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Meat and meat products — Detection and determination of colouring agents

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Contents

Page

Foreword

ISO (the International Organization for Standardization) is a worldwide federation of national standards bodies (ISO member bodies). The work of preparing International Standards is normally carried out through ISO technical committees. Each member body interested in a subject for which a technical committee has been established has the right to be represented on that committee. International organizations, governmental and non-governmental, in liaison with ISO, also take part in the work. ISO collaborates closely with the International Electrotechnical Commission (IEC) on all matters of electrotechnical standardization.

The procedures used to develop this document and those intended for its further maintenance are described in the ISO/IEC Directives, Part 1. In particular, the different approval criteria needed for the different types of ISO documents should be noted. This document was drafted in accordance with the editorial rules of the ISO/IEC Directives, Part 2 (see www.iso.org/directives).

Attention is drawn to the possibility that some of the elements of this document may be the subject of patent rights. ISO shall not be held responsible for identifying any or all such patent rights. Details of any patent rights identified during the development of the document will be in the Introduction and/or on the ISO list of patent declarations received (see www.iso.org/patents).

Any trade name used in this document is information given for the convenience of users and does not constitute an endorsement.

For an explanation of the voluntary nature of standards, the meaning of ISO specific terms and expressions related to conformity assessment, as well as information about ISO's adherence to the World Trade Organization (WTO) principles in the Technical Barriers to Trade (TBT), see www.iso.org/iso/foreword.html.

This document was prepared by Technical Committee ISO/TC 34, *Food products*, Subcommittee SC 6, *Meat, poultry, fish, eggs and their products*.

This second edition cancels and replaces the first edition (ISO 13496:2000), which has been technically revised. The main changes compared with the previous edition are as follows:

— a new test method, high performance liquid chromatography (HPLC), has been added;

— the order of the clauses has been rearranged;

— the title of the document has been modified;

— the Scope has been modified.

Any feedback or questions on this document should be directed to the user's national standards body. A complete listing of these bodies can be found at www.iso.org/members.html.

Meat and meat products — Detection and determination of colouring agents

1 Scope

This document specifies a detection method using thin-layer chromatography and a determination method using high performance liquid chromatography (HPLC) for synthetic colouring agents in meat and meat products.

This document specifies the HPLC method as the reference method.

This document is applicable to meat and meat products, including livestock and poultry products.

The method using thin-layer chromatography can detect the following colouring agents:

- Tartrazine
- Quinoline Yellow
- Sunset Yellow FCF
- Amaranth
- Ponceau 4R
- Erythrosine
- Patent Blue V
- Indigotine
- Brilliant Black PN
- Black 7984
- Fast Green FCF
- Blue VRS

Synonyms and identity numbers of these colouring agents are listed in Annex A. The plant colours and plant extracts which have been observed not to interfere with this method are listed in B.1. Natural colours which in some cases have been shown to interfere with this method are listed in B.2.

The method using HPLC can detect the following colouring agents:

- Tartrazine
- Amaranth
- Ponceau 4R
- Sunset Yellow FCF
- Erythrosine
- Allura Red AC
- Brilliant Blue FCF
- New Red
- Carmoisine
- Indigotine

Chromatograms of these standard reference colours are shown in Annex D.

2 Normative references

The following documents are referred to in the text in such a way that some or all of their content constitutes requirements of this document. For dated references, only the edition cited applies. For undated references, the latest edition of the referenced document (including any amendments) applies.

ISO 3696, *Water for analytical laboratory use — Specification and test methods*

ISO 4793, *Laboratory sintered (fritted) filters — Porosity grading, classification and designation*

AOAC 46.1.08, *Official Methods of Analysis (AOAC International)*

3 Terms and definitions

For the purposes of this document, the following terms and definitions apply.

ISO and IEC maintain terminology databases for use in standardization at the following addresses:

— ISO Online browsing platform: available at https://www.iso.org/obp

— IEC Electropedia: available at http://www.electropedia.org/

3.1
detection of colouring agents
detection of the presence or absence of colouring agents in accordance with the method specified in this document

4 Principle

4.1 Thin-layer chromatography

The colouring agents are extracted from a test portion with hot water and adsorbed onto polyamide powder. The extracted colouring agents are purified by column chromatography and the colours are eluted from the column. The colouring agents are identified by thin-layer chromatography.

4.2 HPLC

The colouring agents are extracted from a test portion with hot water and adsorbed onto polyamide powder. The extracted colouring agents are injected into the column and chromatographed in HPLC in reverse phase (RP). The colouring agents are identified according to retention time and quantified with external standard method.

5 Sampling

It is important that the laboratory receive a sample which is truly representative and has not been damaged or changed during transport or storage.

Proceed from a representative sample of at least 200 g. Store the sample in such a way that deterioration and change in composition are prevented.

6 Preparation of test sample

Homogenize the laboratory sample with the appropriate equipment (7.2.1). Take care that the temperature of the sample material does not rise above 25 °C. If a mincer is used, pass the sample at least twice through the equipment.

Fill a suitable airtight container with the prepared sample. Close the container and store in such a way that deterioration and change in the composition of the sample are prevented. Analyse the sample as soon as practicable, but always within 24 h after homogenization.

7 Test method of thin-layer chromatography

7.1 Reagents

Use only reagents of recognized analytical grade, unless otherwise specified.

7.1.1 **Water**, conforming to at least grade 3 in accordance with ISO 3696.

7.1.2 Petroleum ether, boiling range 40 °C to 60 °C.

7.1.3 Methanol.

7.1.4 Ammonia, 25 % aqueous solution, ρ_{20} = 0,910 g/ml.

7.1.5 Acetic acid, 100 % mass fraction, ρ_{20} = 1,050 g/ml.

7.1.6 Trisodium citrate dihydrate.

7.1.7 Propan-1-ol.

7.1.8 Ethyl acetate.

7.1.9 2-Methyl-2-propanol.

7.1.10 Propionic acid.

7.1.11 Eluent solution for column chromatography.

Mix 95 volumes of methanol (7.1.3) with five volumes of ammonia solution (7.1.4).

7.1.12 Acetic acid, 50 % solution in methanol.

Mix one volume of acetic acid (7.1.5) with one volume of methanol (7.1.3).

7.1.13 Polyamide powder, of particle size 0,05 mm to 0,16 mm.

7.1.14 Sand, fine granular, hydrochloric acid-washed, neutralized and calcinated.

7.1.15 Standard reference colours.

The purities of the standard colours can vary so it is necessary to know the purity of the colours to be used as standards. The purity shall be determined by the method given in AOAC 46.1.08.

NOTE Certified food colours can also be used as standards.

7.1.16 Standard reference solutions for thin-layer chromatography.

Separately make solutions in water of each of the standard reference colours (7.1.15) with a standard colour content of about 1 g/l.

Prepare solutions of Indigotine on the day of use. Other solutions will keep for at least three months (solutions of Erythrosine for one month) when stored in the dark.

7.1.17 Eluent for thin-layer chromatography: solution I.

Weigh, to the nearest 0,1 g, 25 g of trisodium citrate dihydrate (7.1.6) into a 1 000 ml one-mark volumetric flask. Dissolve in water, dilute to the mark with water and mix.

Mix 80 volumes of this citrate solution with 20 volumes of ammonia solution (7.1.4) and 12 volumes of methanol (7.1.3).

To avoid or reduce interference from safflor or saffran, it is advisable to use chromatography solution II (7.1.18).