INTERNATIONAL STANDARD

ISO 8451

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Tobacco — Determination of starch content — Enzymatic method

Tabac — Détermination de la teneur en amidon — Méthode enzymatique

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International Standard ISO 8451 was prepared by Technical Committee ISO/TC 126, *Tobacco and tobacco products*, Sub-Committee SC 2, *Leaf tobacco*.

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Tobacco — Determination of starch content — Enzymatic method

1 Scope

This International Standard specifies a method for the determination of natural starch in tobacco and tobacco products. The method is applicable to all types of tobacco and tobacco products.

NOTES

1 Modified kinds of starch (phosphorylized or oxidized) do not react.

2 If this method is used for tobaccos or tobacco products that have been cased with sugars, determine glucose before hydrolysis and then correct the glucose determination after analysis for the proper determination of starch content.

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2 Definition

For the purposes of this International Standard, the following definition applies.

2.1 starch content of tobacco: The content of the substances determined by the procedure specified in this International Standard and expressed as a percentage of starch (m/m).

3 Reactions

In the presence of the enzyme amyloglucosidase (AGS), starch is hydrolyzed to glucose at pH 4,6 [equation (1)].

$$\text{Starch} + (n-1)\,(H_2O) \xrightleftarrows{\text{AGS}} n\ \text{glucose} \qquad \ldots (1)$$

The glucose formed is determined with hexokinase (HK) and glucose-6-phosphate dehydrogenase (G6P-DH) at pH 7,6. Glucose is phosphorylated to glucose-6-phosphate (G-6-P) by adenosine-5′-triphosphate (ATP) [equation (2)] in the presence of hexokinase.

$$\text{Glucose} + \text{ATP} \xrightleftarrows{\text{HK}} \text{G-6-P} + \text{ADP} \qquad \ldots (2)$$

In the presence of G6P-DH, glucose-6-phosphate is oxidized by nicotinamide adenine dinucleotide phosphate (NADP) to gluconate-6-phosphate (G-6-P) with formation of the reduced form of NADP (NADPH) [equation (3)].

$$\text{G-6-P} + \text{NADP}^+ \xrightleftarrows{\text{G6P-DH}} \text{gluconate-6-phosphate} + \text{NADPH} + \text{H}^+ \qquad \ldots (3)$$

The amount of NADPH formed in the above reaction is stoichiometric with the amount of glucose. NADPH is determined by means of its absorbance at 334 nm, 340 nm or 365 nm.

4 Reagents and preparation of the solutions

4.1 Reagents

Use only reagents of recognized analytical grade and freshly redistilled water in glassware.

4.1.1 Citric acid monohydrate, $C_6H_8O_7.H_2O$.

4.1.2 Trisodium citrate dihydrate, $C_6H_5Na_3O_7.2H_2O$.

4.1.3 Amyloglucosidase, AGS, about 6 units/mg lyophilisate.

4.1.4 Triethanolamine hydrochloride, $C_6H_{15}NO_3.HCl$.

4.1.5 Magnesium sulfate heptahydrate, $MgSO_4.7H_2O$.

4.1.6 Sodium hydroxide solution, $c(NaOH) = 5$ mol/l.

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