SCANDINAVIAN PULP, PAPER AND BOARD
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Paper, board and process waters

Starch content

0 Introduction

Methods for determination of starch content are mainly grouped into acid hydrolysis or enzymatic procedures. Acid hydrolysis procedures can not be applied to all kind of modified starches and thus they have limited applications.

This SCAN-test Method specifies a procedure for the determination of starch content in paper, board and process waters, based on an enzymatic hydrolysis followed by determination of free glucose using either a HPLC-instrument/ IC-instrument or a spectrophotometer.

In Annex A, a procedure for the use of a commercial fast method (a kit method) is described.

Annex B describes how the result is dependent on the reference starch used.

The choice of procedure depends mainly on the availability of apparatus. On the other hand, some samples give rise to disturbing peaks on the HPLC/IC-chromatogram, and these peaks make it difficult to obtain correct results. In these cases, it is better to use the spectrophotometric procedure. The pretreatment is the same in both procedures.

The HPLC/IC method, the spectrophotometric method and the fast method (the kit method) give comparable results.

1 Scope

This SCAN-test Method describes a procedure for the determination of total starch content in paper, board and process waters.

For paper and board samples, the method does not differentiate between starch inside the sample and starch on the surface of the sample. All cationic starches are not fully hydrolyzed by the enzymes used in this method, and it is therefore important to analyse also a reference starch. The calculation shall then be based upon the yield from the reference starch.

This method is intended for all kinds of paper and board samples having a starch content exceeding 1 g/kg or process waters having a starch content exceeding 5 mg/l.

2 Reference

ISO 638	Paper, board and pulps – Determination of dry matter content – Oven-drying method
SCAN-P 52:84	Starch – Dry matter content

Note – SCAN-test has withdrawn a number of test methods and refers instead to the corresponding ISO and/or EN Standards.

3 Definition

For the purpose of this Method, the following definition applies:

3.1 *Starch content* – The total starch content present in paper, board or process water.

4 Principle

The starch is extracted from the paper or board sample. The extracted starch or the starch in the process water is hydrolyzed to glucose using two enzymes. After filtration, the glucose content is determined using either a HPLC/IC-instrument or a spectrophotometer.

5 Reagents

All chemicals shall be of analytical grade.

5.1 *Biocide,* common paper mill biocide, e.g. Rocima 520S (Acima).

5.2 *Water,* of high purity, distilled or deionized.

5.3 *Potassium hydroxide solution*, *c*(KOH) = 5 mol/l.

5.4 *Acetic acid solution, c*(HC_3COOH) = 5 mol/l.

5.5 *Calcium chloride dihydrate, c*($CaCl_2 \cdot 2H_2O$) = 50 wt-%.

Note 1 – Commercially available standard solutions may be used.

5.6 *α-amylase enzyme,* e.g. Termamyl 300 L DX (Novozymes), activity 300 KNU/g.

5.7 *Amyloglucosidase (AMG)*, e.g Roche Diagnostics Scand AB, activity 14 U/mg and 140 U/ml.

It is very important that the enzymes are glucose-free. A blank test shall be done every time.

5.8 *Glucose standard.* Prepare the glucose standard solution as recommended by the supplier of the HPLC/IC column.

Note 2 – Commercially available glucose standard solutions may be used.

5.9 *Eluent solution,* for HPLC/IC. The composition of this solution depends on the type of HPLC/IC column to be used. Therefore, follow the recommendations given by the HPLC/IC column supplier.

For spectrophotometric determination, also use the following reagents:

5.10 *Sodium hydroxide solution*, *c*(NaOH) = 5 mol/l.

5.11 *Triethanolamine buffer,* Dissolve 14,0 g of triethanolamine hydrochloride and 0,25 g of magnesium sulphate ($MgSO_4$ $7H_2O$) in 80 ml water (5.2) in a 100 ml volumetric flask. With 5 ml NaOH (5.10), adjust the pH-value to 7,6. Fill to the mark with water (5.2).

Note 3 – If stored at + 4 ºC, this solution is stable for approximately 4 weeks.

5.12 *NADP solution* (nicotinamid-adenine dinucleotide phosphate)*,* Dissolve 60 mg air-dry NADP-Na_2H_2 in 6 ml water (5.2).

Note 4 – If stored at + 4 ºC, this solution is stable for approximately 4 weeks.

5.13 *ATP solution* (adenosine-5-triphosphate*,* $C_{10}H_{14}N_5O_{13}P_3Na_2 \cdot 3H_20$) Dissolve 300 mg air-dry ATP-Na_2H_2 and 300 mg air-dry $NaHCO_3$ in 6 ml water.

Note 5 – If stored at + 4 ºC, this solution is stable for approximately 4 weeks.

5.14 *Hexokinase/Glucose-6-phosphatedehydrogenase (HK/G6P-DH),* suspension in 3,2 M ammonium sulphate. This solution is normally bought ready to use.

Note 6 – If stored at + 4 ºC, this solution is stable for approximately 1 year.

6 Apparatus

Ordinary laboratory equipment and the following:

6.1 *HPLC, High-performance liquid chromatography* or *IC, ion-chromatography,* including a suitable column and detector for glucose analysis
or
Spectrophotometer, for absorption measurement at 340 nm.

6.2 *Micropipettes,* volume 20 µl, 100 µl and 1000 µl.

6.3 *Balance,* with a resolution of 0,1 mg.

6.4 *Shaking water bath,* equipped with a thermostat.

6.5 *Filtration device,* consisting of a filtration flask, a funnel and a vacuum pump.

6.6 *Glass-fibre filter,* without any addition of binders, having a grammage between 50 g/m^2 and 100 g/m^2, circular with a diameter to fit the funnel (6.5).

7 Sample preparation

The sampling procedure is not covered by this method. Make sure that the test portions taken are representative of the sample received.

Preserve the process water samples by adding biocide (5.1) according to the instructions given by the supplier. Tear the air-dry sample into pieces of suitable size.

Weigh a separate test portion of the paper and board sample for determination of dry matter content in accordance with ISO 638.

Weigh a separate test portion of the reference starch for determination of dry matter content in accordance with SCAN-P 52:84.

8 Procedure

Run the determination in duplicate: weigh out two parallel samples. Suitable amounts are:

- *Reference starch:* approx. 20 mg dry starch;
- *Paper and board:* 1 g of dry sample. The minimum amount is 200 mg;
- *Process water*: Total volume 100 ml, i.e. 50 ml for total starch analysis and 50 ml for dissolved starch analysis.

Note – If the reference starch is in a slurry form, then drying is recommended before weighing.

8.1 Blank test

Carry out the whole determination without any sample.

8.2 Paper and board samples

Carry out the whole procedure with both the reference starch and the sample.

If the type of starch in the sample is unknown, use glucose as a reference in the end determination.

Weigh to the nearest 1 mg a test portion of approx. 1 g (or 20 mg of the reference starch) into a 100 ml flat bottom flask. Add 50 ml of water (5.2) and 5 ml of potassium hydroxide solution (5.3) into the flask and place it in the shaking water bath (6.4) at 95 ºC for 5 min.

Adjust the pH to 5,0 using acetic acid (5.4). Then add 5 drops of calcium chloride (5.5), and 100 µl of α-amylase (5.6) and place the flask in the shaking water bath at 95 ºC for 1 h.

Cool the sample to a temperature below 60 ºC. Add 100 µl of amyloglucosidase, AMG (5.7) into the flask and place it in the shaking water bath at 60 ºC for 1 h.

Filter the contents of the flat bottom flask using the filtration device (6.5), the glass fibre filter (6.6) and vacuum. Wash the sample with 50 ml of water (5.2) and rinse the flask and the filtration device with water. Use vacuum suction until all water has been removed from the sample.

Transfer the filtrate, combined with the washing waters, to a 200 ml volumetric flask and fill up to the mark with water (5.2).

Check that the filtrate is free from fibres and other visible particles. If fibres or particles are visible in the filtrate, filter it once more through a new filter.

8.3 Process waters

Carry out the procedure with the reference starch as described in section 8.2.

8.3.1 *Total starch content.* Before taking the test portion to analysis, shake the bottle of the process water sample well. Take 50 ml of the sample into a 100 ml flat bottom flask and add 5 ml of potassium hydroxide solution (5.3) and place it in the shaking water bath at 95 ºC for 5 min. Then, adjust the pH and follow the instructions given in section 8.2 from paragraph 5.

8.3.2 *Dissolved starch content.* Using the filtration device (6.5), the glass-fibre filter (6.6) and vacuum, filter 50 ml of the process water sample. Transfer the filtrate into a 100 ml flat bottom flask and add 5 ml of potassium hydroxide solution (5.3) and place it in the shaking water bath at 95 ºC for 5 min. Then, adjust the pH and follow the instructions given in section 8.2 from paragraph 5.

8.4 End determination

8.4.1 *HPLC/IC instrument.* The optimum conditions depend on the apparatus and the column. Use the conditions recommended by the manufacturer or determine the optimum conditions empirically.

Perform the chromatography with a standard glucose solution (5.8). Check from the chromatogram that the separation is adequate. Measure either the area or the height of the peak.

Run the sample solution in the same manner.

8.4.2 *Spectrophotometer.* After diluting to 200 ml, continue as follows: add 1,0 ml triethanolamine buffer solution (5.11), 100 µl ATP (5.13), 100 µl NADP (5.12), 100 µl sample (or max. 2,0 ml) and 1,9 ml water (5.2) to a cuvette. For mixing, gently turn the cuvette upside down. After 3 minutes, measure the absorbance at 340 nm for the sample (A1) and the blank ($A1_{blank}$) using water (5.2) as reference.

Add 20 µl of HK/G6P-DH (5.14) into both cuvettes and mix. After 15 minutes, measure the absorbance at 340 nm for the sample (A2) and the blank ($A2_{blank}$) using water (5.2) as reference.

9 Calculation

9.1 Paper and board samples

9.1.1 HPLC/IC procedure

Calculate the starch content according to the expression:

$$X_1 = \frac{(A_x - A_0) \cdot V \cdot C_{ref} \cdot D}{m \cdot (A_{ref} - A_0)} \quad [1]$$

where

X_1 is the starch content in the dry sample, in grams per kilogram;
V is the volume of the sample solution (here 200 ml); in millilitres;
A_x is the area or height of the glucose peak for the sample solution;
A_0 is the area or height of the glucose peak for the blank solution;
A_{ref} is the area or height of the glucose peak for the reference starch solution;
C_{ref} is the concentration of the reference starch solution, in grams per litre;
m is the mass of the test piece, as dry, in grams
D is the dilution factor (= 1, if no dilution has been made).

If the reference starch is unknown then $A_{ref} = A_{glu}$ and $C_{ref} = C_{glu}$, where

A_{glu} is the area or height of the glucose peak for the standard glucose solution;
C_{glu} is the concentration of the standard glucose solution (see 8.2, second paragraph).

In these cases the yield is the theoretical yield (100 %).

***9.1.2** Spectrophotometric procedure*

Calculate the yield of the reference starch according to the expression:

$$X_{ref} = \frac{\Delta A_{ref} \cdot M_w \cdot D \cdot V}{\varepsilon \cdot d \cdot m_{ref}} \quad [2]$$

where

X_{ref} is the yield of the reference starch, in grams per kilogram;
ΔA_{ref} is $(A2_{ref}\text{-}A1_{ref}) - (A2_{blank}\text{-}A1_{blank})$;
M_w is the molecular weight of anhydroglucose = 162 g/mol;
V is the volume of the sample solution (here 0,2 l); in litres;
ε is the molar absorptivity of NADPH at 340 nm = 6,3 l/mmol·cm;
d is the cuvette length = 1 cm;
m_{ref} is the mass of the reference starch, as dry, in grams;
D is the dilution factor = total end volume in the cuvette (ml) / added sample volume in the cuvette (ml).

Calculate the correction factor, f:

$$f = \frac{1000}{X_{ref}} \quad [3]$$

where

1000 is the theoretical yield of the reference starch, in grams per kilogram;
X_{ref} is the yield of the reference starch, in grams per kilogram.

In the cases where the reference starch is unknown, the yield is the theoretical yield, $f = 1$.

Calculate the starch content using the expression:

$$X_1 = f \cdot \frac{\Delta A_{sample} \cdot M_w \cdot D \cdot V}{\varepsilon \cdot d \cdot m_{sample}} \quad [4]$$

where

X_1 is the starch content in the dry sample, in grams per kilogram;
f is the correction factor;
ΔA_{sample} is $(A2_{sample}\text{-}A1_{sample}) - (A2_{blank}\text{-}A1_{blank})$;
M_w is the molecular weight of anhydroglucose = 162 g/mol;
V is the volume of the sample solution (here 0,2 l); in litres;
ε is the molar absorptivity of NADPH at 340 nm = 6,3 l/mmol·cm;
d is the cuvette length = 1 cm;
m_{sample} is the mass of the test piece, as dry, in grams
D is the dilution factor = total end volume in the cuvette (ml) divided by added sample volume in the cuvette (ml).

9.2 Process water samples

9.2.1 HPLC/IC procedure

Calculate the starch content according to the expression:

$$X_1 = \frac{(A_x - A_0) \cdot V \cdot C_{ref} \cdot D}{V_{sample} \cdot (A_{ref} - A_0)} \times 1000 \qquad [5]$$

where
X_1 is the starch content in the sample, in milligrams per litre;
V is the volume of the sample solution (here 200 ml); in millilitres;
A_x is the area or height of the glucose peak for the sample solution;
A_0 is the area or height of the glucose peak for the blank solution;
A_{ref} is the area or height of the glucose peak for the reference starch solution;
C_{ref} is the concentration of the reference starch solution, in grams per litre;
V_{sample} is the volume of the sample, in millilitres
D is the dilution factor (= 1, if no dilution has been made).
1000 is a factor to convert the result to mg/l.

9.2.2 Spectrophotometric procedure

Calculate the starch content according to the expression:

$$X_1 = f \cdot \frac{\Delta A_{sample} \cdot M_w \cdot D \cdot V}{\varepsilon \cdot d \cdot V_{sample}} \qquad [6]$$

where
X_1 is the starch content in the sample, in milligrams per litre;
f is the yield from the reference starch (= correction factor);
ΔA is $(A2_{sample}\text{-}A1_{sample}) - (A2_{blank}\text{-}A1_{blank})$;
M_w is the molecular weight of anhydroglucose = 162 g/mol;
V is the volume of the sample solution (here 200 ml), in millilitres;
ε is the molar absorptivity of NADPH at 340 nm = 6,3 l/mmol·cm;
d is the cuvette length = 1 cm;
V_{sample} is the volume of the sample, in millilitres;
D is the dilution factor = total end volume in cuvette (ml) divided by added sample volume in cuvette (ml).

9.3 Mean

Calculate the mean starch content of the parallel determinations. Report the result with two significant figures. The results of the parallel determinations should not deviate by more than 5 % from their mean.

10 Precision

10.1 Repeatability

One laboratory analysed three different samples using two techniques, the HPLC and the spectrophotometric procedures, according to this SCAN-test Method. Five parallel determinations have been made in each case.

Sample Starch type	Method	Mean starch content, g/kg	Coeff. of variation, %
Board Surface starch	HPLC	7,0	1,5
Board Surface starch	Spectro-photometer	6,9	0,7
Board Wet end starch	HPLC	5,9	1,5
Board Wet end starch	Spectro-photometer	5,6	1,6
Fine paper Wet end starch	HPLC	38,5	1,1
Fine paper Wet end starch	Spectro-photometer	38,5	0,9
Fine paper Surface starch	HPLC	36,1	2,5
Fine paper Surface starch	Spectro-photometer	37,8	0,9
Kraft liner Cationic starch	HPLC	11,4	1,5
Kraft liner Cationic starch	Spectro-photometer	11,3	1,4

10.2 Reproducibility

Different kinds of paper, board and white water samples have been tested in six different laboratories according to this SCAN-test Method. In the test, the HPLC/IC procedure, the spectophotometric procedure and the fast method (kit method) have been used.

Sample Starch type	Mean starch content, g/kg	Coeff. of variation %
Board, Wet end starch	5,7	11,9
Board, Wet end + surface starch	6,4	16,0
Fine paper Wet end starch	36,7	5,2
Fine paper Surface starch	36,7	4,3
Kraftliner, white top Cationic starch	11,2	7,1
Kraftliner, Native starch	4,1	11,0
LWC Cationic starch	5,9	21,2

White water sample	Mean starch content, mg/l	Coeff. of variation %
Fine Paper Wet end starch	46	5,6
Filtrate fine paper Wet end starch	13	40,8
Fine paper Surface starch	38	49,3
Filtrate fine paper Surface paper	14	43,3
Kraftliner Cationic starch	51	16,9
Filtrate kraftliner Cationic starch	46	14,8

The reproducibility is quite poor in some cases due to the quite low starch content in those samples.

11 Report

The test report shall include a reference to this SCAN-test Method and the following particulars:

a) date and place of testing;
b) identification of the sample tested;
c) the results as stated in clause 9;
d) whether a reference starch has been used and the identity of the reference starch;
e) for water samples, state whether the total or the dissolved starch content is reported;
f) any deviation from the procedure described in this Method and any other circumstances that may have affected the result.

Annex A
Determination of starch content using a fast method

Commercial methods (kit methods) have been developed to enable fast determination of starch content in different kinds of samples. If a commercial kit is used for starch determination, the method must follow the principles of this SCAN-method and give the same results. The commercial fast method must also be reproducible and reliable, and give quantitative results even if the sample contains high levels of resistant starch.

One commercial fast procedure for determination of total starch content in different kinds of samples has been developed by Megazyme International Ireland Ltd. This starch assay kit is based on the use of α-amylase and amyloglucosidase enzymes followed by a spectrometric end determination.

Annex B
Starch sources and the problems in choosing the right reference

The problem related to the determination of starch content in samples from the paper mill is to select the correct reference starch. There are several sources of starch and the starches differ from each other.

Starch sources

- *Recycled fibre*
  - Wet end starch from magazine papers and newspapers.
- *Liner and recycled corrugated board*
  - Wet end starch, spray starch, surface sizing starch and starch used producing the corrugated board.
- *Broke*
  - Wet end starch, spray starch, surface sizing starch, coating starch.
- *White water*
  - Starch on fines and starch circulating in the system.
- *Starch addition*
  - Wet end starch, spray starch, surface sizing starch, coating starch.

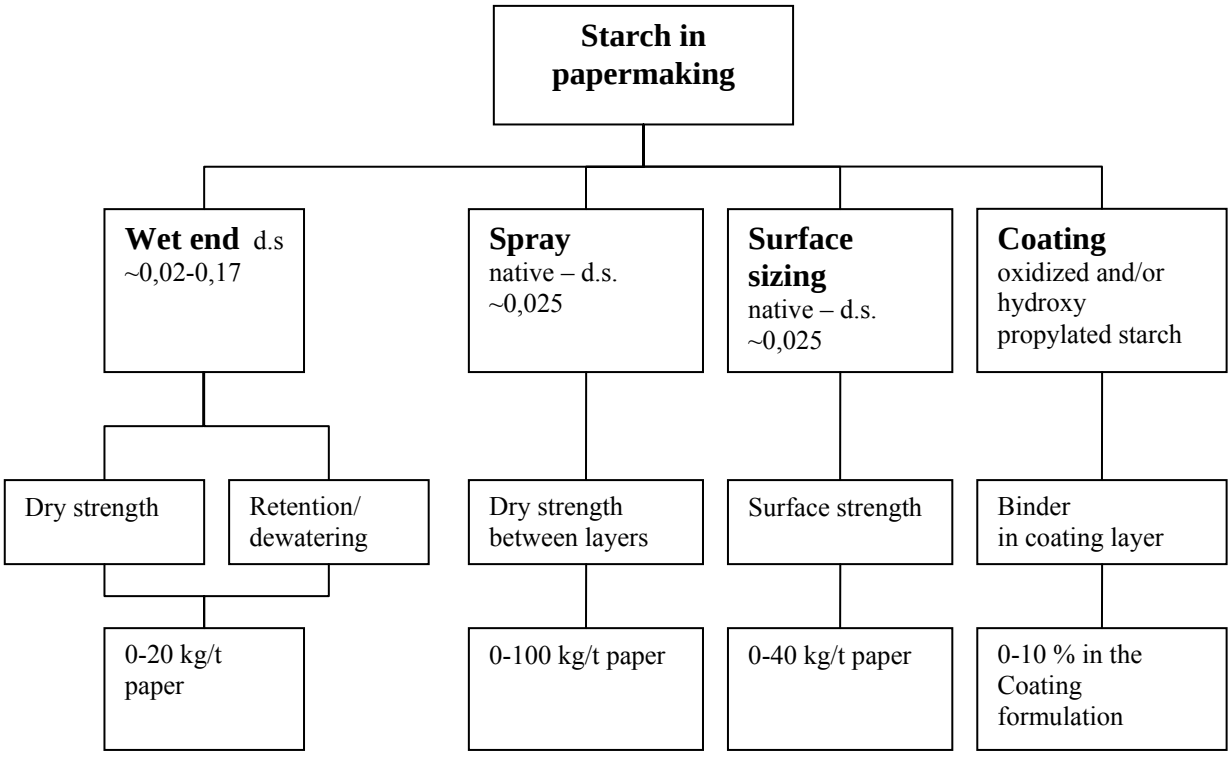

Figure B1 Overview of starches used in papermaking (d.s. = Degree of Substitution)

Different starches give different yields in the determination of starch content. Native starch has 100 % yield and the yield decreases with increased degree of substitution. Figures below were obtained with an HPLC-system with a BioRad Aminex HPX-87H at Lyckeby Industrial.

Example:
Paper from a liner mill. A wet end starch, with a degree of substitution of 0,07 and a dosage of ~5 kg/t, is used. The starch content in the paper is 30 kg/t with the wet end starch as reference starch and 20 kg/t with a native starch as reference starch. The incoming raw material, the recycled fibre, contains a high amount of starch and it could be both native starch and cationic starch. The result depends consequently on the choice of reference starch. It is important to be aware of this problem since a paper sample often contains a mixture of starches.

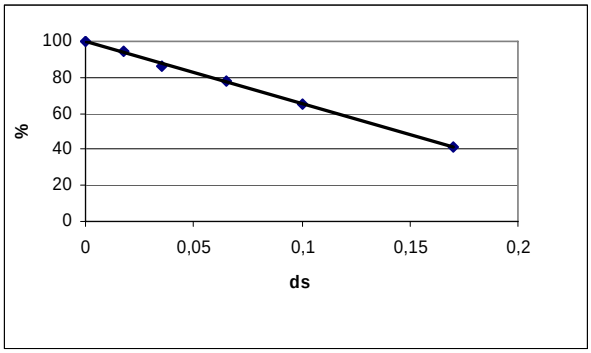

Figure B2 The yield of the starch determined as a function of DS (Degree of Substitution).

Table B1 Data for figure B2.

DS	Yield, %
0	100
0,02	95
0,04	86
0,07	78
0,10	65
0,17	42

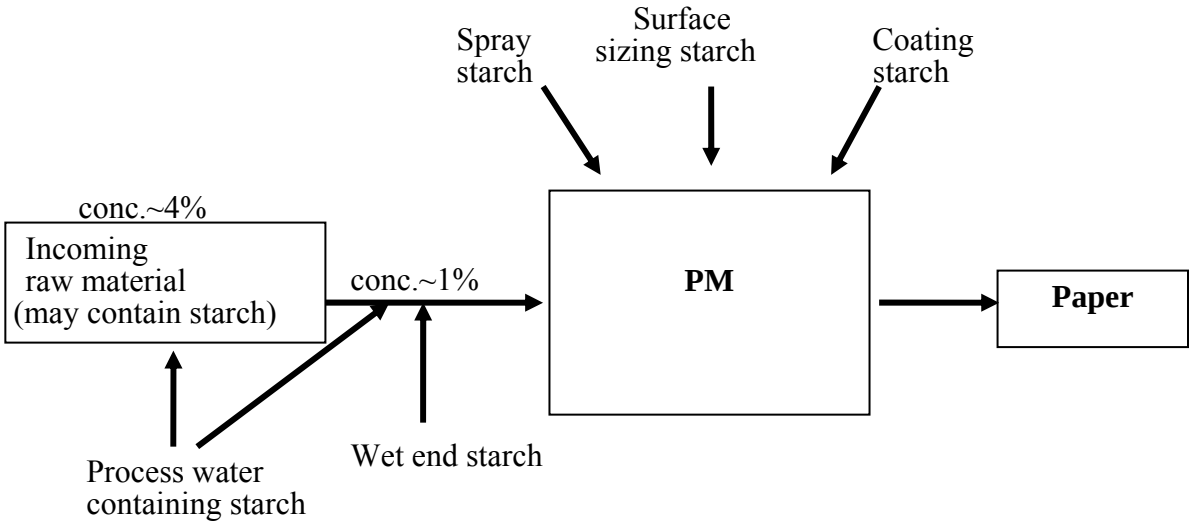

Figure B3 Example of different starch sources on a paper machine.

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