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中华人民共和国进出口商品检验行业标准

SN 0290—93

出口肉类中稻瘟净残留量检验方法

Method for the determination of kitazin residues in meat for export

1993-12-28发布　　1994-05-01实施

中华人民共和国国家进出口商品检验局　发布

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1 主题内容与适用范围

本标准规定了出口肉类中稻瘟净残留量检验的抽样、制样和气相色谱测定方法。

本标准适用于出口分割猪肉中稻瘟净残留量的检验。

2 抽样和制样

2.1 检验批

以不超过 2 500 件为一检验批。

同一检验批的商品应有相同特征，如包装、标记、产地、规格和等级等。

2.2 抽样数量

批量，件	最低抽样数，件
1～25	1
26～100	5
101～250	10
251～500	15
501～1 000	17
1 001～2 500	20

2.3 抽样方法

按 2.2 规定的抽样件数随机抽取，逐件开启。

肉及肉制品（罐头除外）：从每件中取一袋作为原始样品，其总量不少于 2 kg，放入清洁容器内，加封后，标明标记，及时送交实验室。

如每件中无小包装，或有小包装但每袋重量超过 2 kg 者，则可用锋利刀（用酒精灭菌过）在抽出的包件中，每件割取不少于 100 g，混合后置于清洁容器内，作为混合原始样。混合原始样的总量不少于 2 kg。加封后，标明标记，及时送交实验室。

2.4 试样制备

从每袋原始样品中取出部分有代表性样品，经高速组织捣碎机捣碎均匀，充分混匀。用四分法缩分出不少于 1 000 g 试样，混匀，均分成两份。装入清洁的容器内，加封后，标明标记。

2.5 试样保存

将试样于－18℃以下冷冻保存。

注：在抽样及制样过程中，必须防止样品受到污染或发生残留物含量的变化。

3 测定方法

3.1 方法提要

中华人民共和国国家进出口商品检验局 1993-12-28 批准　　1994-05-01 实施

以乙腈提取试样中稻瘟净，经二氯甲烷液液分配，中性氧化铝柱净化。用配有火焰光度检测器的气相色谱仪测定，外标法定量。

3.2 试剂和材料

3.2.1 乙腈：分析纯，用二氯甲烷饱和。

3.2.2 二氯甲烷：分析纯。

3.2.3 氯化钠溶液：浓度为10%，用蒸馏水配制。

3.2.4 氧化铝：中性，层析用，100～200目，经650℃灼烧4 h，在130℃烘箱中活化2 h，贮藏于密闭容器中。

3.2.5 丙酮：分析纯。

3.2.6 滤纸：快速（用丙酮提取3 h）。

3.2.7 稻瘟净(kitazin)标准品：纯度≥99%。

3.2.8 稻瘟净标准溶液：称取适量的稻瘟净标准品，用丙酮配成浓度为1.0 mg/mL的标准储备液，根据需要再配成适当浓度的标准工作溶液。

3.3 仪器和设备

3.3.1 气相色谱仪，配有火焰光度检测器，磷滤光片(526 nm)。汽化室的石英管和柱前石英棉需要经常更换，以达到净化操作的要求。

3.3.2 锥形瓶：具塞：250 mL。

3.3.3 分液漏斗：500 mL。

3.3.4 蒸发瓶：150 mL。

3.3.5 刻度试管：具塞，10 mL。

3.3.6 振荡器。

3.3.7 水浴锅。

3.3.8 高速组织捣碎机。

3.3.9 旋转蒸发器。

3.3.10 氧化铝柱：60 mm×10 mm(id)玻璃柱，柱底部填约2 mm高脱脂棉（脱脂棉经丙酮提取3 h），装入约50 mm高的氧化铝(3.2.4)。用前经10 mL二氯甲烷淋洗。

3.3.11 微量注射器：10 μL。

3.4 测定步骤

3.4.1 提取

取试样约10 g(精确到0.1 g)于锥形瓶中，加30 mL乙腈(3.2.1)，振荡30 min，用滤纸(3.2.6)过滤于分液漏斗中。滤渣用20 mL乙腈(3.2.1)再提取10 min，滤液一并收集于前一分液漏斗中。

3.4.2 净化

在滤液中加入100 mL氯化钠溶液(3.2.3)和30 mL二氯甲烷(3.2.2)。振摇2 min，放置分层，收集下层二氯甲烷提取液于另一分液漏斗中。水层用20 mL二氯甲烷(3.2.2)再提取一次。合并二氯甲烷提取液，用50 mL氯化钠溶液(3.2.3)洗涤，弃去水层。将二氯甲烷提取液浓缩到10 mL，并通过氧化铝柱(3.3.10)(流速2 mL/min)，并收集于蒸发瓶中。再用10 mL二氯甲烷淋洗此氧化铝柱，淋洗液一并收集于同一蒸发瓶中。然后在40℃水浴中用旋转蒸发器蒸发至干。用少量丙酮溶解残渣，将溶液移入刻度试管，再用少量丙酮洗涤此蒸发瓶数次，并入同一刻度试管，用丙酮定容至10 mL，供测定。

3.4.3 测定

3.4.3.1 色谱条件

a. 色谱柱：1 m×3 mm(id)，填充物为4%(*m*/*m*)SE30+6%(*m*/*m*)OV-210涂于Chromosorb W HP(80～100目)；

b. 色谱柱温度：195℃；

c. 进样口温度:245℃;

d. 检测器温度:245℃;

e. 氮气:纯度≥99.99%,50 mL/min;

f. 氢气:50 mL/min;

g. 空气:50 mL/min。

3.4.3.2 色谱测定

根据试样中稻瘟净含量情况,选定峰高相近的标准工作溶液,标准工作溶液和样液中稻瘟净响应值均应在仪器检测的线性范围内。对标准工作溶液和样液等体积参插进样测定。在上述色谱条件下,稻瘟净保留时间约为 5 min。

3.4.4 空白试验

除不加试样外,按上述测定步骤进行。

3.5 结果计算和表述

用色谱数据处理机或按下式计算试样中稻瘟净残留含量:

$$X = \frac{h \cdot c \cdot V}{h_s \cdot m}$$

式中:X——试样中稻瘟净含量,mg/kg;

h——样液中稻瘟净峰高,mm;

c——标准工作溶液中稻瘟净浓度,μg/mL;

V——样液最终定容体积,mL;

h_s——标准工作溶液中稻瘟净峰高,mm;

m——称取的试样量,g。

注:空白值应从计算结果中扣除。

4 测定低限、回收率

4.1 测定低限

本方法测定低限为 0.01 mg/kg。

4.2 回收率

回收率的实验数据:稻瘟净添加浓度在 0.01～1.00 mg/kg 范围内,回收率为 83%～111%。

附加说明:

本标准由中华人民共和国国家进出口商品检验局提出。

本标准由中华人民共和国四川进出口商品检验局负责起草。

本标准主要起草人袁琼绮。

Professional Standard of the People's Republic of China for Import and Export Commodity Inspection

SN 0290—93

Method for the determination of kitazin residues in meat for export

1 Scope and field of application

This standard specifies the methods of sampling, sample preparation and determination by gas chromatography (GC) of kitazin residues in meat for export.

This standard is applicable to the determination of kitazin residues in frozen pork.

2 Sampling and sample preparation

2.1 Inspection lot

The quantity of an inspection lot should not be more than 2 500 packages.

The characteristics of the cargo within the same inspection lot, such as packing, mark, origin, specification and grade, should be the same.

2.2 Quantity and sample taken

Number of packages in each inspection lot	Minimum number of packages to be taken
1—25	1
26—100	5
101—250	10
251—500	15
501—1 000	17
1 001—2 500	20

2.3 Sampling procedure

A number of packages specified in 2.2 are taken at random and opened one by one.

Meat and meat products (except canned foods): From each, at least one bag shall be taken as a primary sample. The total weight of all the primary samples should be not less than 2 kg, which shall be placed in a clean container, sealed, labeled and sent to laboratory in time.

In case the frozen meat-pieces are not contained small bags inside each package, or if there are small bags inside the package, but the content of the bag exceeds 2 kg, cut out a part from the meat in each package of not less than 100 g with a sharp knife (disinfected with alcohol). Mix the parts of the meat as the mixed primary sample, which shall not be less than 2 kg. Place in a clean container, seal, label and sent to laboratory in time.

Approved by the State Administration of Import and Export Commodity Inspection of the People's Republic of China on Dec. 28, 1993

Implemented from May. 1, 1994

2.4 Preparation of test sample

Part of representative sample is taken from each bag of the primary sample, and all the parts are homogenized by grinding in a blender. The homogenized sample is thoroughly mixed and divided into two equal portions. Each portion is placed in a clean container, which is then sealed and labeled.

2.5 Storage of test sample

The test samples should be stored below −18℃.

Note: In the course of sampling and sample preparation, precaution must be taken to avoid contamination or any factors which may cause the change of residue content.

3 Method of determination

3.1 Principle

The kitazin in test sample is extracted from the meat with acetonitrile. Clean up by liquid-liquid partition with methylene chloride and passing through alumina column. The kitazin is determined by gas chromatography with FPD, using external standard method.

3.2 Reagents and materials

3.2.1 Acetonitrile: A. R., saturated with methylene chloride.

3.2.2 Methylene chloride: A. R..

3.2.3 Sodium chloride solution: 10%, prepared with distilled water.

3.2.4 Aluminum oxide: Neutral, used for chromatography, ignited at 650℃ for 4 h, then heated at 130℃ for 2 h in a oven. Keep in a tightly closed container.

3.2.5 Actone: A. R..

3.2.6 Filter paper: High speed (extracted with acetone for 3 h).

3.2.7 Kitazin: Purity≥99%.

3.2.8 Kitazin standard solution: Accurately weigh an adequate amount of kitazin standard, dissolve in acetone and prepare a solution of 1.0 mg/mL as the standard stock solution. According to the requirement, prepare the standard working solution of appropriate concentration.

3.3 Apparatus and equipment

3.3.1 Gas chromatograph: Equipped with flame photometric detector and phosphorous filter (526 nm). The quartz pipe in the vaporizing chamber and the quartz wool in the column should be replaced frequently in order to satisfy the requirements of the cleaning operation.

3.3.2 Conical flask: 250 mL, with ground stopper.

3.3.3 Separatory funnel: 500 mL.

3.3.4 Evaporating flask: 150 mL.

3.3.5 Graduated test tube: 10 mL, with ground stopper.

3.3.6 Shaker.

3.3.7 Water-bath.

3.3.8 High speed blender.

3.3.9 Rotary evaporator.

3.3.10 Aluminum oxide column: 60 mm × 10 mm (id) glass column, packed with ca 2 mm of absorbent cotton (extracted with acetone for 3 h) at the bottom of the column and filled with ca 50 mm of aluminum oxide (3.2.4). Rinse the column with 10 mL of methylene chloride before use.

3.3.11 Syringe: 10 μL.

3.4 Procedure

3.4.1 Extraction

Weigh ca 10 g of the sample (accurate to 0.1 g) into a conical flask. Add 30 mL of acetonitrile (3.2.1), stopper and shake for 30 min with a shaker. Filter the extract with a filter paper (3.2.6) into a separatory funnel. The residue is extracted again with 20 mL of acetonitrile (3.2.1) for 10 min. Combine the filtrates in the same separatory funnel.

3.4.2 Cleanup

Add 100 mL of NaCl solution (3.2.3) and 30 mL of methylene chloride (3.2.2) into the filtrate. Shake for 2 min and let stand to separate clearly. Transfer the lower methylene chloride extract into another separatory funnel and extract the water phase with 20 mL of methylene chloride (3.2.2) once more. Combine the methylene chloride extracts and wash the extract with 50 mL of NaCl solution (3.2.3). Discard the water phase. Concentrate the methylene chloride extract to 10 mL and let pass through an aluminum oxide column (3.3.10) (flow rate ca 2 mL/min), collect the effluent in an evaporating flask. Then wash the aluminum oxide column with 10 mL methylene chloride and collect the effluent in the same evaporating flask. The combined solution is evaporated to dryness by a rotary evaporator in a 40℃ water-bath. Dissolve the residue and wash the evaporating flask several times with a small amount of acetone. Collect the solution and washings into a graduated test-tube and dilute exactly to 10 mL with acetone for determination.

3.4.3 Determintation

3.4.3.1 GC operating condition

a. Column: Glass column, 1 m×3 mm(id), packed with 4%(m/m) SE30+6%(m/m)OV-210 on chromosorb W HP (80—100 mesh);
b. Column temperature: 195℃;
c. Injection port temperature: 245℃;
d. Detector temperature: 245℃;
e. Nitrogen: Purity≥99.99%, flow rate 50 mL/min;
f. Hydrogen: 50 mL/min;
g. Air: 50 mL/min.

3.4.3.2 GC determination

According to the approximate concentration of kitazin in the sample solution, select the standard working solution with similar peak height to that of sample solution. The response values of kitazin in the standard solution and sample solution should be in the linear detecting range of the instrument. The standard working solution should be determined in-between occasionally with the sample solution of equal volume.

Under the above chromatographic condition, the retention time of kitazin is about 5 min.

3.4.4 Blank test

The operation of the blank test is the same as that described in the method of determination, but with omission of sample addition.

3.5 Calculation and expression of result

The residue content of kitazin is calculated by GC data processor or according to the following equation:

$$X = \frac{h \cdot c \cdot V}{h_s \cdot m}$$

where

X—the content of kitazin in test sample, mg/kg;

h—the peak height of kitazin in sample solution, mm;

h_s—the peak height of kitazin in standard working solution, mm;

c—the concentration of kitazin in standard working solution, μg/mL;

V—the final volume of sample solution, mL;

m—the mass of the test sample, g.

Note: The blank value should be subtracted from the above result of calculation.

4 Limit of determination and recovery

4.1 Limit of determination

The limit of determination of this method is 0.01 mg/kg.

4.2 Recovery

According to the experimental data, when the fortifying concentration of kitazin is in the range of 0.01—1.00 mg/kg, the recovery is 83%—111%.

Additional explanations:

This standard was proposed by the State Administration of Import and Export Commodity Inspection of the People's Republic of China.

This standard was drafted by the Sichuan Import and Export Commodity Inspection Bureau of the People's Republic of China.

This standard was mainly drafted by Yuan Qongqi.

Note: This English version, a translation from the Chinese text, is solely for guidance.