
**Starch acetates — Specifications and
test methods**

Acétates d'amidon — Spécifications et méthodes d'essai

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ISO copyright office
CP 401 • Ch. de Blandonnet 8
CH-1214 Vernier, Geneva
Phone: +41 22 749 01 11
Email: copyright@iso.org
Website: www.iso.org

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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 document 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).

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This document was prepared by Technical Committee ISO/TC 93, *Starch (including derivatives and by-products)*.

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.

Introduction

Starch consists mainly of amylose and amylopectin. Amylose is a linear molecule of α -D-glucopyranosyl units, linked by (1-4)- α -linkages. Amylopectin is a highly branched polymer of α -D-glucopyranosyl units, linked by (1-4)- α -linkages and by (1-6)- α - linkages that constitute the branch points. In general, each glucose unit possesses a maximum of three hydroxyls that can undergo chemical substitution. A fourth substitution is also possible at carbon four (4) if that carbon is not involved in a glycosidic bond. Native starches can be chemically modified for improved functionality. The most common sources of native starch used in these modifications are various roots, tubers, cereals and legumes. Modified starches are used in applications requiring special properties that are not attainable by their respective native starches.

Acetylated forms of food starches (including those extracted from hybrid crops such as high-amylose maize) are widely accepted additives that are used in the food industry globally. Starch acetate (INS¹⁾ No. 1420), is produced by esterification of food starch with acetic anhydride or vinyl acetate, with the acetyl groups not exceeding more than 2,5 % of the acetylated product.

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1) International Numbering System for Food Additives.

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Starch acetates — Specifications and test methods

1 Scope

This document specifies the physical, chemical and microbiological requirements for, and test methods of, starch acetates.

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 1666, *Starch — Determination of moisture content — Oven-drying method*

ISO 3188, *Starches and derived products — Determination of nitrogen content by the Kjeldahl method — Titrimetric method*

ISO 3947, *Starches, native or modified — Determination of total fat content*

ISO 11212-1, *Starch and derived products — Heavy metals content — Part 1: Determination of arsenic content by atomic absorption spectrometry*

ISO 11212-2, *Starch and derived products — Heavy metals content — Part 2: Determination of mercury content by atomic absorption spectrometry*

ISO 11212-3, *Starch and derived products — Heavy metals content — Part 3: Determination of lead content by atomic absorption spectrometry with electrothermal atomization*

ISO 11212-4, *Starch and derived products — Heavy metals content — Part 4: Determination of cadmium content by atomic absorption spectrometry with electrothermal atomization*

ISO 4832, *Microbiology of food and animal feeding stuffs — Horizontal method for the enumeration of coliforms — Colony-count technique*

ISO 4833-1, *Microbiology of the food chain — Horizontal method for the enumeration of microorganisms — Part 1: Colony count at 30 °C by the pour plate technique*

ISO 4833-2, *Microbiology of the food chain — Horizontal method for the enumeration of microorganisms — Part 2: Colony count at 30 °C by the surface plating technique*

ISO 21527-2, *Microbiology of food and animal feeding stuffs — Horizontal method for the enumeration of yeasts and moulds — Part 2: Colony count technique in products with water activity less than or equal to 0,95*

OFFICIAL METHOD AOAC, 2011.14: 2011, Calcium, Copper, Iron, Magnesium, Manganese, Potassium, Phosphorus, Sodium, and Zinc in Fortified Food Products. Microwave Digestion and Inductively Coupled Plasma-Optical Emission Spectrometry

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 <https://www.electropedia.org/>

3.1 starch
 carbohydrate polymer consisting of a large number of glucose units linked together primarily by alpha 1-4 glycosidic bonds

Note 1 to entry: The starch polymers come in two forms: (1) linear (amylose) and (2) branched through alpha 1-6 glycosidic bonds (amylopectin), with each glucose unit possessing a maximum of three hydroxyls that can undergo chemical substitution.

3.2 native starch
 starch extracted from plant cells in its natural state as granules, which has not been subjected to any form of modification resulting in physical and/or chemical change

3.3 starch acetate
 modified starch esterified with acetic anhydride or vinyl acetate

Note 1 to entry: It is gluten free and can be used as a stabilizer, thickener, binder and emulsifier during food and cosmetic processing.

4 Requirements

4.1 Physical indexes

Physical indexes shall comply with the requirements given in [Table 1](#).

Table 1 — Physical requirements of starch acetates

Item	Description
Appearance	Powder, granule, coarse particles
Colour	White or nearly white
Microscopy	Granular structure typical of the starch source Typical polarization cross
Solubility	Insoluble in cold water, ether, alcohol
Smell and taste	No smell and foreign tastes
Foreign material	Free of any foreign matter

4.2 Chemical indexes

Chemical indexes shall comply with the requirements given in [Table 2](#).

NOTE Sulfur dioxide method incorporates the ISBT Manual^[3] with modifications.

Table 2 — Chemical indices of starch acetates

Item	Limit/description		Test method
Iodine stain	dark blue to red colour		subclause 5.1
pH	3,0 – 9,0		subclause 5.2
Sulfur dioxide, mg/kg	≤50 dry weight for modified cereal starches	≤10 dry weight for other modified starches	subclause 5.3
Moisture content	≤14 %		ISO 1666
Copper reduction	Copious red precipitate		subclause 5.4.2
Proportion percentage for dry weight (%)	Acetyl groups NMT 2,5	Ester groups NMT 0,5	subclause 5.4
Key			
NMT Not more than			

4.3 Contaminant limits

Contaminant limit shall comply with the requirements given in [Table 3](#).

Table 3 — Contaminant limits of starch acetates

Item	Limit	Test method
Arsenic (As) mg/kg	1	ISO 11212-1
Lead (Pb) mg/kg	2	ISO 11212-3
Mercury (Hg) mg/kg	0,1	ISO 11212-2
Manganese (Mn) mg/kg	50	AOAC Method 2011.14: 2011
Cadmium (Cd) µg/kg	5	ISO 11212-4
Crude fat, %	0,15	ISO 3947
Protein, %	1	ISO 3188

4.4 Microbiological limit

Pathogenic bacterium limit shall comply with the requirements given in [Table 4](#).

Table 4 — Pathogenic bacterium limit

Pathogenic bacterium CFU/g	Limit	Test method
Aerobic plate count	≤1 000	ISO 4833-1, ISO 4833-2
Yeast and mould	≤1 000	ISO 21527-2
Total Coliform	≤10	ISO 4832
Key		
CFU Colony forming unit		

5 Test methods

5.1 Iodine stain

To an aqueous suspension of the starch acetate sample, add 3 to 5 drops of 0,1 N potassium triiodide. A colour change from dark blue to red should be observed.

5.2 pH

Prepare a homogenous 10 % starch acetate solution [starch acetate (10 g)]: distilled water (90 ml)]. Determine the pH with a pre-calibrated pH meter.

5.3 Sulfur dioxide

5.3.1 Procedure

- Accurately weigh 50 g of starch acetates into a 250 ml Erlenmeyer flask to produce a 20 % (weight per volume) starch solution; weigh 25 g if the sulfur dioxide level is more than 8 mg/l.
- Add sufficient purified water to bring total volume to 250 ml.
- Mix the starch acetates and water until the solution is homogenous.
- Cool to 10 °C or below.
- Place cold sample on a magnetic stirrer and stir at a rate sufficient to produce a small vortex at the solution surface.
- Add 10 ml of cold 1,5 N sodium hydroxide solution and stir for 15 to 20 seconds. Add 10 ml of cold 2,0 N sulfuric acid solution; titrate immediately with 0,005 N standard iodine solution until a light blue colour persists for one minute.
- Perform a blank titration using 200 ml of purified water and all reagents.

5.3.2 Calculation

[Formula \(1\)](#) shows how to determine the concentration of sulfur:

$$C_{\text{sul}} = \frac{(V_{\text{sam}} - V_{\text{blank}}) \times N_{\text{Iod}} \times 0,032 \times 1,000,000}{m_s} \quad (1)$$

where

C_{sul} is the concentration of sulfur, expressed in mg/l;

V_{sample} is the volume of sample titrant, expressed in ml;

V_{blank} is the volume of blank titrant, expressed in ml;

m_s is the mass of sample, expressed in g;

m_{Eq} is the milliequivalent mass of sulfur dioxide expressed in g/mol;

0,032 is calculated by $(m_{\text{Eq}}) = \frac{64,07 \text{ g/mol}}{(2 \times 1\,000)}$;

N is the normality expressed in eq/l.

5.4 Acetyl and ester groups

5.4.1 Qualitative analysis for acetyl groups

5.4.1.1 Procedure of analysis for acetyl groups

- Suspend the sample (10 g) in distilled water (25 ml).
- Add 0,4 molar (M) NaOH (20 ml) and shake for one hour.

- Filter the resultant mix and collect the filtrate.
- Heat the filtrate in a 110 °C oven to achieve complete evaporation.
- Dissolve the remaining residue, from evaporation, with 3 to 5 drops of distilled water and transfer to a test tube.
- Add a spatula of calcium hydroxide and apply heat.
 - If the sample is an acetylated starch, acetate will be liberated upon saponification of acetylated starch (heating with calcium hydroxide) and converted to acetone.
 - The acetone vapours generated will produce a blue colour on a paper strip soaked in a fresh saturated solution of o-nitrobenzaldehyde in 2 molarity (M) NaOH.
 - The blue colour is as a result of the acetone being stained by o-nitrobenzaldehyde. The colour is more distinct when the original yellow colour of the reagents is removed with 1 drop of a 1:10 solution of hydrochloric acid.

5.4.2 Copper reduction

5.4.2.1 Qualitative analysis for acetyl groups

- Wash approximately 2,5 g of sample with distilled water and place in a boiling flask.
- Add 3 % hydrochloric acid (10 ml) and distilled water (70 ml).
- Mix, reflux for approximately three hours and cool.
- Add 0,5 ml of the resulting solution to 5 ml of hot alkaline cupric tartrate test solution (TS).

NOTE Copious red precipitate is observed after addition of hot alkaline cupric tartrate to a test sample refluxed under acidic condition.

5.4.3 Quantitative analysis for ester groups

5.4.3.1 Procedure of analysis for ester groups

The infrared spectrum of a thin film gives a typical absorption band at about 1 720 cm^{-1} which is an indication for ester groups. The limit of detection is approximately 0,5 % acetyl groups in the product.

- Accurately weigh 5 g of the sample and transfer into a 250 ml conical flask.
- Suspend in distilled water (50 ml), add 5 to 8 drops of phenolphthalein test solution (TS), and titrate with 0,1 M sodium hydroxide to a permanent pink end point.
- Add 0,45 M sodium hydroxide (25,0 ml), stopper the flask, and shake vigorously for 30 minutes, preferably with a mechanical shaker.
- The temperatures should not exceed 30 °C as some starches can gelatinize.
- Remove stopper and wash with a small quantity of distilled water, allowing the water that runs off the stopper to flow into the flask and not spill.
- Use 3 ml to 5 ml of distilled water to also wash the sides of the flask.
- Titrate the excess alkali with 0,2 molarity (M) hydrochloric acid to the disappearance of the pink colour.
- Record the volume, in ml of 0,2 molarity (M) hydrochloric acid required as the volume, V .