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Wheat — Identification of varieties by electrophoresis

Blé — Identification des variétés par électrophorèse



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Foreword

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International Standard ISO 8981 was prepared by Technical Committee ISO/TC 34, *Agricultural food products*, Sub-Committee SC 4, *Cereals and pulses*.

Annex A forms an integral part of this International Standard. Annex B is for information only.

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International Organization for Standardization
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Introduction

The protein composition of wheat results from direct genetic control and in general is not affected by environmental conditions (e.g. location or year of growth). In addition, because wheat is essentially a self-pollinating plant, the protein composition of the different varieties of wheat remains stable for several plant generations. Therefore, the protein composition of a wheat can be used to characterize and thus to identify its variety.

Protein profiles can be obtained by carrying out polyacrylamide gel electrophoresis (PAGE) separations of the wheat gliadins. The polyacrylamide gels are stained to make the separated protein components visible. If such protein profiles are established for all wheat varieties which can be expected to occur in a particular region (i.e. a variety catalogue is prepared), the identification of an unknown variety of wheat can be established by reference to such a catalogue. Such a practice has been thoroughly characterized and is in common use in numerous countries.

Wheat — Identification of varieties by electrophoresis

1 Scope

This International Standard specifies a method for the identification of the variety of a given lot of soft or hard wheat, in the form of individual ground kernels, flour farina or semolina, by the separation of gliadin proteins.

2 Principle

The separation of gliadin wheat proteins by polyacrylamide gel electrophoresis (PAGE) in slab gels containing aluminium lactate buffer, pH 3,1.

3 Reagents

Use only reagents of recognized analytical grade unless otherwise specified. The water used shall be deionized water having a resistance greater than 10 M Ω .

3.1 Extraction solution, ethanol, 70 % (V/V).

Dilute 700 ml of ethanol (absolute) with 300 ml of water.

3.2 Buffer solution, aluminium lactate, 2,5 g/l solution.

Dissolve 15,0 g of aluminium lactate in 5,5 litre of water. Adjust the pH to 3,1 with lactic acid. Make up to 6 litre with water. Vacuum filter through a filter of pore size 0,45 μ m.

Store at 4 °C.

NOTE 1 The conductivity of the solution should be approximately 950 μ S/m.

3.3 Sample dilution buffer

Dissolve 60,0 g of sucrose in 50 ml of buffer solution (3.2).

Store at 4 °C.

3.4 Acrylamide, 60,0 g/l gel solution.

WARNING — Acrylamide monomer is a neurotoxic substance which can be absorbed through the skin. Caution should be taken when handling either the crystalline powder or the gel solution.

Dissolve, in a 100 ml one-mark volumetric flask, 6,0 g of acrylamide (electrophoresis grade), 0,300 g of *N,N*-methylene bisacrylamide (electrophoresis grade) and 0,020 g of ascorbic acid in the buffer solution (3.2).

Make up to the mark with the buffer solution.

Store at 4 °C.

3.5 Initiator solution, iron(II) sulfate heptahydrate, 10 g/l solution.

Dissolve, in a 10 ml one-mark volumetric flask, 0,1 g of iron(II) sulfate heptahydrate in water and make up to the mark with water.

Prepare this solution shortly before use.

3.6 Catalyst solution, hydrogen peroxide, 0,99 % (m/m) solution.

Dissolve, in a 10 ml one-mark volumetric flask, 0,33 ml of hydrogen peroxide [30 % (m/m)] in water.

Make up to the mark with water.

Prepare a fresh solution daily and store at 4 °C.

3.7 Trichloroacetic acid, 9,6 % (m/m) solution.

Dissolve, in a 1 000 ml one-mark volumetric flask, 96 g of trichloroacetic acid in water.

Make up to the mark with water.

3.8 Brilliant blue R250, 5,0 g/l stock solution.

Dissolve 5,0 g of Brilliant blue in 1 litre of ethanol, stir for 1 h, and then filter through a filter paper (4.10) to remove any inorganic salts present.