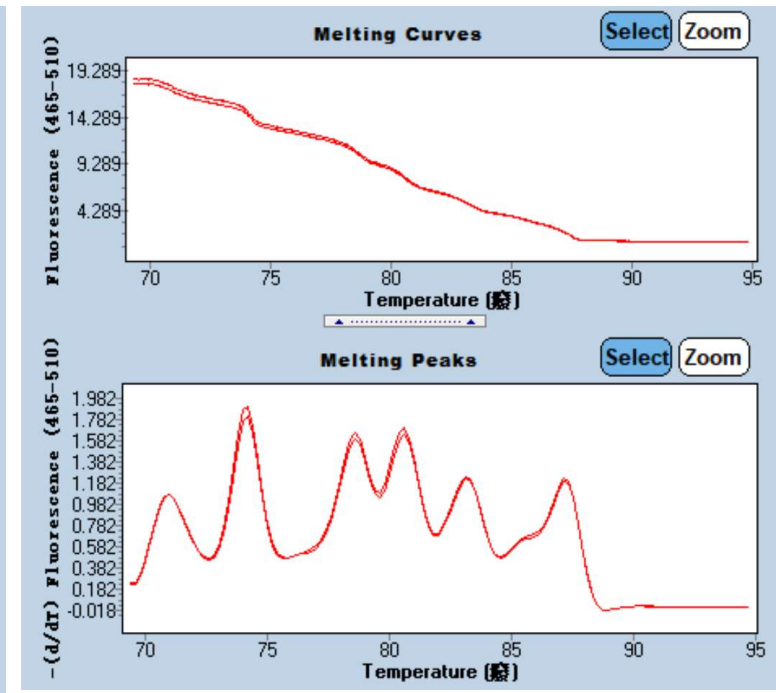
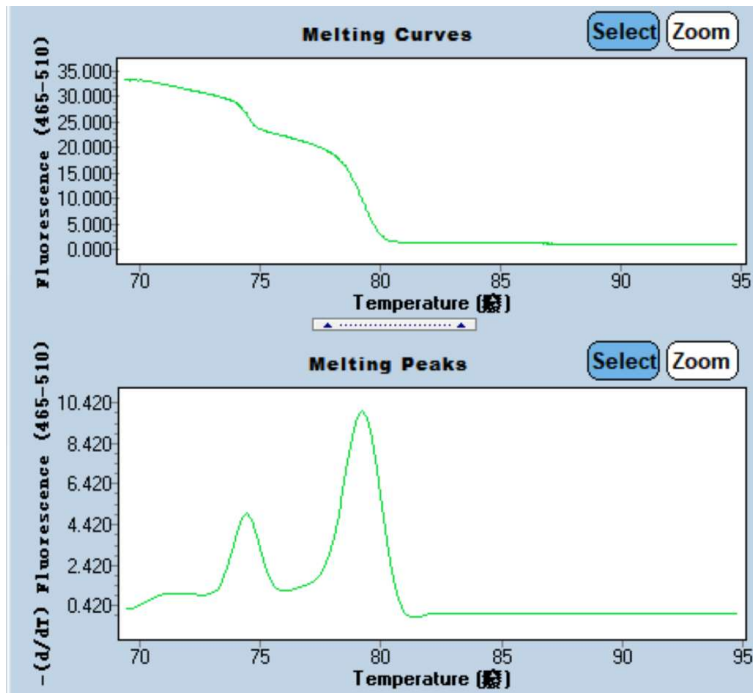
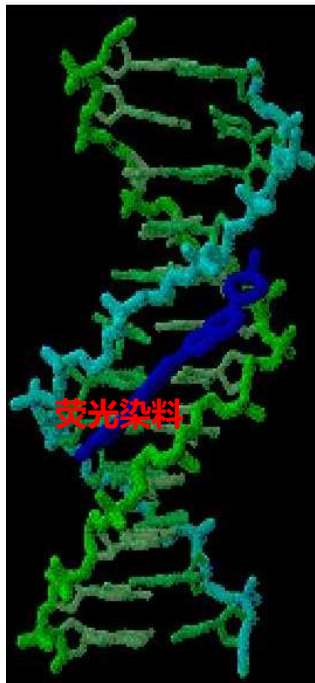
掺假风险 risks of meat adulteration	技术与方法 technology and methods	特点
风险1: 肉种成分掺假 RISK 1: Meat species adulteration	1、基于多重RT-PCR熔解曲线的多物种同步检测技术 Multi species simultaneous detection technology based on multiple RT-PCR fusion curve 2、基于近红外光谱的牛羊肉馅快速鉴定技术 Rapid identification of beef and mutton stuffing based on near infrared spectroscopy	高效 (高信息密度) Efficient (High information density) 无损、快速 Rapid and nondestructive
风险2: 注水药物滥用 RISK 2: RISK 2: Illegal usage of veterinary drugs	3、基于HPLC-MS的注水药物同步检测技术 Simultaneous detection technology of water injection drugs based on HPLC-MS	高效 (高信息密度) Efficient (High information density)
风险3: 超期贮藏肉 RISK 3: RISK 3: Mislabeling of expire date	4、基于机器视觉的冷冻肉冻藏时间判别技术 Discrimination technology of frozen meat storage time based on machine vision	无损、快速 Rapid and nondestructive



1、基于多重RT-PCR溶解曲线的多物种同步检测

1. Multi species synchronous detection technology based on multiple RT-PCR fusion curve

- 溶解曲线是在RT-PCR结合扩增子热力学分析的过程，是理想的肉类掺假检测手段。
- Fusion curve is a process of RT-PCR combined with amplicon thermodynamic analysis. It is an ideal mean for detecting meat adulteration.
- 通过扩增子Tm值多样性设计，可实现在一个荧光波长信号通路下，通过一次多重RT-PCR反应对多种产物的同步定性定量检测，在提升方法信息密度的同时，没有提高对设备和试剂的要求。
- Through the diversity design of amplicon TM value, the simultaneous qualitative and quantitative detection of multiple products through a multi-RT-PCR reaction under one fluorescence wavelength signal path can be achieved, which improves the information density of the method, but the requirements for equipment and reagents.



Slide 7

H27

同前面, 用simultaneous

Hao, 20/10/2021

难点与解决方案 Difficult and Solution

难点 difficulty:

- 如何做好引物设计：特异性好、扩增效率高、扩增子Tm分布合理，相互干扰弱。
- Selection of high-quality species-specific (Universal) DNA sequence sites.
- 反应体系和反应条件优化。
- Primer combination design with strong specificity, high amplification efficiency and weak interference between primers in multiplex RT-PCR system.

方案 Solution:

- 开发出基于python的多重PCR体系智能化引物设计平台，可自动化寻找和筛选特异性或通用性好、引物之间干扰小，扩增靶序列Tm合适的引物组合。 H36
- An intelligent primer design and R&D platform for multiplex PCR system based on Python is developed, which can automatically find and screen primer combinations with good specificity or universality, small interference between primers and appropriate amplification target sequence Tm.
- 靶向鉴定+非定向筛查的策略和扩增子Tm值多态性设计，构建出多重RT-PCR熔解曲线法。
- The multiplex RT-PCR system and amplification conditions were optimized to improve the performance of the method.
- 建立模型和图谱库，提高定性定量检测准确性。
- The models and atlas database were established to improve the accuracy of qualitative and quantitative detection.

Slide 8

H36

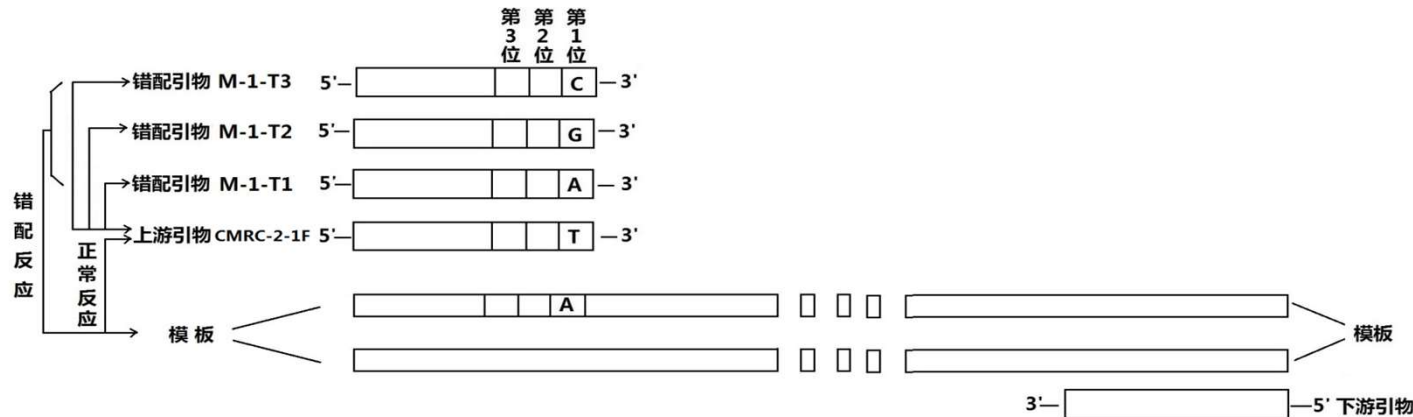
was, which could

Hao, 20/10/2021

平台构建基础：引物性能数字化评价模型体系，以特异性和受热断裂数字化评价为例

Platform construction basis: Construction of primer specific digital prediction model system

- **特异性**是RT-PCR引物的关键性能指标之一，其**数字化评价**是实现自动化和智能化设计的基础。以错配反应与对照反应之间的 ΔCt 值为指示指标，通过研究不同**碱基错配类型、位置与个数**条件下引物3'端与模板之间非特异性扩增的发生规律，构建引物特异性预测模型。
- Specificity is one of the key performance indexes of RT-PCR primers, and its digital evaluation is the basis of automatic and intelligent design. Between mismatch reaction and control reaction ΔCt were used as indicators to construct a primer specificity prediction model by studying the occurrence law of nonspecific amplification between primer 3' end and template under different base mismatch types, positions and numbers.

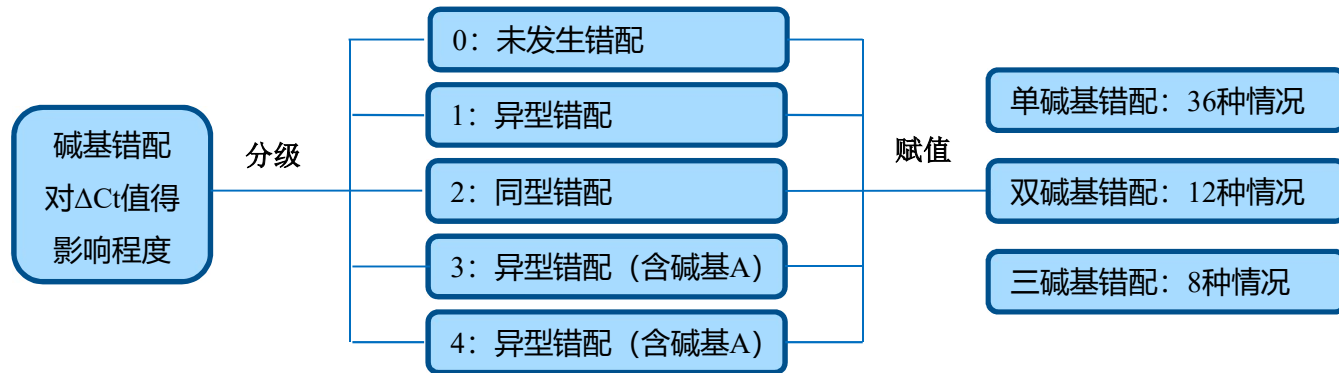


	碱基位置(3'端)			错配类型
	第一位	第二位	第三位	
单碱基错配	TGA/ACA	GAG/CAC	AAG/ATC	同型
	TGG/ACA	GGG/CAC	GAG/ATC	同型
	TGC/ACA	GCG/CAC	CAG/ATC	异型
	CCT/GGT	TTG/ATC	IGC/ICG	同型
	CCG/GGT	TGG/ATC	GGC/ICG	异型
	CCC/GGT	TCG/ATC	CGC/ICG	同型
	AGA/TCG	CAA/GGT	ACA/GGT	同型
	AGI/TCG	CTA/GGT	ICA/GGT	异型
	AGG/TCG	CGA/GGT	GCA/GGT	同型
	TAA/ATC	AAC/TCG	ATG/CAC	异型
	TAT/ATC	ATC/TCG	TTG/CAC	同型
	TAC/ATC	ACC/TCG	CTG/CAC	同型

	错配位置 (3'端)	错配类型
	TAT/AGT	○同同
TTI/AGT	○异同	
TAG/AGT	○同异	
TTG/AGT	○异异	
GAA/AGT	同同○	
CAA/AGT	异同○	
GTA/AGT	同异○	
CTA/AGT	异异○	
GCI/AGI	同○同	
CCT/AGI	异○同	
GCG/AGI	同○异	
CCG/AGI	异○异	

三碱基错配	错配位置 (3'端)	错配类型
	GAT/AGT	同同同
	CAT/AGT	异同同
	GTT/AGT	同异同
	CTT/AGT	异异同
	GAG/AGT	同同异
	CAG/AGT	异同异
	GTG/AGT	同异异
CTG/AGT	异异异	

以 ΔCt 为特异性表征的预测模型



部分碱基
错配赋值
与 ΔCt 值

错配类型	第三位 (x)	第二位 (y)	第一位 (z)	ΔCt
单碱基错配	0	0	4	17.7
	0	0	4	14.12
	0	0	2	3.63
双碱基错配	0	4	3	22.48
	0	1	3	14.19
	0	4	1	7.18
三碱基错配	4	4	3	19.22
	2	4	3	19.04
	2	4	3	21.08

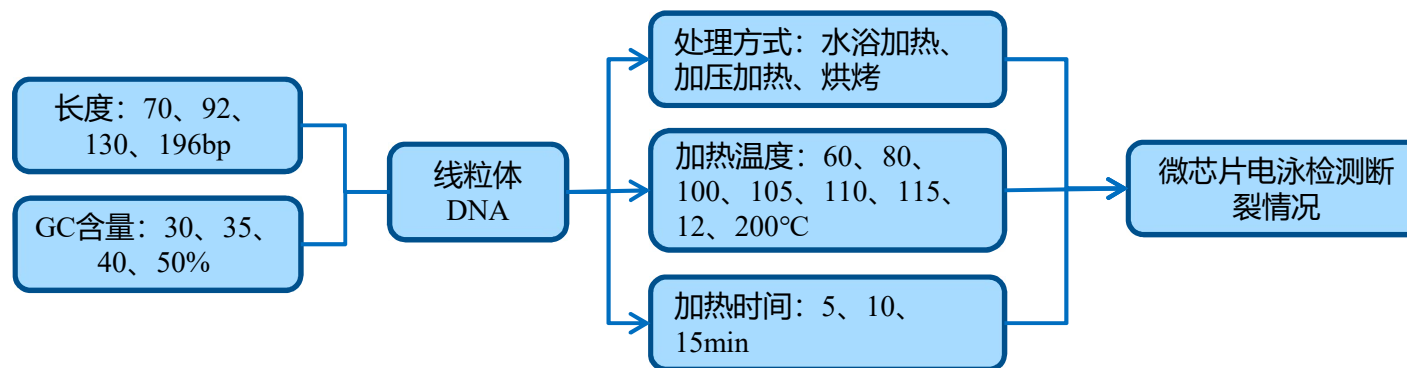
二次多项
式逐步回
归

$$\Delta Ct = -8.49 + 5.41x + 8.32y + 8.62z - 0.52x^2 - 1.26y^2 - 0.76z^2 - 0.21xy - 0.40xz - 0.62yz$$

李金春, 李家鹏*, 等. 引物3'端不同碱基错配情况下实时荧光定量PCR非特异性扩增的发生规律[J]. 食品科学, 2017, 38(10): 277-283.

线粒体DNA受热断裂量化预测模型

- 热加工会造成DNA不同程度的断裂和损伤，研究建立起温度和时间对DNA断裂的量化影响，为扩增片段长度选择提供了判断依据，对定量结果提供了修正方案。
- Heating will cause DNA breakage and damage in varying degrees. The quantitative effects of temperature and time on DNA breakage were studied and established, which provides judgment basis for the selection of amplified fragment length and a correction scheme for the quantitative results.



受热条件	受热时间	断裂主片段	断裂率
100°C	30min	无主片段	1%
105°C	30min	<196bp	55.3%
110°C	30min	<130bp	75.66%
115°C	30min	<92bp	81.98%
121°C	30min	<70bp	95.24%
200°C	30min	不可检测	100%

研发平台：构建同步检测不同物种组合的多重RT-PCR熔解曲线法

R&D platform: construct multiple RT-PCR fusion curve method for simultaneous detection of different species derived materials

```
#!/usr/bin/env python3
# -*- coding: utf-8 -*-
"""
多重RT-PCR熔解曲线法
"""
import sys
import re
import os
import argparse
import glob
import numpy as np
import pandas as pd
import matplotlib.pyplot as plt

def parse_args():
    parser = argparse.ArgumentParser()
    parser.add_argument('-f', '--file', type=str, help='Input file path')
    parser.add_argument('-o', '--output', type=str, help='Output file path')
    parser.add_argument('-c', '--cycling', type=int, help='Number of cycles')
    parser.add_argument('-t', '--temp', type=int, help='Temperature (°C)')
    parser.add_argument('-d', '--dye', type=str, help='Fluorescence dye')
    parser.add_argument('-p', '--primer', type=str, help='Primer sequence')
    parser.add_argument('-m', '--method', type=str, help='Detection method')
    parser.add_argument('-s', '--species', type=str, help='Species list')
    return parser.parse_args()

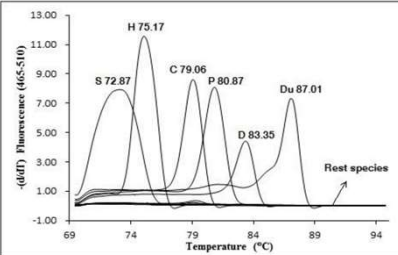
def read_file(file):
    with open(file, 'r') as f:
        return f.read()

def write_file(file, content):
    with open(file, 'w') as f:
        f.write(content)

def main():
    args = parse_args()
    # ... (rest of the code) ...
```



```
mus_mus domestica C A G A C T A C T A C G C C A A C C G C T A G T T A G G T C A A G G T G T A T A T G C C A T
bos_taurus C A G A C T A C T A C G C C A A C A C G C T A G T T A G G T C A A G G T G T A C C T A T G A T
ovis_aries C A G A C T A C T A C G C C A A C A C G C C C G G T T A G G T C A A G G T G T A C C T A T G A T
capra_hircus C A G A C T A C T A C G C C A A C A C G C C G G G T T A G G T C A A G G T G T A C C T A T G A T
ceruus_elaphus C A G A C T A C T A C G C C A A C A A G C T T A G T T A G G T C A A G G T G T A C C T A T G A T
camelus_ferus C A G A C T A C T A C G C C A A C A C G T T A G T T A G G T C A A G G T G T A C C T A T G A T
equus_asinus C A G A C T A C T A C G C C A A C A C G C T A G T T A G G T C A A G G T G T A C C T A T G A T
equus caballus C A G A C T A C T A C G C C A A C A C G C T A G T T A G G T C A A G G T G T A C C T A T G A T
oryzolagus_cuniculus C A G A C T A C T A C G C C A A C A C G C T A G T T A G G T C A A G G T G T A C C T A T G A T
canis_familiaris C A G A C T A C T A C G C C A A C A A G C T T A G T T A G G T C A A G G T G T A C C T A T G A T
vulpes_vulpes C A G A C T A C T A C G C C A A C A C G T T A G T T A G G T C A A G G T G T A C C T A T G A T
martes_sibellina C A G A C T A C T A C G C C A A C A C G C T T A G T T A G G T C A A G G T G T A C C T A T G A T
proterotes_procyonoides C A G A C T A C T A C G C C A A C A C G T T A G T T A G G T C A A G G T G T A C C T A T G A T
felis_catus C A G A C T A C T A C G C C A A C A A G C T T A G T T A G G T C A A G G T G T A C C T A T G A T
mus_musculus C A G A C T A C T A C G C C A A C A C T T A G T T A G G T C A A G G T G T A C C T A T G A T
鸡 gallus_gallus C A G A C T A C T A C G C C A A A C G C T T A G T T A G G T C A A G G T G T A C C T A T G A T
鸭 anas_platyrhynchos C A G A C T A C T A C G C C A A A C G C T T A G T T A G G T C A A G G T G T A C C T A T G A T
猪 sus_scrofa_gallica C A G A C T A C T A C G C C A A C A C G C T T A G T T A G G T C A A G G T G T A C C T A T G A T
火鸡 meleagris_gallus C A G A C T A C T A C G C C A A C A C G C T T A G T T A G G T C A A G G T G T A C C T A T G A T
鹌鹑 coturnix_japonica C A G A C T A C T A C G C C A A A C G C T T A G T T A G G T C A A G G T G T A C C T A T G A T
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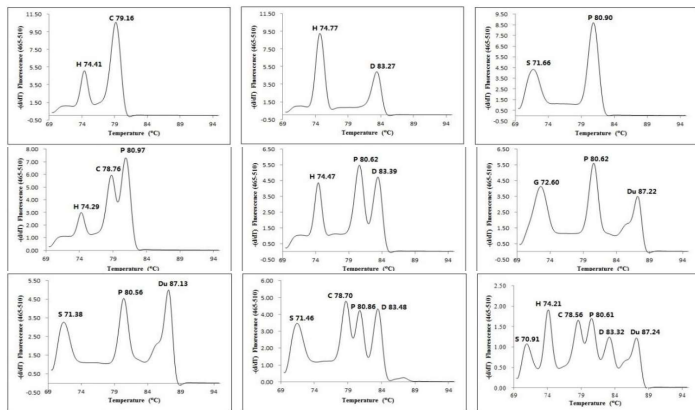


源性成分熔解峰	T _m 值范围 (°C)
山羊/绵羊	71.69 ± 0.60
马	74.47 ± 0.34
牛	78.87 ± 0.33
猪	80.75 ± 0.15
驴	83.36 ± 0.08
鸡/鸭/鹅/鸽/雁	87.16 ± 0.07

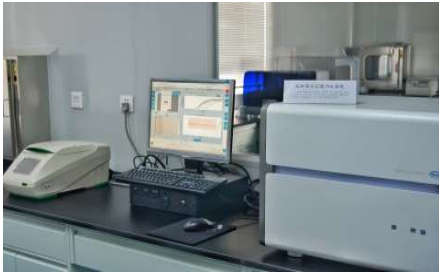
基于模型体系和DNA序列信息库的python智能算法

热力学多态性靶标DNA片段挖掘

引物设计



根据熔解曲线峰个数、位置、形状、大小判定样品中动物源性成分与配比



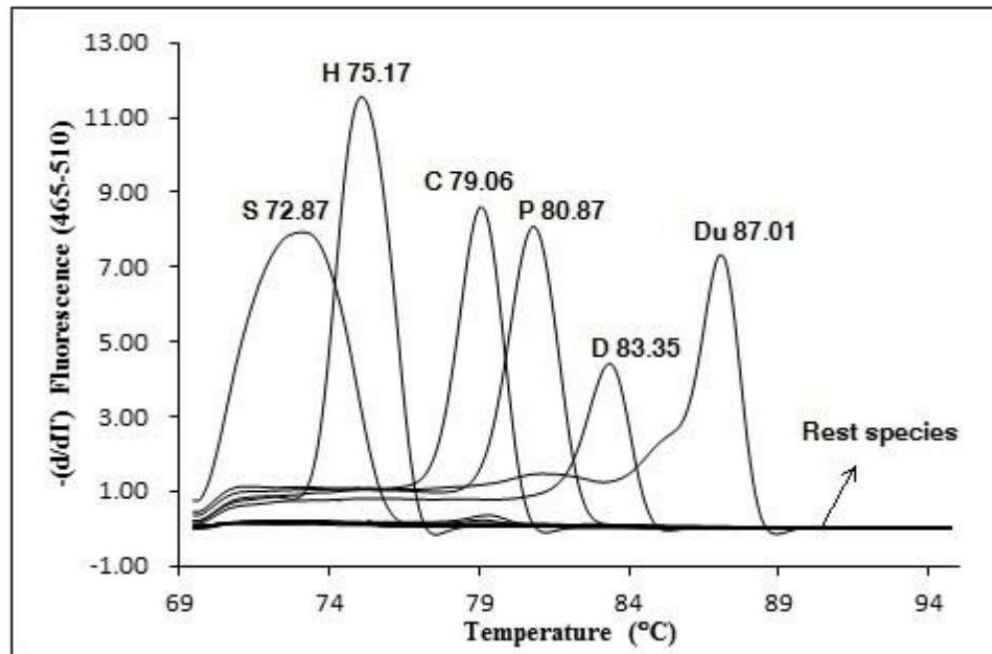
反应体系与扩增条件优化、确立

技术路线与开发流程，满足了多物种源性成分快速鉴定与筛查需求

The technical route, meet the needs of rapid identification and screening of multi species derived materials

6重RT-PCR同步检测11种肉种成分

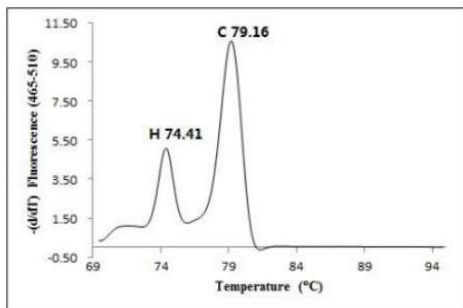
- ◆ 单次PCR反应可同步检测猪、牛、(山羊/绵羊)、马、驴、(鸡/鸭/鹅/鸽/雁...) 6类、11种以上源性成分，检出限0.1~1%。根据峰面积和峰高，可实现半定量。
- ◆ A PCR reaction can simultaneously detect 6 categories and more than 11 species of derived components from pigs, cattle, (goats / sheep), horses, donkeys and (chickens / ducks / geese / pigeons / geese...), with a detection limit of 0.1 ~ 1%. According to the peak area and peak height, semi quantification can be realized.



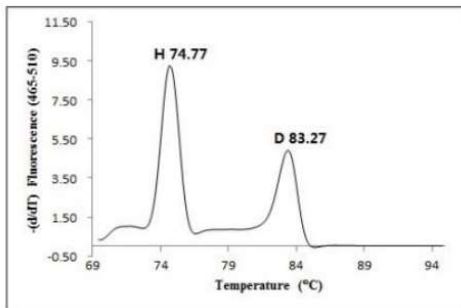
物种	T_m 值范围 (°C)
山羊/绵羊	71.69 ± 0.60
马	74.47 ± 0.34
牛	78.87 ± 0.33
猪	80.75 ± 0.15
驴	83.36 ± 0.08
鸡/鸭/鹅/鸽/雁	87.16 ± 0.07

Fig. 1 Specificity analysis of hexaplex RT-qPCR

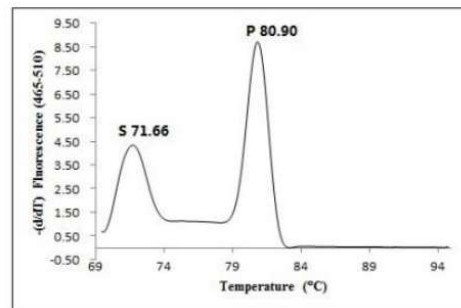
Jinchun Li, Jiapeng Li*, WANG Shouwei, et al. Identification of eleven meat species in foodstuff by a hexaplex real-time PCR with melting curve analysis, 10.1016/j.Food Control.2020.107599



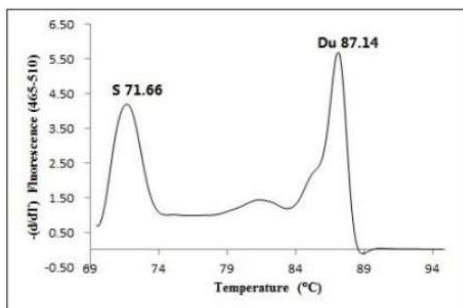
(a)



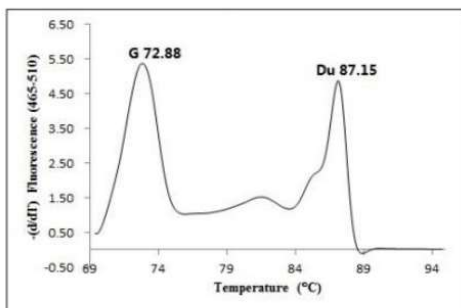
(b)



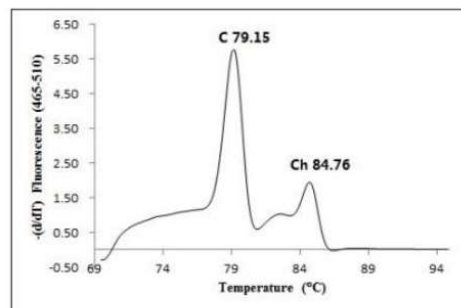
(c)



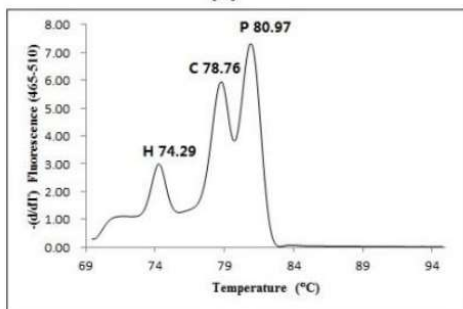
(d)



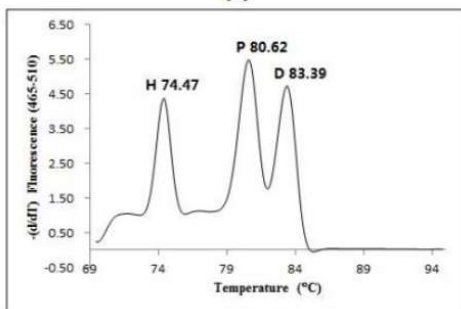
(e)



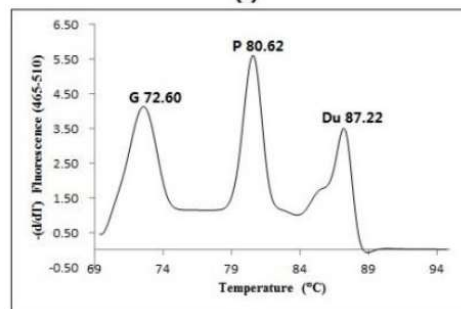
(f)



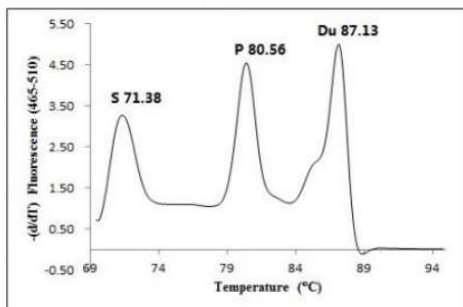
(g)



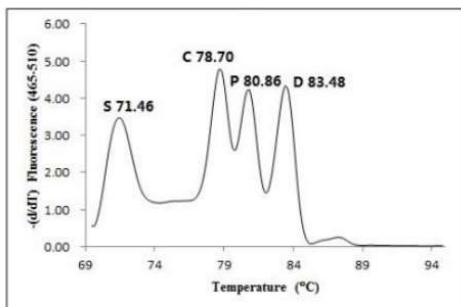
(h)



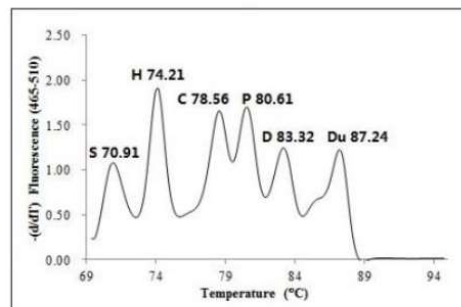
(i)



(j)



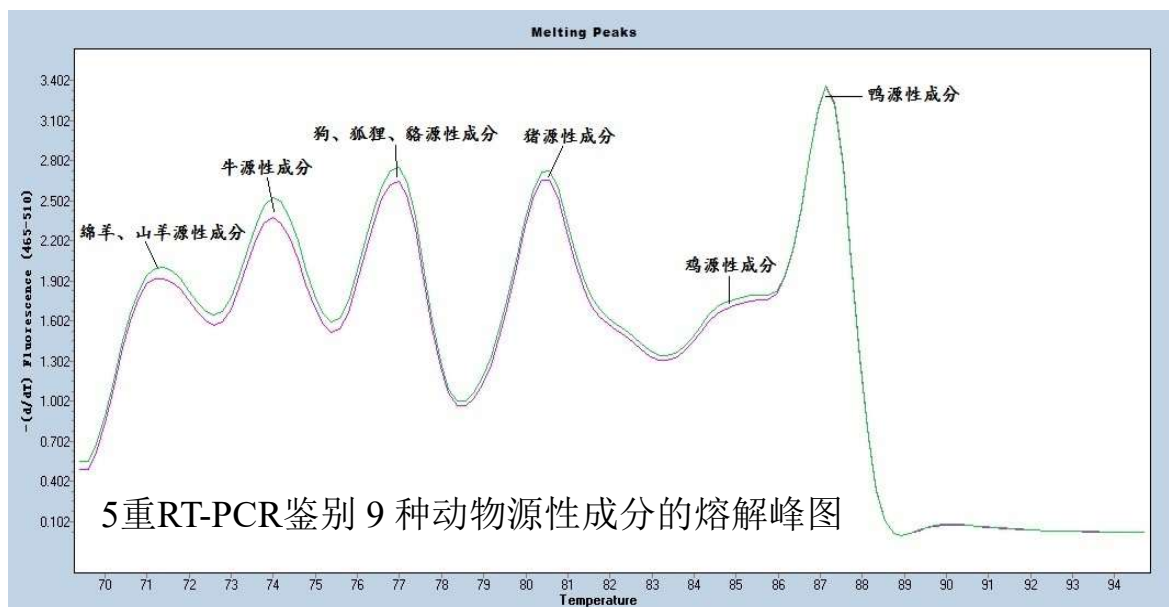
(k)



(l)

5重RT-PCR鉴别 9 种肉种成分

单个PCR反应**检测猪、牛、（山羊、绵羊）、（鸡、鸭）、（狗、狐、貉）**5类9种以上源性成分，其中猪牛用的是特异性引物，另外三个是半特异性（种类特异性）引物，检出限0.1~1%。根据峰面积和峰高，可实现半定量。

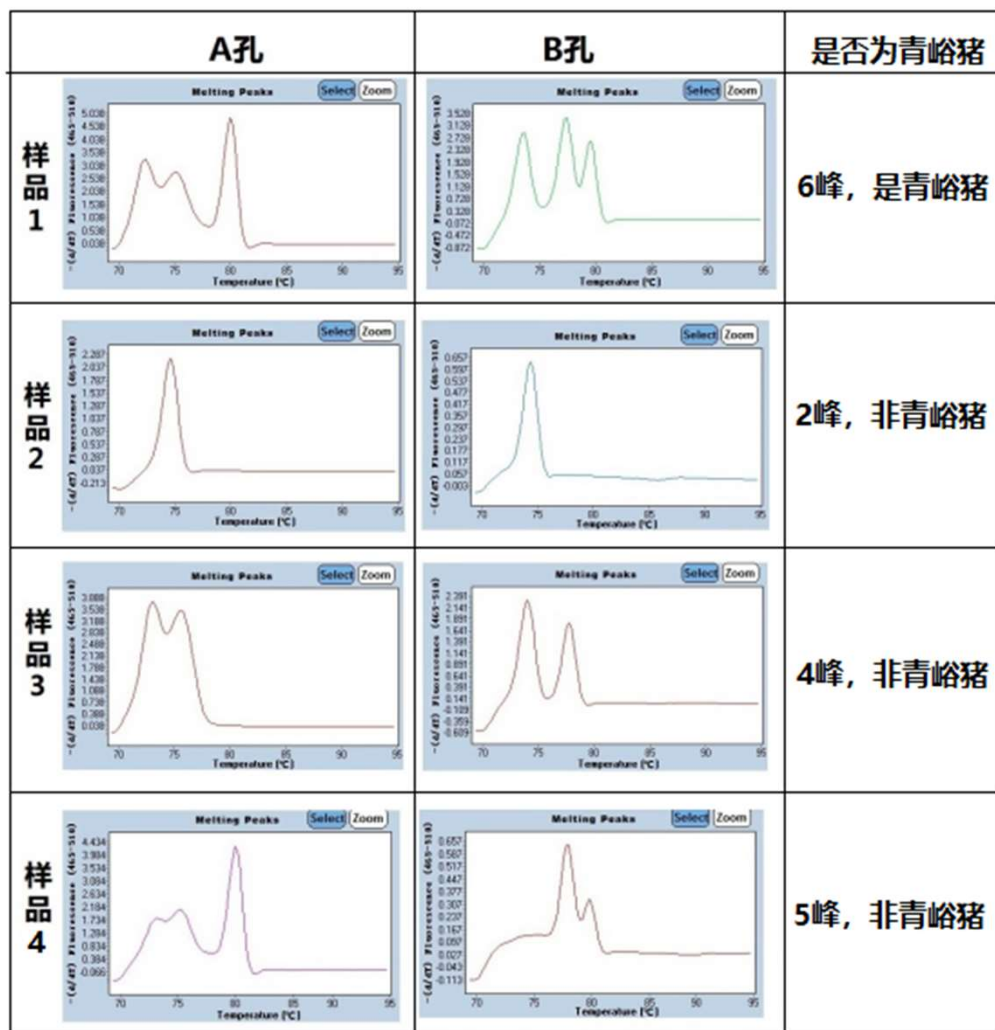


源性成分熔解峰	T_m 值范围 (°C)
绵羊	71.8 ± 0.6
山羊	73.3 ± 0.4
牛	74.7 ± 0.7
狗/狐/貉	77.2 ± 0.9
猪	80.8 ± 0.5
鸡	85.1 ± 0.4
鸭	87.4 ± 0.2

发明专利：一种鉴别肉或肉制品中动物源性成分的方法，ZL 201510061793.2

科技论文：一种基于多重实时荧光聚合酶链式反应熔解曲线分析的肉及肉制品掺假鉴别方法[J].食品科学,2017,38(12):217-222.

2*3重RT-PCR 单倍型SNP位点组合青峪猪鉴定方法



- 筛选出用于鉴定青峪猪品种的19个线粒体SNP位点，识别准确率94.57%。针对其中9个核心位点，建立起2×3重RT-PCR溶解曲线鉴别法。
- 判定方法：对应Tm值上出现6个溶解曲线峰判定为青峪猪，不足6个溶解曲线峰的判定为非青峪猪。
- 采用该方法能同步鉴定受试验品上述位点组合中的9个核心位点的基因型，进而完成对样品青峪猪或青峪猪肉真伪的鉴定。通过对126个猪肉样品进行验证，准确率达到85.71%。
- 本方法解决了肉种鉴别领域中地方特色品种鉴定难题，具有检测准确、快速、成本低的优点，对保护优质地方黑猪品牌，促进黑猪产业健康、快速发展具有重要意义。

线粒体SNP位点基因型溶解曲线峰型图
Mitochondrial SNP genotype melting curve

基于多重RT-PCR熔解曲线的多物种同步检测成果列表

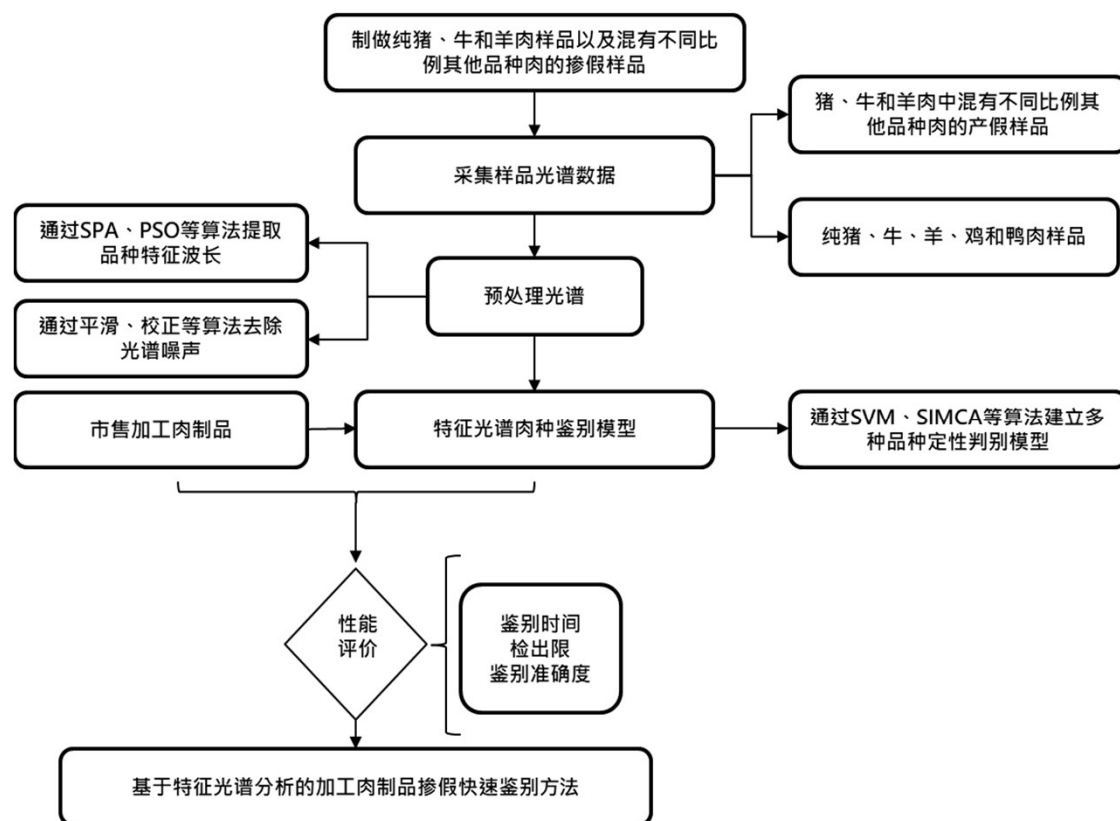
List of research achievements in multi species simultaneous detection based on multiple RT-PCR melting curve analysis

序号	技术名称	发表论文/专利	备注
1	基于 6重RT-PCR熔解曲线 分析的肉及肉制品中11种动物源性成分同步检测技术	Identification of eleven meat species in foodstuff by a hexaplex real-time PCR with melting curve analysis	《Food Control》, 2021
2	基于 5重RT-PCR熔解曲线 分析的肉及肉制品中猪、牛、绵羊、山羊、鸡、鸭、狗、狐、貉同步检测技术	一种鉴别肉或肉制品中动物源性成分的方法	ZL 201510061793.2
		一种基于多重实时荧光聚合酶链式反应熔解曲线分析的肉及肉制品掺假鉴别方法	《食品科学》, 2017
3	基于 3重RT-PCR熔解曲线 分析的蓝鳍金枪鱼、裸盖鱼、异鳞蛇鲭同步检测技术	多重实时荧光 PCR 熔解曲线法同步鉴别蓝鳍金枪鱼、裸盖鱼、异鳞蛇鲭	《食品科学》, 2020
		鉴定鱼类源性成分的引物及鉴定方法	202010586034.9
4	基于 2重RT-PCR熔解曲线 分析的牛肉制品中掺假动物源性成分筛查技术	A novel duplex SYBR Green real-time PCR with melting curve analysis method for beef adulteration detection	《Food Chemistry》, 2021
5	基于 SNP位点和多重RT-PCR熔解曲线 分析的当雄高山牦牛鉴别技术	当雄高山牦牛源性成分的鉴别方法	ZL 202010932120.0
		基于单核苷酸多态性位点的多重实时荧光定量聚合酶链式反应法鉴别当雄高山牦牛肉	《肉类研究》, 2020
6	基于 SNP位点和多重RT-PCR熔解曲线 分析的青峪猪源性成分鉴别技术	青峪猪源性成分的鉴别方法	202010932396.9
7	基于 SNP位点和多重RT-PCR熔解曲线 分析的滩羊源性成分鉴别技术	滩羊源性成分的鉴别方法	202010932153.5

2、基于近红外光谱分析的加工肉制品掺假快速鉴别技术

Rapid identification technology of adulteration of processed meat products based on near infrared spectroscopy

- 针对动物源性成分检测在**现场检测**或**在线快速检测**需求，应用**便携式近红外光谱仪**（Viavi公司Micro NIR OnSite），开发了**生鲜牛羊肉制品快速定量检测方法**。
- According to the needs of on-site detection or on-line rapid detection of animal derived components, a rapid quantitative detection method for fresh beef and mutton products was developed by using portable near infrared spectrometer (micro NIR onsite of viavi company).



技术路线 technology roadmap



便携式近红外仪
Portable near infrared instrument



混合H43儿样品 Mixed meat sample

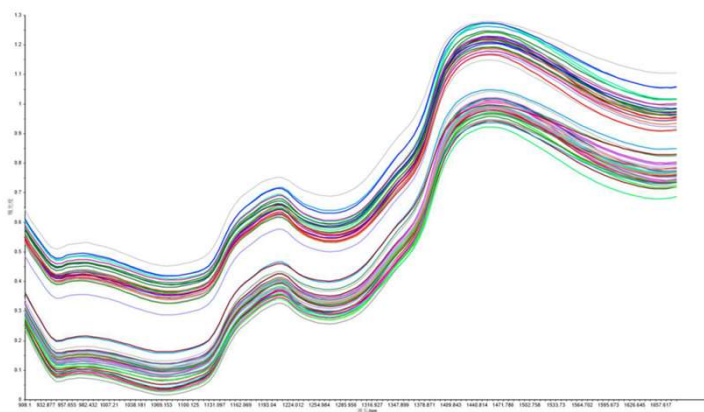
H43

把混合换为adulterated

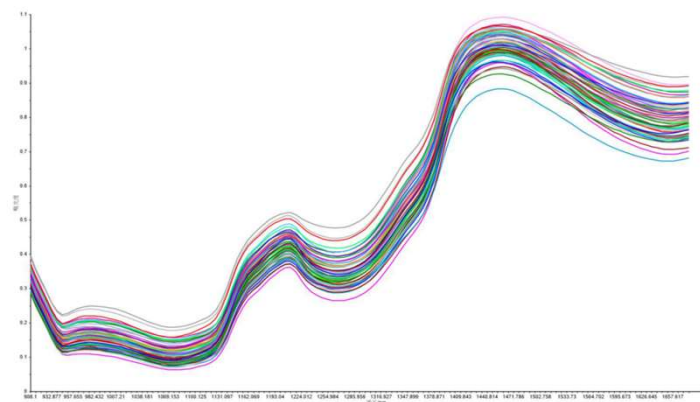
Hao, 20/10/2021

模型构建 Model building

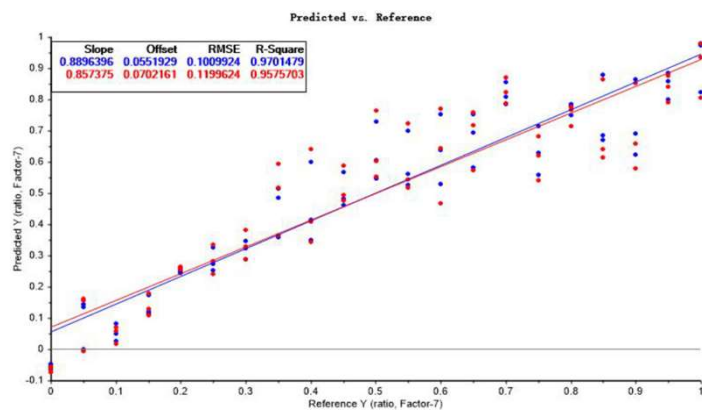
- 通过光谱预处理、主成分分析和偏最小二乘回归算法建立了常见畜禽肉掺假定量检测模型体系，覆盖猪、牛和羊肉中掺杂猪、牛、羊、鸡和鸭肉，并实现模型自适应选择。
- Through spectral preprocessing, principal component analysis and partial least squares regression algorithm, the assumed amount detection model system of common livestock and poultry meat was established, which covered the adulteration of pig, cattle, sheep, chicken and duck in pig, cattle and mutton, and the adaptive selection of the model was realized.



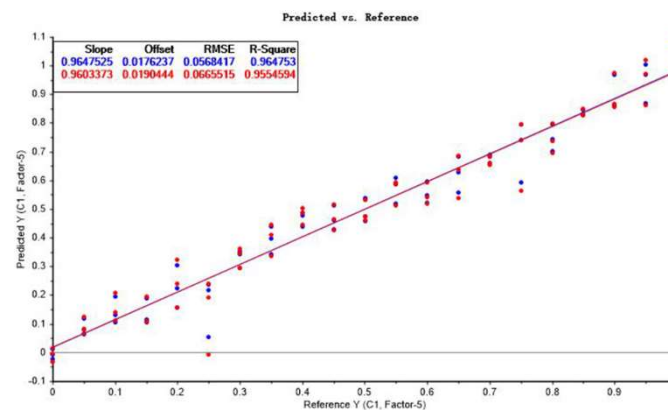
羊肉中掺鸭肉样品原始光谱图



牛肉中掺假猪肉样品原始光谱图



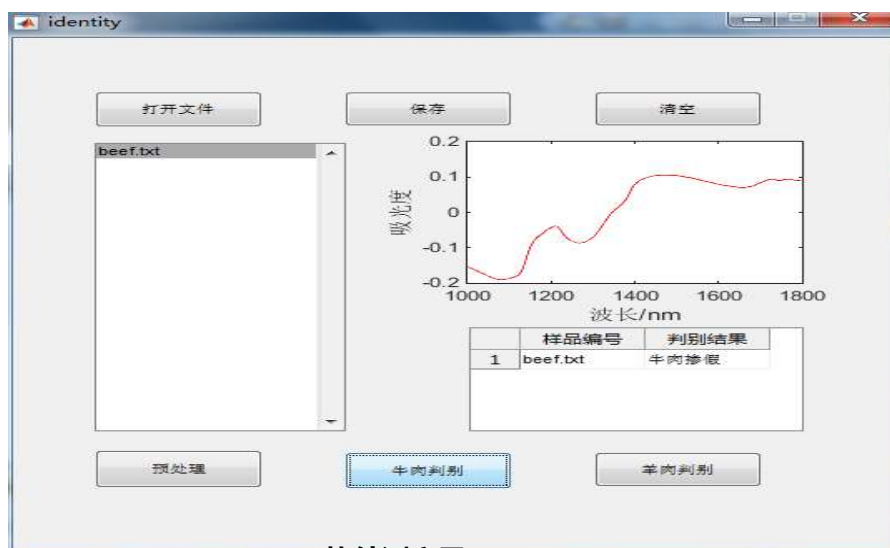
羊肉中掺鸭肉定量检测结果



牛肉中掺假猪肉定量检测结果

模型性能 Model performance

- 模型组合：12个模型校正决定系数和交叉验证决定系数均值分别达到了0.94和0.96；而交叉验证均方根误差均低于0.2，体现了方法较好的准确性和稳定性。
- The mean values of the correction determination coefficients and cross validation determination coefficients of the 12 models reached 0.94 and 0.96 respectively; The root mean square error of cross validation was less than 0.2, reflected that the method had relative good accuracy and stability.



软件测定界面

模型	校正决定系数	交叉验证决定系数	交叉验证均方根误差
羊肉中掺杂牛肉	0.9754	0.9701	0.1032
羊肉中掺杂猪肉	0.8028	0.7621	0.1613
羊肉中掺杂鸭肉	0.9701	0.9576	0.1199
羊肉中掺杂鸡肉	0.9930	0.9905	0.0567
牛肉中掺杂羊肉	0.9584	0.9348	0.1477
牛肉中掺杂猪肉	0.9648	0.9555	0.0666
牛肉中掺杂鸭肉	0.9725	0.9637	0.1103
牛肉中掺杂鸡肉	0.9814	0.9768	0.0886
猪肉中掺杂牛肉	0.9899	0.9855	0.0712
猪肉中掺杂羊肉	0.9609	0.8931	0.1896
猪肉中掺杂鸭肉	0.9566	0.9391	0.1481
猪肉中掺杂鸡肉	0.9677	0.9508	0.1295
平均值	0.9578	0.9400	0.1161

各模型的性能评价参数

科技论文:

白京, 李家鹏*, 邹昊, 等. 近红外光谱定性定量检测牛肉汉堡饼中猪肉掺假[J]. 食品科学, 2019, 40(8): 287-292.

白京, 李家鹏*, 邹昊, 等. 近红外特征光谱定量检测羊肉卷中猪肉掺假比例[J]. 食品科学, 2019, 40(2): 287-292.

软件著作权:

中国肉类食品综合研究中心肉品掺假真伪鉴别判定软件V1.0, 2020SR0295567

3、注水药物和胶体检测技术

Water injection drug and colloid detection technology

注水手段不断迭代 Continuous iteration of water injection means

- 随着注水肉监管的加强，注水手段也在不断更新迭代，越来越隐蔽。
- 第一代：单纯注水，屠宰前水管直接灌水
- 第二代：药物协助注水，注射肾上腺素促进动物吸收水分
- 第三代：复合药物+胶体协助注水，肾上腺素+阿托品等，促进吸收水分的同时缓解疼痛

- 1st generation: simple water injection, direct water injection through water pipes before slaughter
- 2nd generation: drug assisted water injection and injection of adrenaline to promote the absorption of water by animals
- 3rd generation: compound drug injection, epinephrine + atropine, etc. to promote water absorption and relieve pain at the same time



农业农村部公布农产品质量安全执法监管十大典型案例

日期：2018-07-10 13:45

作者：

来源：农业农村部新闻办公室

【字号：大 中 小】

打印本页

本网讯 记者从农业农村部获悉，近年来，各地农业农村部门认真履职、主动出击，围绕农兽药残留、非法添加、违禁使用、私屠滥宰及注水和注入其他物质等突出问题，坚持问题导向，加大巡查检查和监督抽查力度，实行最严格的监

四、安徽省霍邱县畜牧兽医局查处王某某等人向生猪注药、注水案

2016年，安徽省霍邱县畜牧兽医局联合县公安局、市场监管局根据群众举报，经2个月的暗访蹲守，成功端掉一个给待宰生猪注药、注水窝点。执法人员现场查获生猪29头，盐酸异丙嗪7支，无名药水1瓶及作案工具若干。经查，该窝点负责人王某某伙同张某某等人，于2016年7月至9月期间，贩购生猪后注射药物并注水，检测其所注入的无色液体以及生猪尿液中含非食品原料肾上腺素，案件随后移交公安机关查处。2018年5月，王某某、张某某二人犯生产、销售有毒、有害食品罪，一审判判处有期徒刑一年二个月，并处罚金人民币1万元，查扣在案的猪肉4780公斤予以没收、销毁。

常见注水药物种类 Types of common water injection drugs

- ◆ 根据风险交流平台情报、市场调研及大样本分析结果，确定出靶标药物种类：包括 阿托品、利多卡因、普鲁卡因、山莨菪碱、东莨菪碱、沙丁胺醇。
- ◆ According to the information of risk exchange platform, market research and large sample analysis results, the types of target drugs are determined, including atropine, lidocaine, procaine, anisodamine, scopolamine and salbutamol.

药品名称 Drug name	分子式 Molecular formula	功能	在注水中的作用
阿托品 Atropinol Atropine	$C_{17}H_{23}NO_3$	M-受体阻断剂，适用于缓解内脏绞痛。 M-receptor blocker, suitable for relieving visceral colic.	<ul style="list-style-type: none"> ■ 动物体注水以后会产生应激反应，诸如利尿、血管扩张、身体疼痛等反应。 ■ 这些药物协同作用下具有舒缓血管扩张，减少动物体疼痛感觉、减少排尿的作用来达到保水的作用。 ■ After water injection, animals will produce H50s reactions, such as diuresis, vasodilation, physical pain and so on. ■ Under the synergistic action of these five drugs, it can relieve vasodilation, reduce animal pain and reduce urination, so as to achieve the effect of water retention.
山莨菪碱 Anisodamine	$C_{17}H_{23}NO_4$	神经系统药物，用于胃肠道痉挛引起的绞痛。 Nervous system drugs for colic caused by gastrointestinal spasm.	
东莨菪碱 Scopolamine	$C_{17}H_{21}NO_4$	莨菪烷型生物碱，缓解胃肠道痉挛。 Anisodamine alkaloid can relieve gastrointestinal spasm.	
普鲁卡因 Procaine	$C_{13}H_{20}N_2O_2$	局部麻醉药，主要用于浸润麻醉。 Local anesthetic, mainly used for infiltration anesthesia.	
沙丁胺醇 salbutamol	$C_{13}H_{21}NO_3$	β_2 肾上腺素能受体激动剂，防止支气管痉挛 β_2 adrenergic receptor agonists, prevent bronchospasm	
利多卡因 Lidocaine	$C_{14}H_{22}N_2O$	局部麻醉药，用于缓解急性室性心律失常。 A local anesthetic used to relieve acute ventricular arrhythmias.	

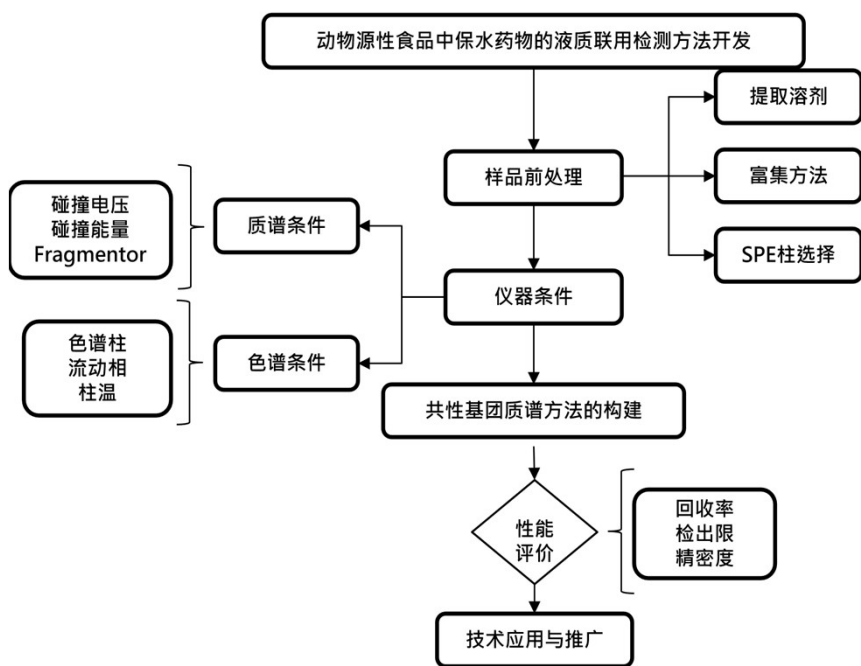
H50

have

Hao, 20/10/2021

肉类食品保水剂的高通量同步检测技术

- 开发出能够同步定性定量检测上述药物分子的高效液相色谱串联三重串联四级杆质谱方法。
- A high performance HPLC-MS method capable of simultaneous qualitative and quantitative detection of the above drug molecules was developed.



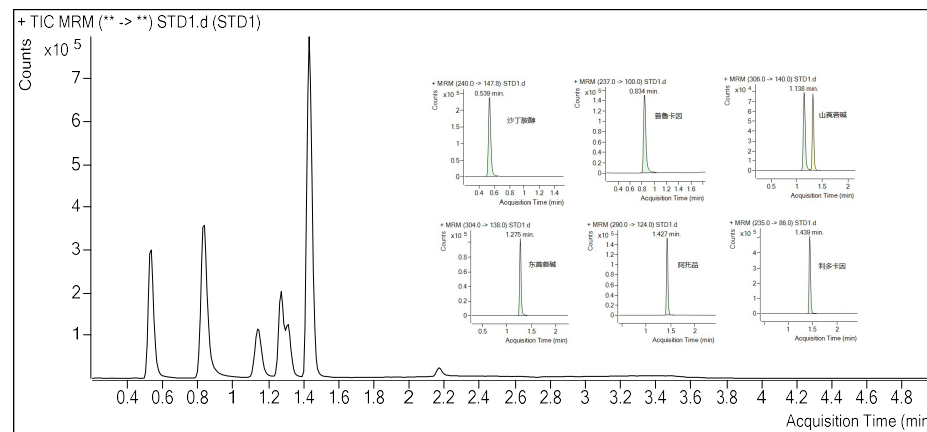
技术路线 Technology roadmap

化合物	母离子	子离子	毛细管电压	碰撞能量
沙丁胺醇	240.0	147.8*	90	25
		222.0	90	15
普鲁卡因	237.0	100*.0	80	20
		120.0	80	20
山莨菪碱	306.0	140.0*	80	20
		122.0	80	25
东莨菪碱	304.0	138.0*	80	20
		156.0	80	18
阿托品	290.0	124.0*	80	22
		93.0	80	30
利多卡因	235.0	86.0*	80	20
		58.1	80	40

*为定量离子。

6种药物的多反应监测质谱参数

Multi reaction monitoring mass spectrometry parameters of six drugs



总离子图

Total ion diagram

方法性能

- 沙丁胺醇、阿托品、利多卡因、普鲁卡因、山莨菪碱、东莨菪碱的检出限为0.5 $\mu\text{g}/\text{kg}$ 。
- 各种化合物的回收率在 75.5% 到 114.5% 之间, 精密度的 RSD 值在 0.5%到 7.4%之间。
- the detection limit of salbutamol, atropine, lidocaine, procaine, anisodamine and scopolamine were 0.5 $\mu\text{g}/\text{kg}$.
- The recoveries of various compounds ranged from 75.5% to 114.5%, and the RSD values of precision ranged from 0.5% to 7.4%.

化合物	线性方程	相关系数	检出限 ($\mu\text{g}/\text{kg}$)	定量限 ($\mu\text{g}/\text{kg}$)
沙丁胺醇	$y=90035x+131687$	0.9994	0.5	2.0
普鲁卡因	$y=71834x-78157$	0.9910	0.5	2.0
山莨菪碱	$y=24742x+97219$	0.9992	0.5	2.0
东莨菪碱	$y=29292x+84895$	0.9991	0.5	2.0
阿托品	$y=37150x+127088$	0.9996	0.5	2.0
利多卡因	$y=163369x+69479$	0.9970	0.5	2.0

六种药物定量检测的相关系数、检出限和定量限

Correlation coefficient, detection limit and quantitative limit of six drugs

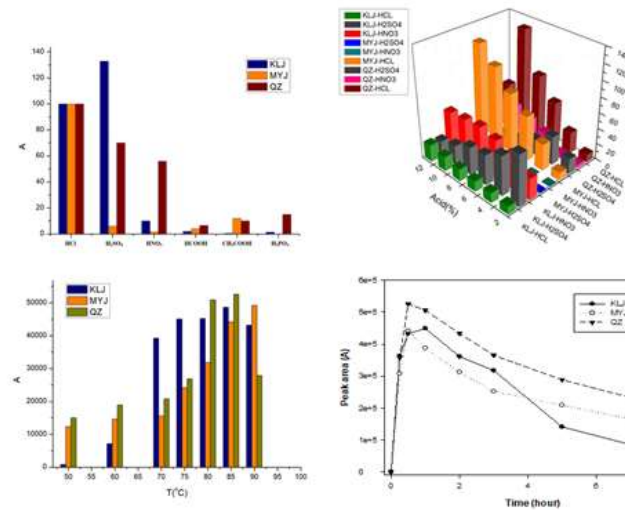
化合物	添加量 ($\mu\text{g}/\text{kg}$)	回收率 (%)		RSD (%)	
		猪肉	牛肉	猪肉	牛肉
沙丁胺醇	6.5	78.07~79.77	80.32~82.50	0.9	1.2
	25	86.39~88.99	86.48~89.52	1.1	1.9
	50	84.87~87.40	83.15~84.21	1.1	0.7
普鲁卡因	6.5	112.1~114.5	101.2~103.5	0.8	0.6
	25	79.07~80.97	88.45~90.21	0.9	1.1
	50	87.23~89.54	86.25~87.89	0.9	0.5
山莨菪碱	6.5	87.94~90.83	89.32~91.22	1.3	1.1
	25	82.86~84.40	90.26~92.54	0.7	0.9
	50	85.44~88.65	87.32~89.30	1.4	1.1
东莨菪碱	6.5	86.19~90.96	91.15~93.20	2.1	1.3
	25	84.83~86.36	86.25~88.29	0.7	0.9
	50	84.36~87.10	80.35~83.96	1.1	1.3
阿托品	6.5	93.20~96.76	82.32~85.18	1.4	1.5
	25	84.86~87.10	89.45~90.25	0.9	0.5
	50	85.11~87.75	86.02~87.11	1.1	0.7
利多卡因	6.5	101.7~103.8	96.35~97.92	0.7	0.6
	25	80.98~81.80	88.20~90.15	0.4	1.0
	50	86.21~88.76	84.25~86.18	1.1	1.1

六种药物不同添加量的回收率评价

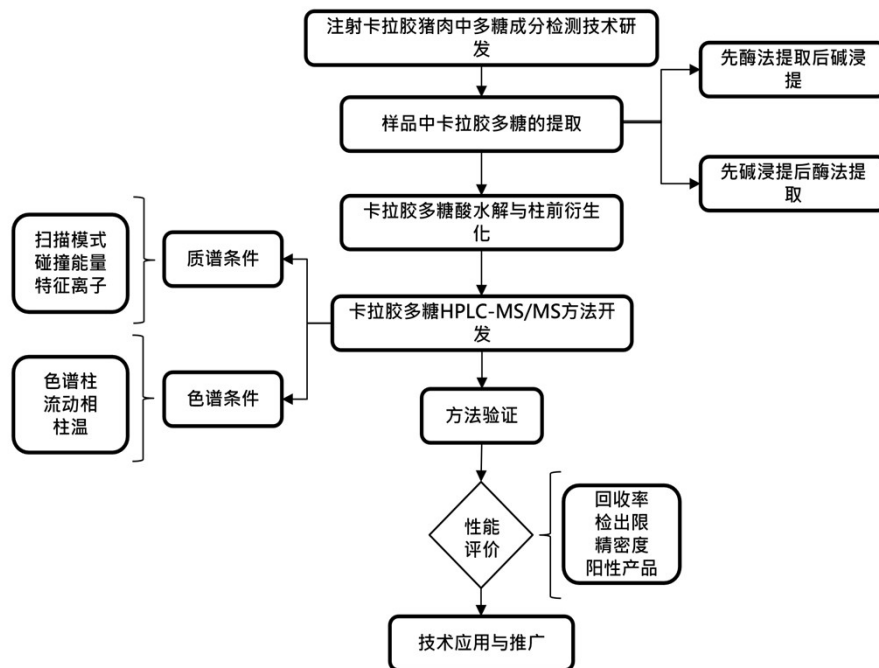
科技论文: 李志刚, 李慧晨, 马燕红, 等. 高效液相色谱-串联质谱法检测动物源性食品中异丙嗪[J].食品科学, 2019, 40(24): 320-324.
发明专利: 一种基于LC-MC/MS同时测定肾上腺素及其代谢产物的方法, 已受理, 申请号, 201910989630.9

注射卡拉胶猪肉中多糖成分检测技术

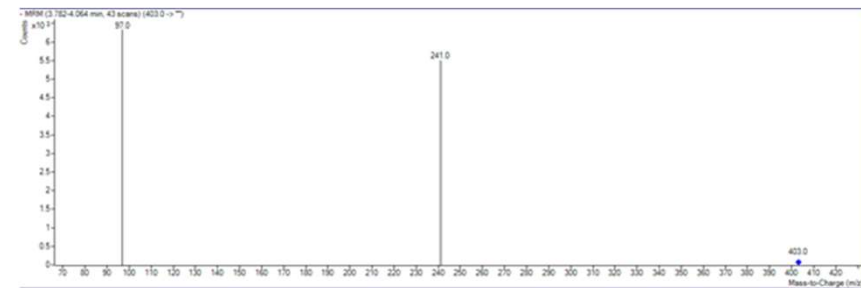
- 通过酸水解的方法将水溶性胶体水解为质谱可以检测的特征性小分子，建立能同步检测样品中卡拉胶、魔芋胶及琼脂三种多糖成分的高效液相色谱串联三重串联四级杆质谱方法。
- The water-soluble colloid was hydrolyzed into characteristic small molecules that could be detected by mass spectrometry by acid hydrolysis. A high performance liquid chromatography tandem triple tandem four-stage rod mass spectrometry method was established for the simultaneous detection of carrageenan, konjac gum and agar.



不同胶体水解条件的优化
Optimization of hydrolysis conditions of different colloids



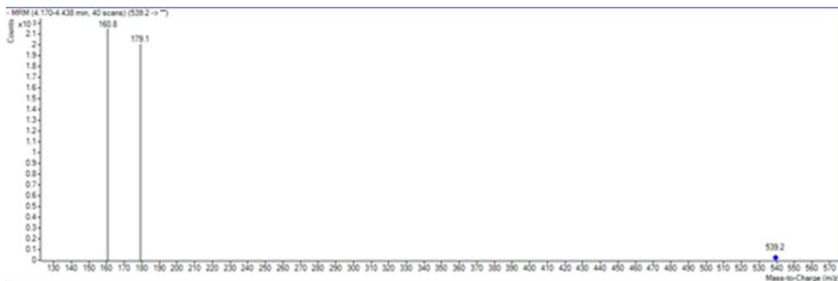
技术路线 Technology roadmap



魔芋胶水解产物MRM质谱图
MRM mass spectrum of konjac gum hydrolysate

方法性能

- 卡拉胶、魔芋胶及琼脂进行检测的回收率范围在80.5%到105.7%之间
- 检出限和定量限分别为25 $\mu\text{g}/\text{kg}$ 和100 $\mu\text{g}/\text{kg}$ 。
- The recoveries of carrageenan, konjac gum and agar ranged from 80.5% to 105.7%
- The detection limit and quantitation limit were 25, respectively $\mu\text{G}/\text{kg}$ and 100 $\mu\text{G}/\text{kg}$.



发明专利：基于液质联用检测动物源性食品中亲水性胶体的方法，已进入实质审查，申请号：201811437066.1

化合物	添加浓度	实测浓度	回收率	平均回收率	相对标准偏差
卡拉胶	50 $\mu\text{g}/\text{mL}$	49.53 $\mu\text{g}/\text{mL}$	99.1%	103.6	4.5
		54.21 $\mu\text{g}/\text{mL}$	108.4%		
		51.62 $\mu\text{g}/\text{mL}$	103.2%		
魔芋胶	50 $\mu\text{g}/\text{mL}$	41.86 $\mu\text{g}/\text{mL}$	83.7%	87.6	4.8
		46.03 $\mu\text{g}/\text{mL}$	92.1%		
		43.55 $\mu\text{g}/\text{mL}$	87.1%		
琼脂	50 $\mu\text{g}/\text{mL}$	52.83 $\mu\text{g}/\text{mL}$	105.7%	97.2	8.5
		44.59 $\mu\text{g}/\text{mL}$	89.2%		
		48.38 $\mu\text{g}/\text{mL}$	96.8%		

高浓度卡拉胶、魔芋胶及琼脂的回收率评价

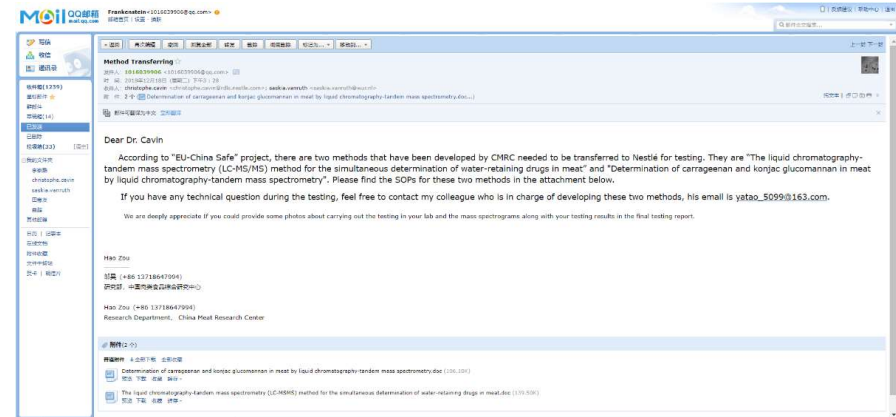
化合物	添加浓度	实测浓度	回收率	平均回收率	相对标准偏差
卡拉胶	10 $\mu\text{g}/\text{mL}$	9.05 $\mu\text{g}/\text{mL}$	90.5%	97.3%	7.1
		10.43 $\mu\text{g}/\text{mL}$	104.3%		
		9.70 $\mu\text{g}/\text{mL}$	97.0%		
魔芋胶	10 $\mu\text{g}/\text{mL}$	8.26 $\mu\text{g}/\text{mL}$	82.6%	84.3%	5.8
		8.97 $\mu\text{g}/\text{mL}$	89.7%		
		8.05 $\mu\text{g}/\text{mL}$	80.5%		
琼脂	10 $\mu\text{g}/\text{mL}$	10.52 $\mu\text{g}/\text{mL}$	105.2%	101.6%	3.9
		10.22 $\mu\text{g}/\text{mL}$	102.2%		
		9.74 $\mu\text{g}/\text{mL}$	97.4%		

低浓度卡拉胶、魔芋胶及琼脂的回收率评价

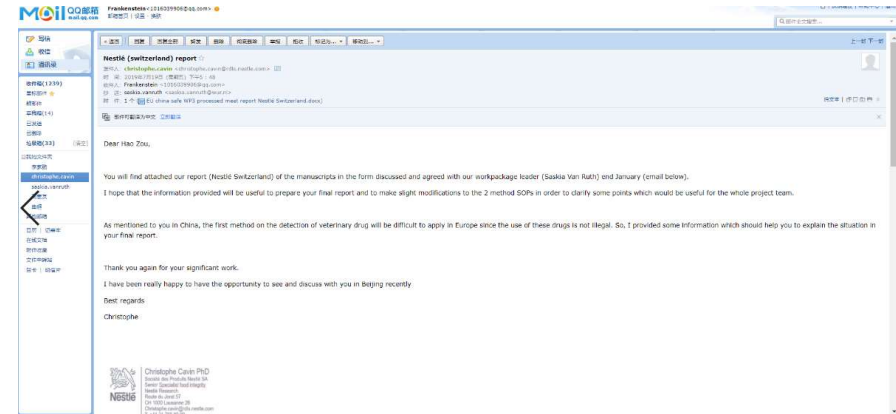
- 上述两种方法都以SOP的形式，输出给雀巢（欧洲）
- The above two methods are exported to Nestle (Europe) in the form of SOP



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 M.+41 79 333 11 75



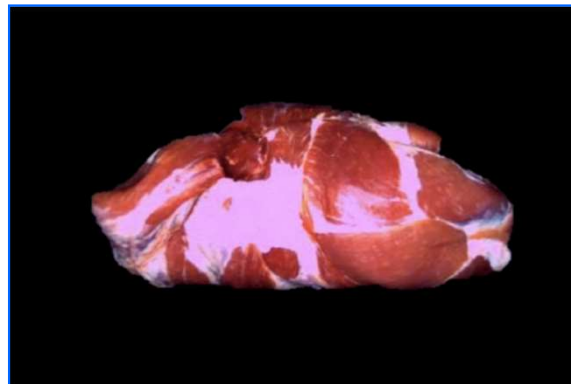
发件人	主题	日期	状态
christophe.cavin	RE: Merry Christmas and Happy New Year - Dear Hao, I am fine Thank you ! I wish you also a merry X-mas and all the best for 2020 Greetings C	2019/12/24	☆
christophe.cavin	Merry Christmas and Happy New Year	2019/12/24	☆
christophe.cavin	EU-China Safe Report draft	2019/09/11	☆
christophe.cavin	Automatic reply: 回复: Nestlé (switzerland) report - I am out of the office with no access to my emails until Tuesday 6th of August	2019/07/22	☆
christophe.cavin	回复: Nestlé (switzerland) report	2019/07/22	☆
christophe.cavin	Nestlé (switzerland) report - Dear Hao Zou, You will find attached our report (Nestlé Switzerland) of the manuscripts in the for	2019/07/19	☆
christophe.cavin	Method evaluation	2019/05/15	☆
christophe.cavin	回复: 回复: RE: Testing for transferred methods	2019/01/10	☆
christophe.cavin	RE: 回复: RE: Testing for transferred methods - Dear Dr Hao, Thank you for your complementary information. I am not totally clear on the meat piece	2019/01/08	☆
christophe.cavin	回复: RE: Testing for transferred methods	2019/01/08	☆
christophe.cavin	Re: RE: Testing for transferred methods	2019/01/04	☆
christophe.cavin	RE: Testing for transferred methods - Dear Dr Zou, Many thanks for your message and Happy New Year 2019 to you. I tried to contact you an	2019/01/04	☆
christophe.cavin	Automatic reply: Testing for transferred methods - I am out of the office until January 7th	2019/01/03	☆
christophe.cavin	Testing for transferred methods	2019/01/03	☆
christophe.cavin	Validation files - Dear Dr Zou, Thank you for having transferred your methods SOPs. Just to follow the EU-China Safe p	2018/12/19	☆
christophe.cavin	Method Transferring	2018/12/18	☆
christophe.cavin	回复: 回复: RE: methods transferring	2018/12/11	☆
saskia.vanruth	回复: RE: methods transferring	2018/12/11	☆
christophe.cavin	RE: methods transferring - Dear Saskia, Thank you for your e-mail. In fact, I didn't receive any feedback to my e-mail from N	2018/12/10	☆
christophe.cavin	RE: methods transferring - Dear Dr Zou, Sorry for my late reply, I was out of office with limited access to my emails. Could y	2018/11/26	☆
christophe.cavin	Automatic reply: methods transferring - I am currently out of the office with limited access to my emails until November 19	2018/11/15	☆
christophe.cavin	methods transferring	2018/11/15	☆
christophe.cavin	Re: RE: Task 3.1.3 processed meat/question	2018/02/11	☆
christophe.cavin	RE: Task 3.1.3 processed meat/question - Dear Dr Zou, Thank you for your feedback. We have never heard about such practices so, do you have	2018/02/06	☆
christophe.cavin	Re: Task 3.1.3 processed meat/question	2018/02/05	☆



4、基于机器视觉的冷冻肉冻龄识别技术

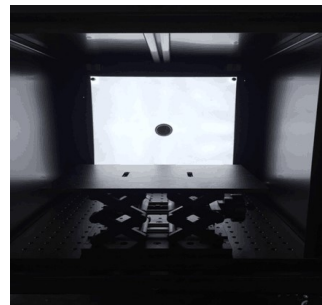
Discrimination technology of frozen meat storage time based on machine vision

- ◆ 开发冷冻猪肉图像自动采集系统和智能算法，研制出基于机器视觉的冷冻猪肉冻龄识别装置，解决了冷冻猪肉冻藏时间无法鉴别的难题。
- ◆ The automatic image acquisition system and intelligent algorithm of frozen pork were developed, and the frozen age recognition device of frozen pork based on machine vision was developed, which solved the problem that the frozen storage time of frozen pork can not be identified.



方法性能

- ◆ **原理：应用颜色空间转换、形态学分割及颜色特征抽提技术，挖掘出样品在不同颜色空间下的特征均值、红度差值与冻藏时间的相关性，可预测该样品的冷冻周期，模型 $R^2=0.91$ ，对于冻藏6个月以上的样品预测准确率可达到96.3%。**
- ◆ **By using color space conversion, morphological segmentation and color feature extraction technology, the correlation between the characteristic mean value, redness difference and frozen storage time of the sample in different color space can be excavated, and the freezing cycle of the sample can be predicted. The model $R^2 = 0.91$. For samples frozen for more than 6 months, the prediction accuracy can reach 96.3%.**



图像采集箱



猪肉样品

分段
阈值

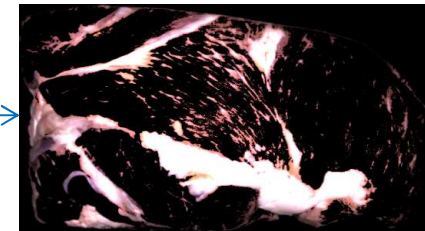
分量
比值

形态学
运算

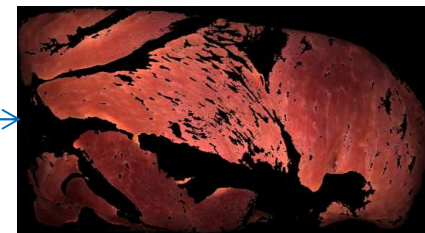
掩模
重建

样品中兴趣区域的分割

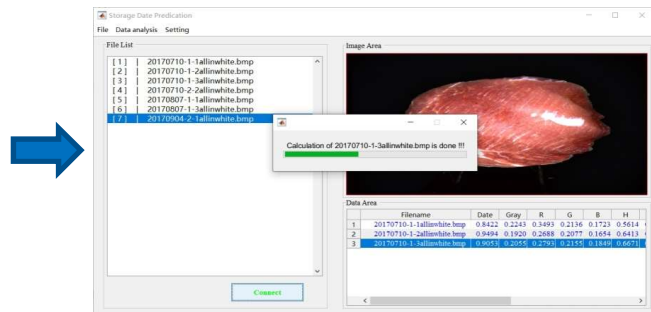
智能算法



脂肪区域



肌肉区域



计算结果

发明专利：冷冻肉新鲜程度的评估方法及系统， ZL 201510874182.X；一种冷冻畜肉储存周期预测装置及方法，申请号：201811641009.5

软件著作权：生鲜和冷冻畜肉色泽判定评价软件，2016SR027791

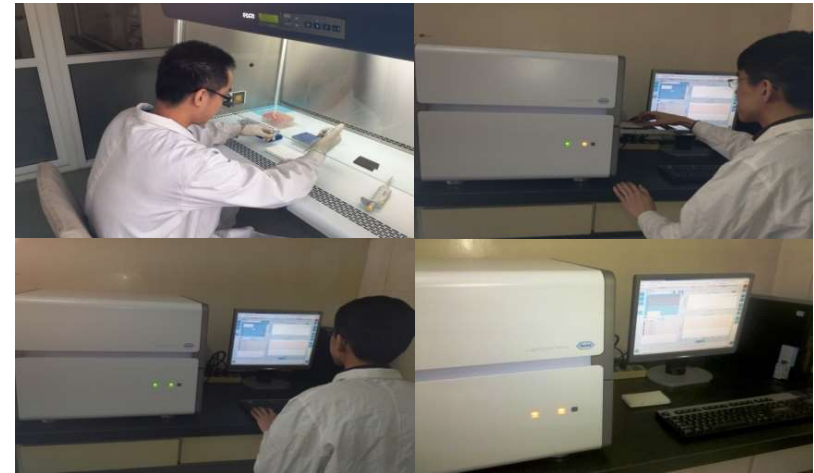
科技论文：颜色空间的冷冻猪肉储藏时间机器视觉判定，农业工程学报，2019，35(3)

三、技术应用 Technology Application

- 上述技术得到了很好的应用，为**食品监管机构、公安部门、肉制品企业、大型商超、电商平台以及消费者**都提供了样品检测服务和技术支持、为食品安全监管、企业和商超对原料和产品的内部质控等作出了贡献。
- The above technologies have been well applied, providing sample testing services and technical support for food regulatory agencies, public security departments, meat products enterprises, large supermarkets, e-commerce platforms and consumers, and contributing to food safety supervision, enterprises and supermarkets' internal quality control of raw materials and products.



肉类食品真伪与掺假检测实验室



工作照片

三、技术应用 Technology Application



部分送检样品 some samples for detection

委托检验协议		CMRCSYSS-9-5	
编号: 20190824	承检方: 北京肉类食品质量监督管理局	委托方: 沧县公安局	地址: 北京市丰台区洋桥70号
名称: 猪肉松	规格/等级: 散装	生产日期: 20190724	样品状态: 散装
检验项目: 菌落总数, 大肠菌群, 金黄色葡萄球菌, 沙门氏菌, 霉菌和酵母, 肠杆菌, 水分, 酸价, 过氧化值, 挥发性盐基氮, 挥发性盐基氮, 挥发性盐基氮, 挥发性盐基氮	检验依据: 委托方提供方法	保存条件: 常温	其他(请注明):
报告格式: 纸质	报告份数: 1份	交付日期: 1个工作日内	备注: 委托方提供方法

中国肉类食品综合研究中心		委托检验协议	
1. 委托方	联系人: 姜玉坤	电话: 18010776269	传真:
2. 被委托方	地址: 北京市丰台区洋桥70号	邮编: 100068	电话: 010-67215033
3. 样品信息	样品名称: 五香牛肉	规格/等级: /	保存条件: 常温
4. 检验项目	牛肉内源性分析(定量)		
5. 检验依据	委托方提供方法		
6. 报告要求	报告格式: 纸质	报告份数: 1份	交付日期: 1个工作日内

国家市场监督管理总局
委托检验检测项目

2018 年生猪屠宰注药注水问题排查
专项抽检委托检验项目
合同书

市场总局任务委托书 Dection tasks from SAMR

技术成果应用证明	
技术名称	肉品真实性高效鉴别技术研究与应
技术开发单位	中国肉类食品综合研究中心
应用单位	江苏雨润食品有限公司
应用成果起止时间	2019年2月-至今
应用单位地址与邮编	江苏省南京市浦口区紫云岭19号 (211806)
联系人电话	025-56677181

应用证明	
“肉品真实性高效鉴别技术研究与应”是中国肉类食品综合研究中心通过参与政府国际科技创新合作重点专项项目“中欧食品安全合作 H2020 EU-China-Safe” (2017YFE0110800) 取得的标志性成果。	
本公司自 2019 年起, 陆续使用该成果技术, 用于原料和产品质量控制, 成果内容包括: (1) “肉类食品保水剂的高通量同步检测技术”, 实现对猪肉内源性成分定量检测, 方法检出限和定量限分别为 0.5 μg/kg 和 2.0 μg/kg, 回收率在 78.07% 到 114.5% 之间; (2) “注射卡拉胶猪肉中多糖成分检测技术”, 实现对猪肉中卡拉胶等多糖成分的定性定量检测, 方法检出限为 25 μg/kg, 回收率在 81.62%~106.3% 范围内; (3) “基于多重 RT-PCR 熔解曲线分析的多物种同步定量检测技术”, 实现同步定量检测 11 种物种, 相对检出限可达到 0.1%; (4) “基于特征光谱分析的加工肉制品快速鉴别技术”, 实现对猪、牛和羊肉制品中添加剂的猪、牛、羊、鸡和鸭肉成分的定性和定量检测, 定性预测模型准确率均达到 95% 以上, 定量预测模型决定系数均达到 0.9 以上, 范围误差比均高于 2.5, 线性范围 0~100%, 检测时间小于 10s。	
特此证明。	

为沧县公安局、老爸评测等任务委托单
Detection tasks from Public Security Bureau and e-commerce platform

雨润集团和大红门集团应用证明
Technical application certificate from large meat Enterprises

四、技术展望 Technology Outlook

下一步研究计划 Research plan

- 进一步丰富技术、方法体系、适应不同的使用需求和场景；
- 构建多技术、多维度的肉类食品真实性检验的技术体系；
- 进一步扩大检测物种数量、提高定量精度、降低检测成本；
- 构建肉类食品成分真实性数据库。

- Further enrich the technology and method system to adapt to **H59**ferent use needs and scenarios;
- Build a multi-technical and multi-dimensional technical system for meat food **H57** authenticity inspection;
- Further expand the number of detection species, improve the quantitative accuracy and reduce the detection cost;
- Construct the authenticity database of meat food **H58**redients.

Slide 32

- H57** **建议删除food**
Hao, 20/10/2021
- H58** **删除food**
Hao, 20/10/2021
- H59** **删除to**
Hao, 20/10/2021

治理对策建议 Regulatory recommendations

- 完善标准体系，优化标准制修订流程；
- 制定基础性、性能参数要求性标准，加快技术与方法的创新与应用进程；
- 加强产品标准与检测标准的协同，对掺假严重情况进行分级管理；
- 加大肉制品及食品掺假风险监控力度，建立企业、平台的信用评分体系。

- Improve the standardization system and optimize the standard development and revision process;
- Develop basic and performance parameter requirements standards, and speed up the innovation and application process of technologies and methods;
- Strengthen the coordination between product standards and testing standards, and carry out hierarchical management based on the severity of the adulteration;
- Strengthen the risk monitoring of meat products and food adulteration, and establish a credit scoring system for enterprises and platforms.



THANKS

