荷兰乳品生产安全质量控制体系/
免疫球蛋白及功能性肽检测方法浅析

于　伟

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2013. 11. 21

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Part 1

荷兰乳品生产质量安全控制体系/The quality and safety control of milk product in Holland

Part 2

免疫球蛋白及功能性肽检测方法研究/The test method research on Ig and functional peptide

Part 1—Dairy industry in Holland

20.000 Dairy Farms
11 billion kg milk

⇩

30 Collectors of farm milk
(mostly departments of dairy companies)

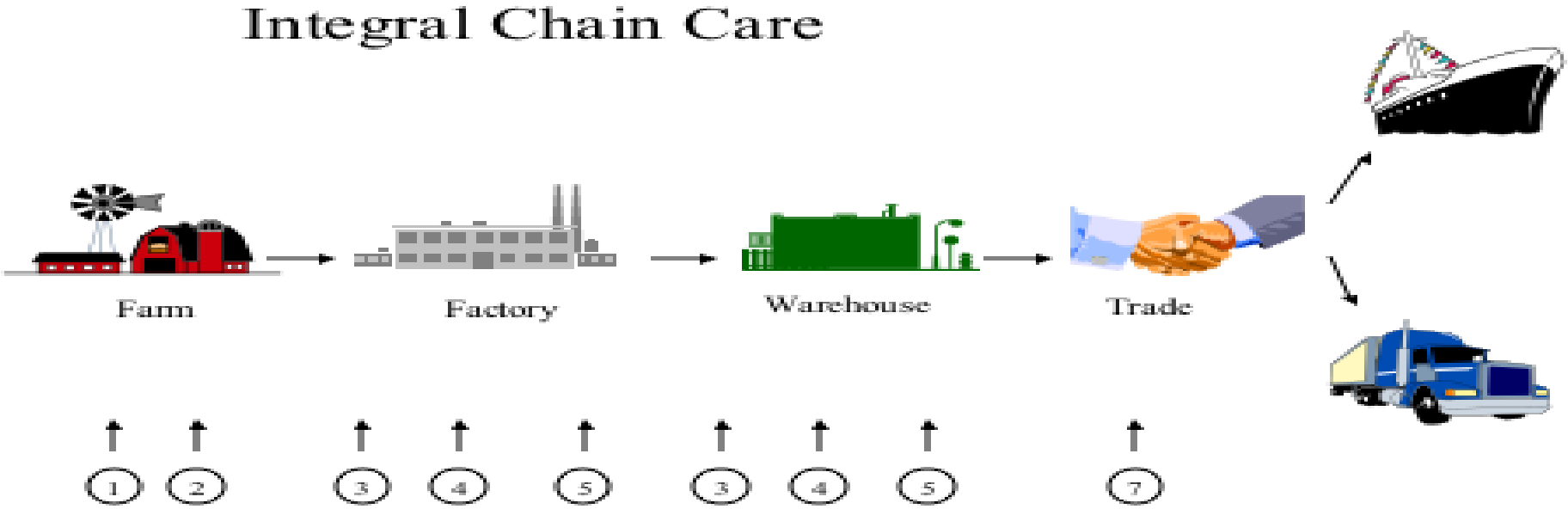
⇩

75 Dairy factories
(dairy establishments which receive farm milk)

⇩

250 processors of dairy products

Part 1—全产业链监控 Integral Chain Care



Part 1—全产业链监控 Integral Chain Care

1. Farm inspection system 农场检验体系
2. Milk payment system 牛乳支付系统
3. Official sampling and analysis 官方取样和分析
4. Quality assurance systems in the production chain 生产过程的质量保证体系
5. （Export) certificates 出口证书
6. Supervision on legislation 监督立法
7. EU hygiene regulation 欧盟卫生监管
8. Dutch Agricultural Quality Act 荷兰农业质量法案

Part 1--主要监管机构及规范

- HACCP
- BRC（英国零售协会食品安全认证标准）
- IFS （国际食品标准）
- ISO 22000
- GMP+

Part 1—COKZ 与Qlip

COKZ is part of the authorities, they control and check the dairy industry from farmer to end-product.

Qlip is a certified laboratory, they do the analyses on all the samples (e.g. COKZ sends them samples)

Ministry of Economic Affairs

Ministry of Public Health, Welfare and Sport

NVWA
(central authority for food production)

RIKILT

COKZ
(sector authority milk and dairy production)

•Assessment and approval of establishments

•Assessment of laboratories

Acceptance of raw milk
•Sampling and analysis of raw milk

•Requirements on milking, equipment

Dairy laboratory (Qlip)
(official samples)

Farm milk laboratory (Qlip)

Dairy Farm Inspection

NVWA
Infectious animal diseases

Animal Health Service

Part 1—COKZ organisation

since 1 July 2012 authority for eggs

- independent
- Under government supervision
- Only active in the dairy and egg sector
- 45 employees + 10 employees

Part 1: Key for improving quality of farm milk

Dairy farm inspection system

Milk payment system

Part 1—Dairy farm inspection system

1. Farm inspection system

- Prudent use of **drugs** **Personnel** **Animal health** and well-being
- **Feed and water** supply **Milking and milk-storage**
- Cleaning and of **milking equipment** **Sampling and analysis**
- Periodical Farm Control by veterinarian
- Continuous Farm Monitoring by veterinarian
- Continuous Animal Health Monitoring based on data
- Inspection hygiene milk collecting truck and unload place farm milk
- Detecting and removing contaminated milk out of the processing chain as early as possible…..

2. Residues and contaminants farm milk

- Antibiotics (抗生素) Antiparasitics（抗寄生物药剂） Aflatoxin M1 （黄曲霉毒素M1） Dioxines and dioxin-like PCB's（二噁英） Organochlorpesticides （农药） Chloroform （氯仿） Heavy metals（重金属） Radioactivitity（X射线活性） Adulterants（掺假成分）





Part 1—Dairy fram inspection system

3. EU hygiene regulation

- ISO 17021(Auditing/management system certification)
- ISO 17020 (Inspections and sampling)
- EN 45011 (Product certification)
- ISO 17025 (Laboratory analysis, 94 methods)
- ISO 17043 (Proficiency testing)

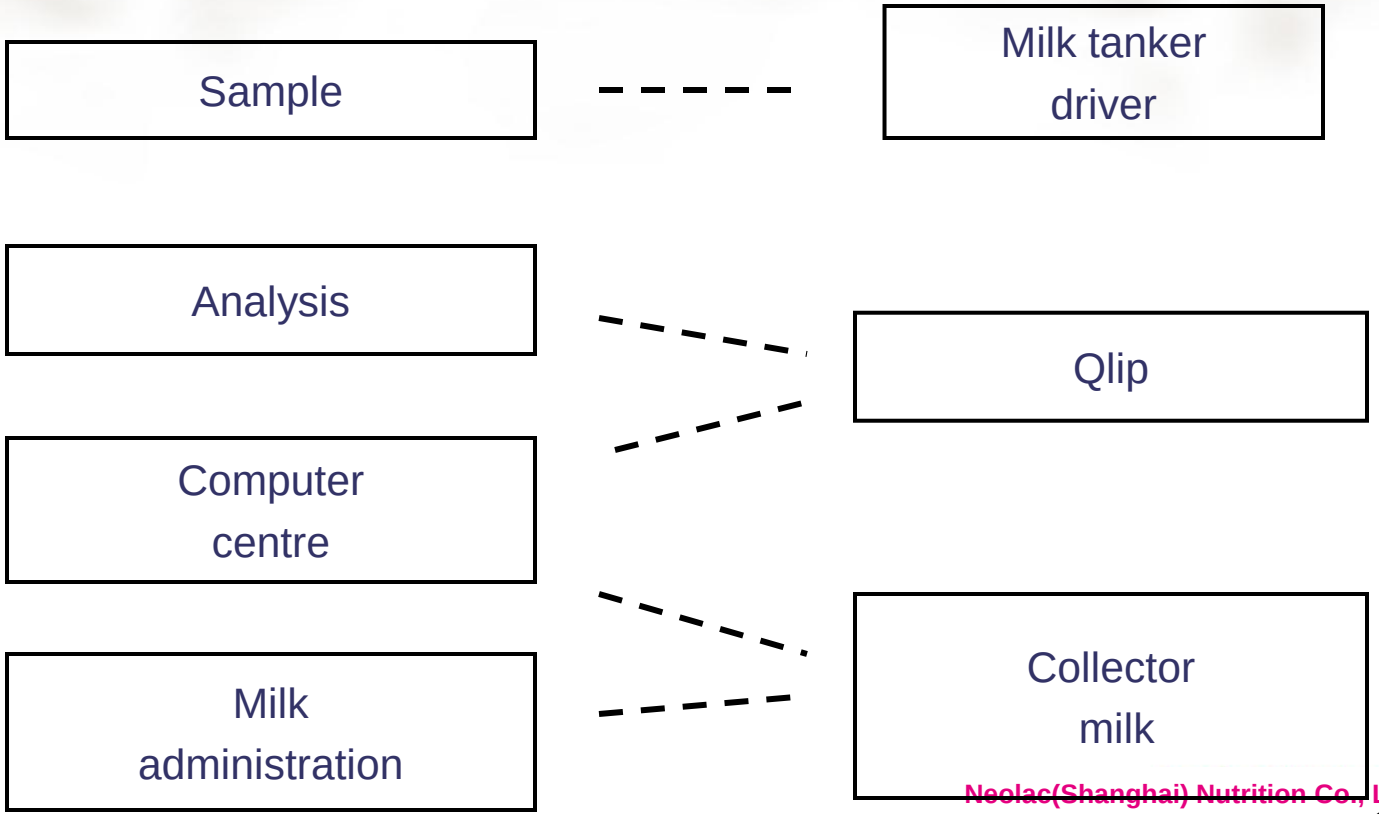
Part 1: Milk payment system

Milk payment system

- Sample of each delivery of tank milk
- Every sample analysed for antibiotics
- Analysis for quality aspects
- Laboratory testing by an independant Milk Control Station (Qlip)
- Education programme for tank lorry drivers for taking samples

Part 1-- Milk payment system

Milk payment system



1. Amount
2.Composition
3. Quality

Part 1--Milk payment system

Milk payment system

Good quality => Bonus per kg milk

Bad quality => Shortage per kg milk

Bad quality during a longer period:

=> Prohibition of delivering raw milk/Farm inspection

No improvement leads to prohibition of delivering raw milk

Quality assurance systems in the production chain

生产过程的质量保证体系

Quality management system

Lypack's quality management system is based on the European Foundation Quality Award (EFQM) model. In this model, the interests of all stakeholders – customers, partners, suppliers, employees,financers,the Board and society at large – are carefully balanced in a way that profits all.

Food Safety

In order to provide our customers with the highest guarantee of food safety, we work according to Hazard Analyses of Critical Control Points (HACCP) standards.

Laboratory

All our products are being checked by an independent laboratory

Quality assurance systems in the production chain

生产过程的质量保证体系

Internal **Audits**

We follow various internal audit and verification procedures to ensure our HACCP is as up to date as possible.

External **Audits**

Our HACCP system is tested twice a year by the Qlip. This is done under the terms of relevant national and/or European legislation, under the auspices of the Dutch Government. Aside from the HACCP audit, the COKZ is also responsible for EU-related inspections. Lastly, Lypack regularly welcomes auditors from its leading customers and partners to inspect our quality management and HACCP systems themselves.

LYPACK
food processing for life

COKZ
Qlip
GMP+
EKO
HACCP CERTIFIED
APPROVED BY
FOOD QUALITY · FDA · FOOD QUALITY
HALAL
BRC FOOD CERTIFICATED
international food standard IFS

Part2-- 免疫球蛋白检测方法研究/The test method research on Ig

免疫球蛋白（immunoglobulin，ig)是一组具有抗体活性的蛋白质。人血浆内的免疫球蛋白大多数存在于丙种球蛋白（γ-球蛋白）中。免疫球蛋白可以分为IgG、IgA、IgM、IgD、IgE五类。

1、IgG的三种检测方法：

-单向免疫扩散法

-聚丙烯酰胺凝胶电泳法

-高效液相色谱法

2、IgA的HPLC法

Part2-- 免疫球蛋白IgG检测方法研究/单向免疫扩散法

原理：

单向免疫扩散法，即利用琼脂凝胶为介质的一种沉淀反应，扩散作用使滴加的抗体与琼脂凝胶中的抗原结合形成抗原-抗体复合物，比例达到合适比例，刚开始出现沉淀现象时进行记录，用以测定产品的IgG含量。

结论：

实验中发现，用单向免疫扩散法对样品IgG进行测定时，免疫扩散沉淀环不易观察，且大小难以准确测量。同一样品两次测定结果IgG含量分别为：14.8%及20.5%。检测结果偏差大，超过30%，故不适合用于样品IgG含量定量检测。

Part2-- 免疫球蛋白IgG检测方法研究/聚丙烯酰胺凝胶电泳法

原理：SDS-page凝胶电泳：在样品介质和聚丙烯酰胺凝胶中加入离子去污剂和强还原剂后，蛋白质亚基的点泳迁移率主要取决于分子量的大小，而电荷因素可以被忽略。当蛋白质的分子量在15KD到200KD之间时，电泳迁移率与分子量的对数呈线性关系，可以用来测定蛋白质亚基的分子量。 根据电泳条带颜色深浅,可对蛋白浓度进行比较。

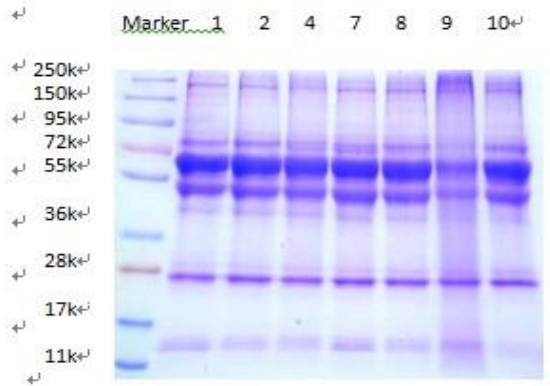
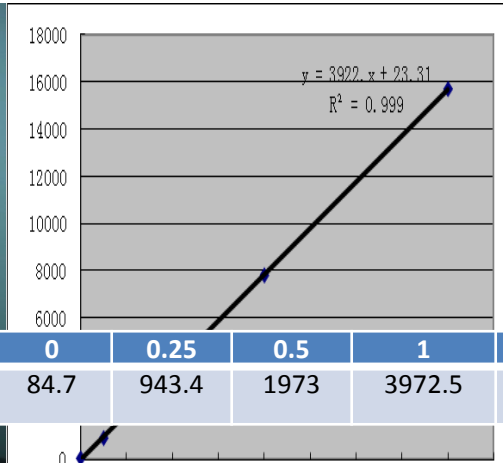


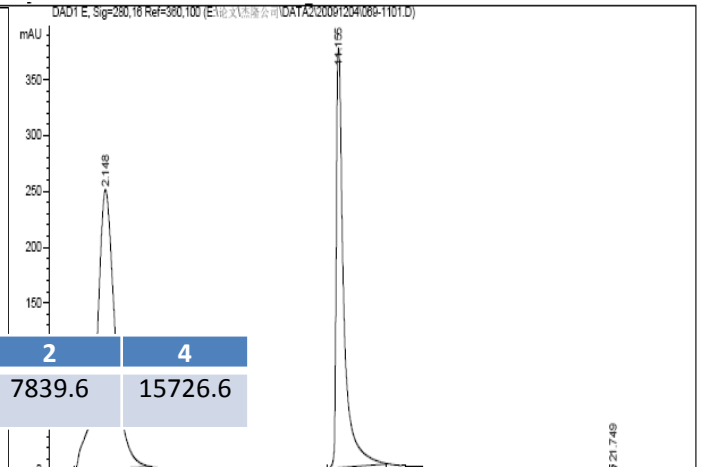
图 1 10%-15%SDS-Page 胶

Part2-- 免疫球蛋白IgG检测方法研究/ HPLC法

原理：在HPLC上，通过蛋白G柱对样品中的免疫球蛋白进行特异性吸附，再洗脱吸附的免疫球蛋白，比较样品洗脱峰面积与标准免疫球蛋白洗脱峰面积计算样品中的免疫球蛋白含量。



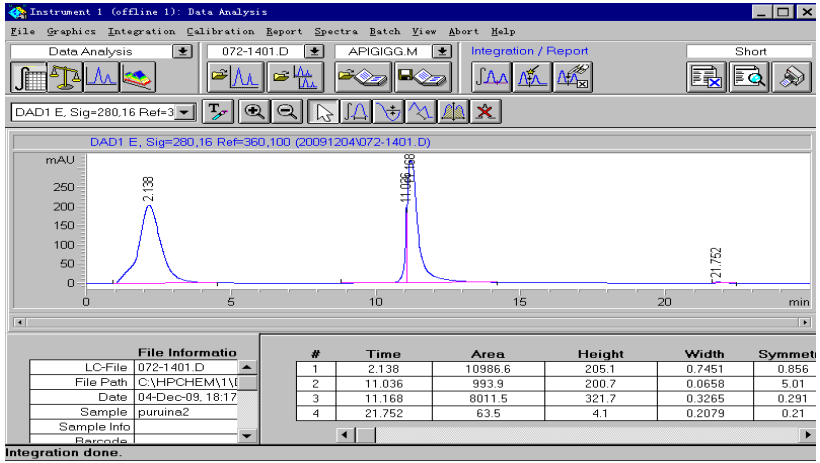
浓度(mg/ml)	0	0.25	0.5	1	2	4
峰面积	84.7	943.4	1973	3972.5	7839.6	15726.6

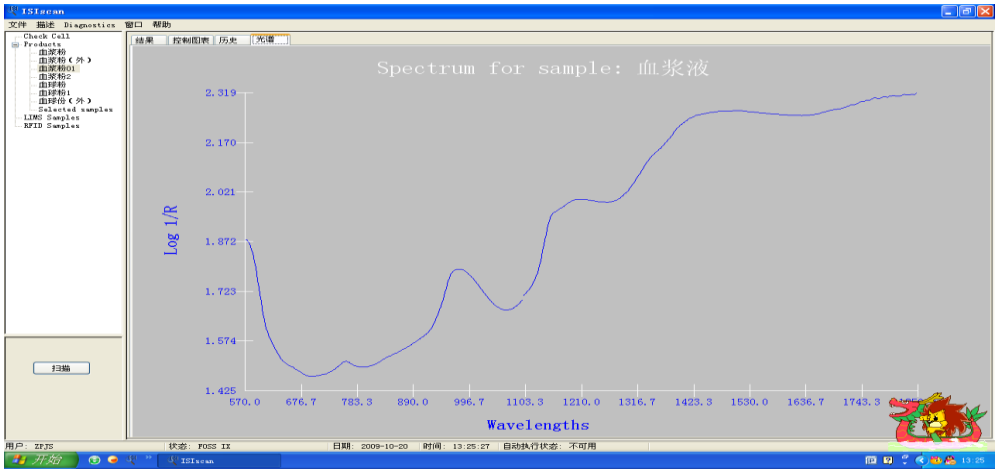


Part2-- 免疫球蛋白IgG检测方法研究/近红外光谱快速检测

1、利用高效液相色谱仪对样品的IgG进行测定，然后通过对产品进行光谱扫描，建立IgG的特征吸收光谱数据库。实现产品功能性成份的快速检测。

2、有利于建立在线监测、适时质量控制体系。





Part2-- 免疫球蛋白IgG检测方法研究

- 结论：通过对单向免疫扩散法、电泳法、高效液相法检测血浆中IgG研究比较，发现单向免疫扩散法中，免疫扩散沉淀环不易观察，沉淀环直径难以准确测量，导致检测结果偏差大，超过30%，故此方法不适合用IgG含量定量检测。根据电泳法的电泳图，我们发现，根据条带的位置及Marker，我们只能对样品IgG进行定性，无法精确定量。
- HPLC法，通过蛋白G柱对IgG的专一性结合，不仅能够对样品IgG进行定性研究，而且可以准确检测出其含量，此方法操作简单，准确度高，重复性好，可作为样品I gG定性定量检测

Part2– 功能性肽检测方法研究/The test method research on peptide

1、小肽的功能：

促进氨基酸吸收，消除游离氨基酸的吸收竞争；吸收速度快、加快蛋白质合成；一些具有特殊生理活性的肽，参与机体生理活动和代谢调节，有降血脂降血压等功能，有些含有具有金属结合性的结构，能促进矿物元素的被动转运及在体内的储存；促进体内双歧杆菌、乳酸杆菌繁殖、提高抗病力，刺激消化酶分泌，提高机体免疫力，促进肌体营养性康复；某些肽还具有抗氧化等作用。

2、肽的检测方法：

-TCA

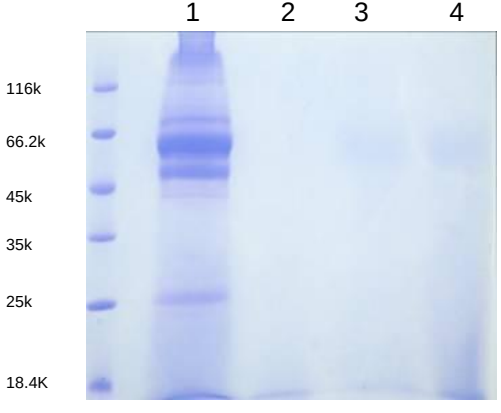
-凝胶层析法

-质谱

Part2– 肽检测方法研究

原理：

利用三氯乙酸（TCA）作蛋白质沉淀剂，将小肽产品中的蛋白质和肽链较长的肽沉淀，并将其中的短链小肽用酸溶解出来，经过过滤、离心、消化、蒸馏，测定其含氮量，并以其占样品粗蛋白质的百分数来表示含量。检测出的结果需减去非蛋白氮后得出小肽含量。



1：小肽产品的水溶液。
2：TCA处理后的上清

$$寡肽\% = \frac{离心上清粗蛋白含量*50}{样品总蛋白粗蛋白含量*V} *100\%$$

Part2– 肽检测方法研究-凝胶过滤法

将小肽产品溶解于水，用HPLC进行凝胶过滤分析，依据样品组分分子体积大小的差别进行分离，在波长220nm条件下检测，使用GPC软件，对色谱图及其数据进行处理，获得小肽产品的分子量分布。

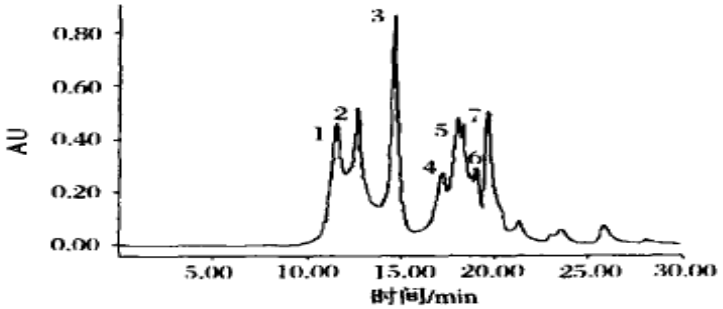
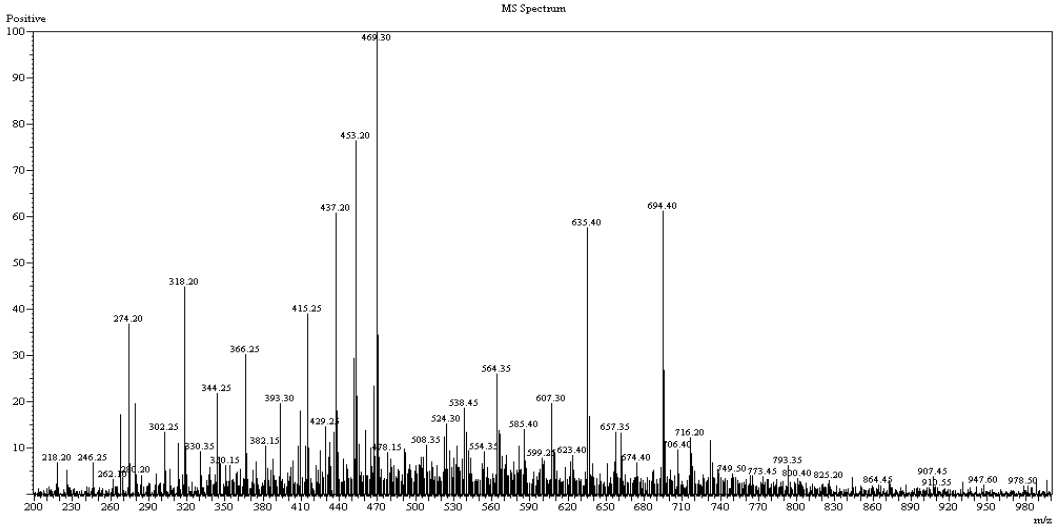


图 凝胶过滤色谱图

表 珠蛋白肽分子量分布

分子质量范围/ u	峰面积百分比/ %（$\lambda_{220\ nm}$）	数均分子质量/ Da	重均分子质量/ Da
12 826~ 2 168	5. 70	3 047	3 334
2 168~ 1 452	4. 61	1 762	1 785
1 452~ 802	12. 26	1091	1111
802~ 321	26. 46	537	551
321~ 104	41. 97	176	175
< 104	9. 00	—	—

Part2– 肽检测方法研究-质谱



- 图：水解珠蛋白粉粉质谱图

Part2– 肽检测方法研究

- 结论：
- TCA处理法测试水解珠蛋白粉的肽含量（1000分子量以下）是可行的，且此方法操作简单、分析时间短、设备要求低，适合一般检测；利用高效凝胶过滤色谱法、质谱法可对水解珠蛋白粉不同分子量的肽进行分析验证，可进一步验证TCA法的检测结果的可靠性。
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Thank you
for your attention!