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Flour from wheat (*Triticum aestivum* L.) — Amperometric method for starch damage measurement

*Farine de blé tendre (*Triticum aestivum *L.) — Méthode ampérométrique pour le mesurage de l'endommagement de l'amidon*

Reference number
ISO 17715:2013(E)

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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.

International Standards are drafted in accordance with the rules given in the ISO/IEC Directives, Part 2.

The main task of technical committees is to prepare International Standards. Draft International Standards adopted by the technical committees are circulated to the member bodies for voting. Publication as an International Standard requires approval by at least 75 % of the member bodies casting a vote.

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ISO 17715 was prepared by Technical Committee ISO/TC 34, *Food products*, Subcommittee SC 4, *Cereals and pulses*.

Introduction

Damaged starch content is an important parameter in flour quality as it directly impacts the flour water absorption capacity and therefore its use in the agri-food industry.

In the past, a number of methods based on various principles were developed to estimate such content, but comparing the results is difficult due to the different principles and units of measurement used.

A laboratory device is dedicated to the determination of damaged starch content using an amperometric method and which offers a choice of units of measurement according to individual references.

Flour from wheat (*Triticum aestivum* L.) — Amperometric method for starch damage measurement

1 Scope

This International Standard specifies the determination of the damage to starch using an amperometric method.

It is applicable to all flour samples from industrial or laboratory milling of wheat (*Triticum aestivum* L.).

NOTE 1 Wheat can be milled in the laboratory according to the methods described in ISO 27971[9] or in BIPEA guidance document BY.102.D.9302.[10]

NOTE 2 In the absence of validity studies, the results on semi-wholemeal or wholemeal flour, although able to meet the conditions of repeatability given in Clause 9, require careful interpretation.

2 Terms and definitions

For the purposes of this document, the following terms and definitions apply.

2.1
damaged starch
starch granules present in wheat flour mechanically damaged during milling, leading to a greater capacity to absorb water and increasing susceptibility to amylolytic enzymes

Note 1 to entry: Too high a damaged starch content has a negative effect on quality of flours.

3 Principle

Determination of damaged starch content of a flour sample by measurement of iodine absorption kinetics in an aqueous medium using an amperometric electrode.

The amperometric method is based on the existing proportionality between iodine absorption capacity and starch damage content.

4 Reagents

Use only reagents of recognized analytical grade, unless otherwise specified.

4.1 Water, osmosed or demineralized or at least equivalent grade.

4.2 Boric acid or **citric acid**, powdered, for testing.

WARNING — The use of boric acid involves hazardous operations. This document does not purport to address all the safety problems, if any, associated with its use. It is the responsibility of the user to establish appropriate safety and health practices and to ensure complaince with any national regulatory conditions.

4.3 Potassium iodide, powdered, for testing.

4.4 Sodium thiosulfate, solution in water at 0,1 mol/l prepared from a ready-to-use vial containing 0,1 mol sodium thiosulfate, to be diluted with water (4.1) in a 1 l flask. Powdered sodium thiosulfate can

also be used where the concentration of the final solution is 0,1 mol/l. Protect the solution from light and use within 3 months.

5 Equipment

Usual laboratory apparatus and, in particular, the following.

5.1 Chopin SDmatic®,[1)] equipped with a reaction vessel and sample holder.

NOTE This International Standard has been developed using the Chopin SDmatic®.[1)] It does not apply to the SD4 Chopin and Rapid FT devices which also measure damaged starch content, but using different technology.

5.2 Laboratory scales, with a display accuracy of 10^{-2} g enabling weighing at 10^{-1} g accuracy.

5.3 Laboratory scales, with a display accuracy of 10^{-4} g enabling weighing at 10^{-3} g accuracy.

5.4 Piston distributor, delivering 120 ml distilled water to the nearest 0,5 ml.

5.5 One-mark volumetric flask, capacity 1 000 ml, ISO 1042,[2] class A.

6 Sampling

Sampling is not part of the method specified in this International Standard. A recommended sampling method is given in ISO 24333.[8]

It is important the laboratory receive a truly representative sample which has not been damaged or changed during transport or storage.

7 Procedure

7.1 Reagent weighing and dissolution

Weigh (5.2), to the nearest 0,5 g, 3,0 g boric acid (4.2) or 1,5 g citric acid (4.2) and 3,0 g (4.3) potassium iodide and add to a clean and dry reaction vessel (5.1). Add one drop (about 0,04 ml) of sodium thiosulfate solution (4.4) and dispense (5.4) 120 ml distilled water (4.1) into the vessel.

As the test begins with a heating and stirring phase, it is not necessary to obtain full dissolution of the reagents at this stage. In order to minimize losses during transfer, add powdered reagents directly to the reaction vessel.

7.2 Sample weighing

Weigh (5.3), to the nearest 10^{-3} g, 1,000 g ± 0,100 g of the test sample of flour and place it in the pre-cleaned sample holder (5.1).

7.3 Test

Place the reaction vessel in the well on the device.

Lower the head of the device, and insert the sample holder containing the flour (7.2) into the compartment.

1) Chopin SDmatic® is the trade name of a product supplied by Chopin Technologies. This information is given for the convenience of users of this document and does not constitute an endorsement by ISO of the product named. Equivalent products may be used if they can be shown to lead to the same results.

Start the test. Indicate the exact mass of the test portion of flour weighed to the nearest 0,001 g. It is also possible to indicate the water and protein content of the sample if a result corrected on this basis is to be obtained, otherwise the default values should be left (mass fractions of 14 % and 12 %, respectively) for the two components. Confirm the start of the test.

The test lasts 6 min to 7 min. Ensure that all of the flour descends into the reaction vessel when the vibrator starts up. Use the tip of a brush or lightly blow to enable any remaining flour to fall.

Wait for the beep at the end of the test at which point the result is displayed.

7.4 Cleaning

Raise the head of the device and remove the reaction vessel. Rinse, then carefully and gently wipe the measuring electrode, the heating element and the stirrer.

Remove any residue from the vessel (do not dispose of in the sink). Carefully wash and wipe dry the reaction vessel, which shall be free of all traces of reagent, sample or moisture, and which shall be left ready for use in a later test.

7.5 Number of determinations

Perform two determinations on the same test sample.

8 Expression of results

The result is expressed as A_I % (iodine absorption percentage) converted into UCD (Chopin–Dubois units). Equations provided by the manufacturer can be used to calculate the equivalence in another unit.

The arithmetic mean of the two determinations (7.5) shall be taken as the result if they meet the conditions of repeatability specified in 9.2 or in Table A.5. Otherwise, perform two new determinations.

NOTE It can be useful to calculate starch damage on a constant water and protein content basis. In this case, flour moisture content and protein content can be determined in accordance with ISO 712[1] for moisture and ISO 20483[7] or ISO/TS 16634-2[6] for protein.

9 Precision

9.1 Interlaboratory tests

Two interlaboratory tests established the repeatability and reproducibility limits of the method. The statistical results of the study are given in Annex A.

The values of each of the studies apply to the concentration ranges and flours from wheat (*Triticum aestivum* L.*).*

9.2 Repeatability limits, *r*

Repeatability limit is the value below which the absolute value, of the difference between two test results obtained in conditions of repeatability is located, with a probability of 95 %.

The repeatability limits, *r*, are obtained from Formulae (1) and (2). Some repeatability limit values are listed in Table A.5.

For A_I %:

$$r = \left(-0{,}007\mu_{A_I\ \%} + 0{,}787\,1\right) \times 2{,}8 \qquad (1)$$

where $\mu_{A_I\ \%}$ is the mean iodine absorption capacity.

For UCD:

$$r = (-0{,}007\mu_{UCD} + 0{,}473\ 9) \times 2{,}8 \quad (2)$$

where μ_{UCD} is the mean Chopin–Dubois unit value.

9.3 Reproducibility limits, R

Reproducibility limit is the value below which the absolute value, of the difference between two test results obtained in conditions of reproducibility is located, with a probability of 95 %.

The reproducibility limits, R, are obtained from Formulae (3) and (4). Some reproducibility limit values are listed in Table A.6.

For A_I %:

$$R = \left(-0{,}03\mu_{A_I}\ \% + 3{,}074\,5\right) \times 2{,}8 \quad (3)$$

For UCD:

$$R = (-0{,}041\mu_{UCD} + 1{,}522\ 2) \times 2{,}8 \quad (4)$$

9.4 Critical difference, d_C

The critical difference is the deviation between two average values obtained from two test results under repeatability conditions.

9.4.1 Comparison of two measurement groups in the same laboratory

The critical difference for comparing two average values obtained from two test results in the same laboratory under repeatability conditions, $d_{C,\,r}$, is given by:

$$d_{C,r} = 2{,}8\, s_r \sqrt{\frac{1}{2n_1} + \frac{1}{2n_2}} = 2{,}8\, s_r \sqrt{\frac{1}{2}} = 1{,}98\, s_r \quad (5)$$

where

s_r is the repeatability standard deviation;

n_1, n_2 are the number of test results for each of the average values — here, n_1 and n_2 equal 2.

9.4.2 Comparison of two measurement groups in two different laboratories

The critical difference for comparing two average values obtained from two test results in two different laboratories under repeatability conditions, $d_{C,\,R}$, is equal to:

$$d_{C,R} = 2{,}8\sqrt{s_R^2 - s_r^2\left(1 - \frac{1}{2n_1} - \frac{1}{2n_2}\right)} = 2{,}8\sqrt{s_R^2 - 0{,}5s_r^2} \tag{6}$$

where:

s_r is the repeatability standard deviation;

s_R is the reproducibility standard deviation;

n_1, n_2 are the number of test results for each of the average values — here, n_1 and n_2 equal 2.

Some critical difference values between two laboratories are listed in Table A.7.

9.5 Uncertainty, u

Uncertainty, u, is a parameter characterizing the dispersion of values that may reasonably be attributed to the result. Uncertainty is established from statistical distribution of the results from the interlaboratory test and characterized by the experimental standard deviation.

For each parameter, uncertainty is equal to more or less twice the reproducibility standard deviation quoted in this International Standard.

For A_I %:

$$u = \left(-0{,}03\mu_{A_\mathrm{I}\ \%} + 3{,}074\,5\right) \times 2 \tag{7}$$

For UCD:

$$u = (-0{,}041\mu_{\mathrm{UCD}} + 1{,}522\ 2) \times 2 \tag{8}$$

10 Test report

The test report shall contain at least the following information:

a) all information necessary for the complete identification of the sample;

b) the sampling method used, if known;

c) the test method used, with reference to this International Standard (ISO 17715:2013);

d) all operating details not specified in this International Standard, or regarded as optional, together with details of any incidents which may have influenced the test result(s);

e) the test result(s) obtained;

f) if the repeatability has been checked, the final quoted result obtained.

Annex A
(informative)

Data from interlaboratory tests on wheat flour

Two interlaboratory tests have been done on this method. The first was organized in 2004-04 by Chopin Technologies and involved 15 internationally recognized laboratories. The second was conducted in 2012-02 in China by the State Administration of Grain and Chopin (Beijing) Trading Co., and involved 12 Chinese laboratories.

All the participants used a Chopin SDmatic®.[1)]

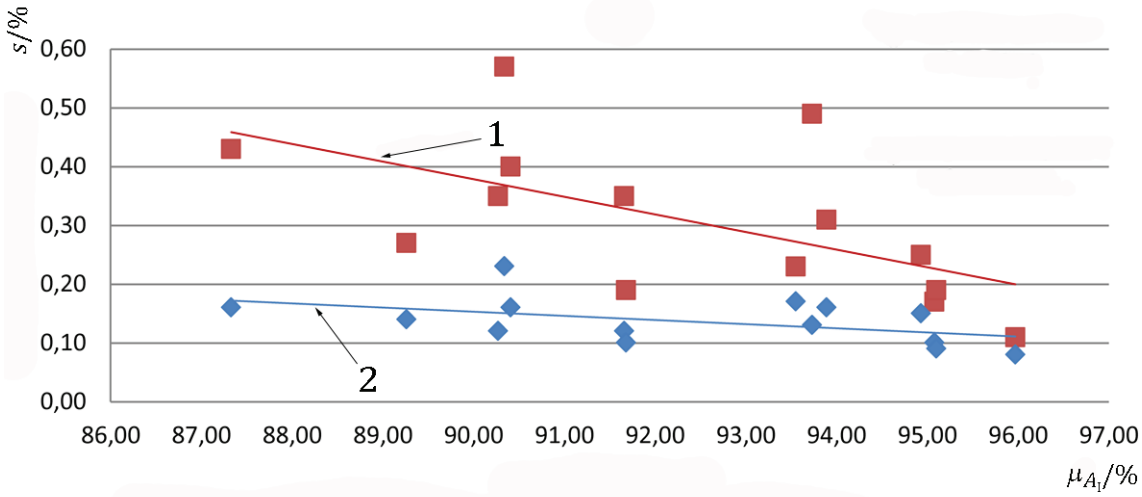
Testing was conducted according to the recommendations set out in ISO 5725–2,[3] ISO 5725–3,[4] and ISO 5725–6,[5] on eight wheat flour samples for the first interlaboratory test and six for the second, selected to cover a broad range of values for starch damage. The results of statistical analysis are given in Tables A.1 to A.7 and Figures A.1 and A.2.

Table A.1 — Statistical results for A_I % on wheat flour, 2004

Parameter	Flour							
	5	1	4	7	2	6	8	3
Number of laboratories or tests	15	15	15	15	15	15	15	15
Mean value, μ_{A_I} %	87,33	89,26	90,27	90,41	91,66	93,55	95,08	95,10
Repeatability standard deviation, s_r,%	0,16	0,14	0,12	0,16	0,12	0,17	0,1	0,09
Coefficient of variation, $C_{V,r}$ (s_r/μ_{A_I}), %	0,2	0,2	0,1	0,2	0,1	0,2	0,1	0,1
Repeatability limit, r (2,8 × s_r)	0,43	0,4	0,32	0,45	0,33	0,48	0,27	0,25
Reproducibility standard deviation, s_R, %	0,43	0,27	0,35	0,4	0,35	0,23	0,17	0,19
Coefficient of variation, $C_{V,R}$ (s_R/μ_{A_I}), %	0,5	0,3	0,4	0,5	0,4	0,3	0,2	0,2
Reproducibility limit, R (2,8 × s_R)	1,19	0,75	0,96	1,12	0,98	0,65	0,48	0,54

Table A.2 — Statistical results for A_I % on wheat flour, 2012

Parameter	Flour					
	1	5	2	3	4	6
Number of laboratories or tests	11	11	11	10	10	9
Mean value, μ_{A_I} %	90,34	91,68	93,73	93,90	94,93	95,97
Repeatability standard deviation, s_r,%	0,23	0,1	0,13	0,16	0,15	0,08
Coefficient of variation, $C_{V,r}$ (s_r/μ_{A_I}), %	0,3	0,1	0,1	0,2	0,2	0,1
Repeatability limit, r (2,8 × s_r)	0,6	0,3	0,4	0,4	0,4	0,2
Reproducibility standard deviation, s_R, %	0,57	0,19	0,49	0,31	0,25	0,11
Coefficient of variation, $C_{V,R}$ (s_R/μ_{A_I}), %	0,6	0,2	0,5	0,3	0,3	0,1
Reproducibility limit, R (2,8 × s_R)	1,6	0,5	1,4	0,9	0,7	0,3



Key

s	standard deviation	1	standard deviation of reproducibility, $s_R = -0{,}03\ \mu_{A_I} + 3{,}074\ 5 \quad R^2 = 0{,}351$
μ_{A_I}	mean iodine absorption capacity	2	standard deviation of repeatability, $s_r = -0{,}007\ \mu_{A_I} + 0{,}787\ 1 \quad R^2 = 0{,}215\ 6$

Figure A.1 — Relationship between precision standard deviation and mean iodine absorption capacity

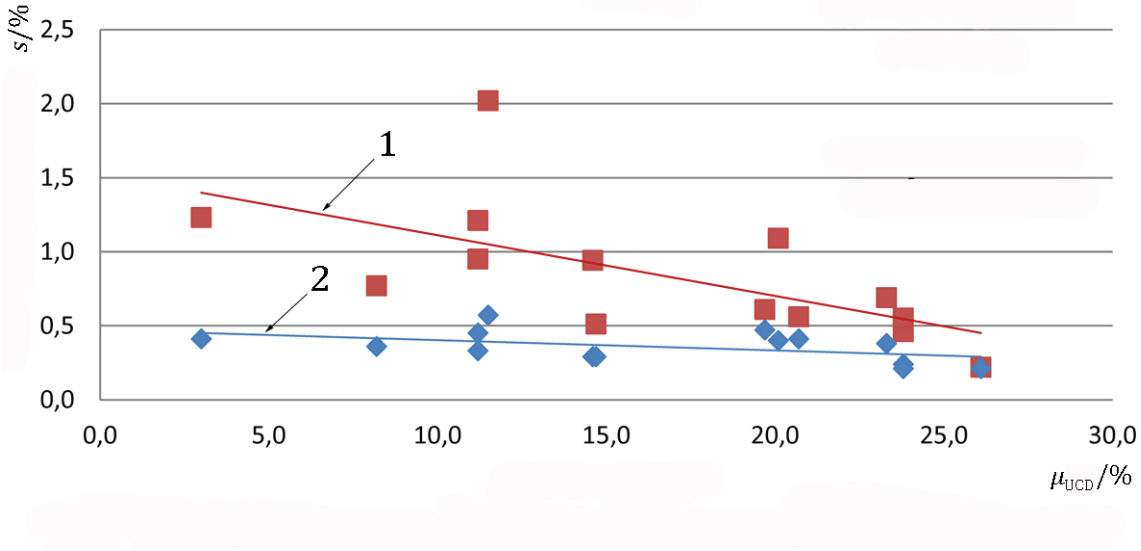
Repeatability and reproducibility standard deviations are inversely proportional to the average value.

Table A.3 — Statistical results for UCD on wheat flour, 2004

Parameter	Flour							
	5	1	7	4	2	6	8	3
Number of laboratories or tests	15	15	15	15	15	15	15	15
Mean value, μ_{UCD}, %	3,0	8,2	11,2	11,2	14,6	19,7	23,8	23,8
Repeatability standard deviation, s_r,%	0,4	0,4	0,5	0,3	0,3	0,5	0,2	0,2
Coefficient of variation, $C_{V,r}$ (s_r/μ_{UCD}), %	13,5	4,4	4,0	2,9	2,0	2,4	1,0	0,9
Repeatability limit, r (2,8 × s_r)	1,1	1,0	1,2	0,9	0,8	1,3	0,7	0,6
Reproducibility standard deviation, s_R, %	1,2	0,8	1,2	1	0,9	0,6	0,5	0,6
Coefficient of variation, $C_{V,R}$ (s_R/μ_{UCD}), %	40,5	9,4	10,8	8,4	6,5	3,1	1,9	2,3
Reproducibility limit, R (2,8 × s_R)	3,4	2,1	3,4	2,6	2,6	1,7	1,3	1,5

Table A.4 — Statistical results for UCD on wheat flour, 2012

Parameter	Flour					
	1	5	2	3	4	6
Number of laboratories or tests	11	11	11	10	10	9
Mean value, μ_{UCD}, %	11,5	14,7	20,1	20,7	23,3	26,1
Repeatability standard deviation, s_r,%	0,57	0,29	0,40	0,41	0,38	0,21
Coefficient of variation, $C_{V,\,r}$ (s_r/μ_{UCD}), %	5,0	2,0	2,0	2,0	1,6	0,8
Repeatability limit, r (2,8 × s_r)	1,6	0,8	1,1	1,1	1,1	0,6
Reproducibility standard deviation, s_R, %	2,02	0,51	1,09	0,56	0,69	0,22
Coefficient of variation, $C_{V,\,R}$ (s_R/μ_{UCD}), %	17,6	3,5	5,4	2,7	3,0	0,8
Reproducibility limit, R (2,8 × s_R)	5,6	1,4	3,0	1,6	1,9	0,6



Key

s	standard deviation	1	standard deviation of reproducibility, $s_R = -0{,}041\mu_{UCD} + 1{,}522\,2$ $R^2 = 0{,}396\,4$
μ_{UCD}	mean Chopin Dubois unit value	2	standard deviation of repeatability, $s_r = -0{,}007\mu_{UCD} + 0{,}473\,9$ $R^2 = 0{,}214\,4$

Figure A.2 — Relationship between precision standard deviation and mean Chopin–Dubois unit value

Repeatability and reproducibility standard deviations are inversely proportional to the average value.

Table A.5 — Derived repeatability limits, *r*, on wheat flour

Iodine absorption capacity, % Validity range: 87,33 to 95,97 $s_r = -0{,}007\ \mu_{A_I} + 0{,}787\ 1$		**Chopin–Dubois unit value** Validity range; 3,0 to 26,1 $s_r = -0{,}007\mu_{UCD} + 0{,}473\ 9$	
μ_{A_I} %	Repeatability limit ($r = s_r \times 2{,}8$)	μ_{UCD}	Repeatability limit ($r = s_r \times 2{,}8$)
87,30	0,49	3,0	1,3
87,50	0,48	3,5	1,2
87,70	0,48	4,0	1,2
87,90	0,48	4,5	1,2
88,10	0,47	5,0	1,2
88,30	0,47	5,5	1,2
88,50	0,46	6,0	1,2
88,70	0,46	6,5	1,2
88,90	0,46	7,0	1,2
89,10	0,45	7,5	1,2
89,30	0,45	8,0	1,2
89,50	0,44	8,5	1,1
89,70	0,44	9,0	1,1
89,90	0,44	9,5	1,1
90,10	0,43	10,0	1,1
90,30	0,43	10,5	1,1
90,50	0,43	11,0	1,1
90,70	0,42	11,5	1,1
90,90	0,42	12,0	1,1
91,10	0,41	12,5	1,1
91,30	0,41	13,0	1,1
91,50	0,41	13,5	1,1
91,70	0,40	14,0	1,0
91,90	0,40	14,5	1,0
92,10	0,39	15,0	1,0
92,30	0,39	15,5	1,0
92,50	0,39	16,0	1,0
92,70	0,38	16,5	1,0
92,90	0,38	17,0	1,0
93,10	0,38	17,5	1,0
93,30	0,37	18,0	1,0
93,50	0,37	18,5	1,0
93,70	0,36	19,0	0,9
93,90	0,36	19,5	0,9
94,10	0,36	20,0	0,9
94,30	0,35	20,5	0,9
94,50	0,35	21,0	0,9
94,70	0,34	21,5	0,9
94,90	0,34	22,0	0,9
95,10	0,34	22,5	0,9
95,30	0,33	23,0	0,9

Table A.5 *(continued)*

Iodine absorption capacity, % Validity range: 87,33 to 95,97 $s_r = -0{,}007\ \mu_{A_I} + 0{,}787\ 1$		**Chopin–Dubois unit value** Validity range; 3,0 to 26,1 $s_r = -0{,}007\mu_{UCD} + 0{,}473\ 9$	
μ_{A_I} %	Repeatability limit ($r = s_r \times 2{,}8$)	μ_{UCD}	Repeatability limit ($r = s_r \times 2{,}8$)
95,50	0,33	23,5	0,9
		24,0	0,8

Table A.6 — Derived reproducibility limits, *R*, on wheat flour

Iodine absorption capacity, % Validity range: 87,33 to 95,97 $s_R = -0{,}03\ \mu_{A_I} + 3{,}074\ 5$		**Chopin–Dubois unit value** Validity range; 3,0 to 26,1 $s_R = -0{,}033\ 2\mu_{UCD} + 1{,}319\ 1$	
μ_{A_I} %	Reproducibility limit ($R = s_R \times 2{,}8$)	μ_{UCD}	Reproducibility limit ($R = s_R \times 2{,}8$)
87,30	1,26	3,0	3,9
87,50	1,25	3,5	3,8
87,70	1,23	4,0	3,8
87,90	1,21	4,5	3,7
88,10	1,20	5,0	3,6
88,30	1,18	5,5	3,6
88,50	1,16	6,0	3,5
88,70	1,15	6,5	3,5
88,90	1,13	7,0	3,4
89,10	1,11	7,5	3,4
89,30	1,10	8,0	3,3
89,50	1,08	8,5	3,3
89,70	1,06	9,0	3,2
89,90	1,05	9,5	3,1
90,10	1,03	10,0	3,1
90,30	1,01	10,5	3,0
90,50	1,00	11,0	3,0
90,70	0,98	11,5	2,9
90,90	0,96	12,0	2,9
91,10	0,95	12,5	2,8
91,30	0,93	13,0	2,7
91,50	0,91	13,5	2,7
91,70	0,90	14,0	2,6
91,90	0,88	14,5	2,6
92,10	0,86	15,0	2,5
92,30	0,85	15,5	2,5
92,50	0,83	16,0	2,4
92,70	0,81	16,5	2,3
92,90	0,80	17,0	2,3
93,10	0,78	17,5	2,2
93,30	0,76	18,0	2,2
93,50	0,75	18,5	2,1

Table A.6 *(continued)*

Iodine absorption capacity, % Validity range: 87,33 to 95,97 $s_R = -0{,}03\ \mu_{A_I} + 3{,}074\ 5$		**Chopin–Dubois unit value** Validity range; 3,0 to 26,1 $s_R = -0{,}033\ 2\mu_{UCD} + 1{,}319\ 1$	
μ_{A_I} %	Reproducibility limit ($R = s_R \times 2{,}8$)	μ_{UCD}	Reproducibility limit ($R = s_R \times 2{,}8$)
93,70	0,73	19,0	2,1
93,90	0,71	19,5	2,0
94,10	0,70	20,0	1,9
94,30	0,68	20,5	1,9
94,50	0,66	21,0	1,8
94,70	0,65	21,5	1,8
94,90	0,63	22,0	1,7
95,10	0,61	22,5	1,7
95,30	0,60	23,0	1,6
95,50	0,58	23,5	1,5
		24,0	1,5

Table A.7 — Derived critical differences, d_C, in two laboratories

Iodine absorption capacity, % Validity range: 87,33 to 95,97		**Chopin–Dubois unit value** Validity range; 3,0 to 26,1	
μ_{A_I} %	Critical difference between two laboratories	μ_{UCD}	Critical difference between two laboratories
	d_c		d_c
87,3	1,23	3,0	3,81
87,5	1,21	3,5	3,76
87,7	1,19	4,0	3,70
87,9	1,18	4,5	3,64
88,1	1,16	5,0	3,58
88,3	1,14	5,5	3,53
88,5	1,13	6,0	3,47
88,7	1,11	6,5	3,41
88,9	1,09	7,0	3,35
89,1	1,08	7,5	3,30
89,3	1,06	8,0	3,24
89,5	1,04	8,5	3,18
89,7	1,03	9,0	3,12
89,9	1,01	9,5	3,07
90,1	0,99	10,0	3,01
90,3	0,98	10,5	2,95
90,5	0,96	11,0	2,89
90,7	0,94	11,5	2,84
90,9	0,93	12,0	2,78
91,1	0,91	12,5	2,72
91,3	0,89	13,0	266
91,5	0,88	13,5	2,61
91,7	0,86	14,0	2,55

Table A.7 *(continued)*

Iodine absorption capacity, % Validity range: 87,33 to 95,97		**Chopin–Dubois unit value** Validity range; 3,0 to 26,1	
μ_{A_1} %	Critical difference between two laboratories	μ_{UCD}	Critical difference between two laboratories
	d_c		d_c
91,9	0,84	14,5	2,49
92,1	0,83	15,0	2,43
92,3	0,81	15,5	2,38
92,5	0,79	16,0	2,32
92,7	0,77	16,5	2,26
92,9	0,76	17,0	2,20
93,1	0,74	17,5	2,14
93,3	0,72	18,0	2,08
93,5	0,71	18,5	2,03
93,7	0,69	19,0	1,97
93,9	0,67	19,5	1,91
94,1	0,66	20,0	1,85
94,3	0,64	20,5	1,79
94,5	0,62	21,0	1,73
94,7	0,61	21,5	1,68
94,9	0,59	22,0	1,62
95,1	0,57	22,5	1,56
95,3	055	23,0	1,50
95,5	0,54	23,5	1,44
		24,0	1,38

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