Commission Regulation (EC) No 152/2009 of 27 January
2009 laying down the methods of sampling and analysis
for the official control of feed (Text with EEA relevance)

ANNEX III

METHODS OF ANALYSIS TO CONTROL THE COMPOSITION
OF FEED MATERIALS AND COMPOUND FEED

L.DETERMINATION OF STARCH

POLARIMETRIC METHOD

1.   **Purpose and scope**

This method makes it possible to determine the levels of starch and of high molecular weight starch degradation products in feed for the purpose of checking compliance with the declared energy value (provisions in Annex VII) and Council Directive 96/25/EC[1].

2.   **Principle**

The method comprises two determinations. In the first, the sample is treated with dilute hydrochloric acid. After clarification and filtration the optical rotation of the solution is measured by polarimetry.

In the second, the sample is extracted with 40 % ethanol. After acidifying the filtrate with hydrochloric acid, clarifying and filtering, the optical rotation is measured as in the first determination.

The difference between the two measurements, multiplied by a known factor, gives the starch content of the sample.

3.   **Reagents**

3.1.   Hydrochloric acid, solution 25 % (w/w) density: 1,126 g/ml.

3.2.   Hydrochloric acid. solution 1,13 % (w/v)

The concentration must be checked by titration using a sodium hydroxide solution 0,1 mol/litre in the presence of 0,1 % (w/v) methyl red in 94 % (v/v) ethanol. For the neutralisation of 10 ml, 30,94 ml of NaOH 0,1 mol/litre is needed.

3.3.   Carrez solution I: dissolve 21,9 g of zinc acetate $Zn(CH_3COO)_2$ $2H_2O$ and 3 g of glacial acetic acid in water. Make up to 100 ml with water.

3.4.   Carrez solution II: dissolve 10,6 g of potassium ferrocyanide K_4 $Fe(CN)_6$ $3H_2O$ in water. Make up to 100 ml with water.

3.5.   Ethanol, solution 40 % (v/v), density: 0,948 g/ml at 20 $^{\circ}$C.

4.   **Apparatus**

4.1.   250 ml Erlenmeyer flask with standard ground-glass joint and with reflux condenser.

4.2.   Polarimeter or saccharimeter.

5.   **Procedure**

5.1.   Preparation of the sample

Crush the sample until it is fine enough for all of it to pass through a 0,5 mm round-meshed sieve.

5.2.   Determination of the total optical rotation (P or S) (see observation 7.1)

Weigh 2,5 g of the crushed sample to the nearest mg and place in a 100 ml graduated flask. Add 25 ml of hydrochloric acid (3.2), shake to obtain even distribution of the test sample and add a further 25 ml of hydrochloric acid (3.2). Immerse the flask in a boiling water bath shaking vigorously and steadily for the first three minutes to prevent the formation of agglomerates. The quantity of water in the water bath must be sufficient for the bath to remain at boiling point when the flask is introduced into it. The flask must not be taken out of the bath whilst being shaken. After exactly 15 minutes, remove from the bath, add 30 ml of cold water and cool immediately to 20 $^{\circ}$C.

Add 5 ml of Carrez solution I (3.3) and shake for approximately 30 seconds. Then add 5 ml of Carrez solution II (3.4) and shake again for approximately 30 seconds. Make up to volume with water, mix and filter. If the filtrate is not perfectly clear (which is rare), repeat the determination using a larger quantity of Carrez solutions I and II, for example 10 ml.

Measure the optical rotation of the solution in a 200 mm tube with the polarimeter or saccharimeter.

5.3. Determination of the optical rotation (P' or S') of substances soluble in 40 % ethanol

Weigh 5 g of the sample to the nearest mg, place in a 100 ml graduated flask and add about 80 ml of ethanol (3.5) (see observation 7.2). Leave the flask to stand for 1 hour at room temperature; during this time, shake vigorously on six occasions so that the test sample is thoroughly mixed with the ethanol. Make up to volume with ethanol (3.5), mix and filter.

Pipette 50 ml of the filtrate (corresponds to 2,5 g of the sample) into a 250 ml Erlenmeyer flask, add 2,1 ml of hydrochloric acid (3.1) and shake vigorously. Fit a reflux condenser to the Erlenmeyer flask and immerse the latter in a boiling water bath. After exactly 15 minutes, remove the Erlenmeyer flask from the bath, transfer the contents to a 100 ml graduated flask, rinsing with a little cold water, and cool to 20 $^{\circ}$C.

Clarify using Carrez solutions I (3.3) and II (3.4), make up to volume with water, mix, filter and measure the optical rotation as indicated in the 2nd and 3rd paragraphs of 5.2.

6. **Calculation of results**

The starch content (%) is calculated as follows:

6.1. Measurement by polarimeter

$$\text{Starch content (\%)} = \frac{2\,000(P\text{-}P')}{[\alpha]_{20^\circ}^{D}}$$

P	=	Total optical rotation in angle degrees
P'	=	Optical rotation in angle degrees of the substances soluble in 40 % (V/V) ethanol
$[\alpha]_{20^\circ}^{D}$	=	Specific optical rotation of pure starch. The numerical values conventionally accepted for this factor are the following:

185,9°:	rice starch
185,7°:	potato starch
184,6°:	maize starch
182,7°:	wheat starch
181,5°:	barley starch

181,3°:	oat starch
184,0°:	other types of starch and starch mixtures in compound feed

6.2. **Measurement by saccharimeter**

$$\text{Starch content (\%)} = \frac{2\,000}{[\alpha]_{20}^{D}} \times \frac{(2\,N \times 0{,}665) \times (S\text{-}S')}{100} - \frac{26{,}6\,N \times (S\text{-}S')}{[\alpha]_{20}^{D}}$$

S   = Total optical rotation in saccharimeter degrees

S'   = Optical rotation in saccharimeter degrees of the substances soluble in 40 % (v/v) ethanol

N   = weight (g) of saccharose in 100 ml of water yielding an optical rotation of 100 saccharimeter degrees when measured using a 200 mm tube

       16,29 g for the French saccharimeters

       26,0 g for the German saccharimeters

       20,0 g for mixed saccharimeters.

$[\alpha]_{20}^{D}$ = Specific optical rotation of pure starch (see 6.1)

6.3. Repeatability

The difference between the results of two parallel determinations carried out on the same sample must not exceed 0,4 in absolute value for a starch content lower than 40 % and 1 % relative for starch contents equal to or greater than 40 %.

7. **Observations**

7.1. If the sample contains more than 6 % of carbonates, calculated in terms of calcium carbonate, they must be destroyed by treatment with an exactly appropriate quantity of dilute sulphuric acid before determination of the total optical rotation.

7.2. In the case of products with a high lactose content, such as powdered milk serum or skimmed milk powder, proceed as follows after adding 80 ml of ethanol (3.5). Fit a reflux condenser to the flask and immerse the latter in a water bath at 50 °C for 30 minutes. Leave to cool and continue the analysis as indicated in 5.3.

7.3. The following feed materials, where they are present in significant amounts in feed, are known to give rise to interferences when determining the starch content by the polarimetric method and thereby incorrect results could be yielded:

— (sugar) beet products such as (sugar)beet pulp, (sugar) beet molasses, (sugar) beet pulp — molassed, (sugar) beet vinasse, (beet) sugar,

— citrus pulp,

— linseed; linseed expeller; linseed extracted,

— rape seed; rape seed expeller; rape seed extracted; rape seed hulls,

— sunflower seed; sunflower seed extracted; sunflower seed, partially decorticated, extracted,

— copra expeller; copra extracted,

— potato pulp,

— dehydrated yeast,

— products rich in inulin (e.g. Chips and meal of Jerusalem artichokes),

— greaves.

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(1)   OJ L 125, 23.5.1996, p. 35.