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**Instant coffee — Determination of free
and total carbohydrate contents —
Method using high-performance
anion-exchange chromatography**

*Café soluble — Détermination des teneurs en hydrates de carbone libres
et totaux — Méthode par chromatographie d'échange d'anions à haute
performance*



Reference number
ISO 11292:1995(E)

Foreword

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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.

International Standard ISO 11292 was prepared by Technical Committee ISO/TC 34, *Agricultural food products*, Subcommittee SC 15, *Coffee*.

Annexes A and B of this International Standard are for information only.

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Instant coffee — Determination of free and total carbohydrate contents — Method using high-performance anion-exchange chromatography

1 Scope

This International Standard specifies a method for the determination of free and total carbohydrate contents in instant coffee using high-performance anion-exchange chromatography. In particular, it determines the content of individual monosaccharides, sucrose and mannitol.

2 Normative references

The following standards contain provisions which, through reference in this text, constitute provisions of this International Standard. At the time of publication, the editions indicated were valid. All standards are subject to revision, and parties to agreements based on this International Standard are encouraged to investigate the possibility of applying the most recent editions of the standards indicated below. Members of IEC and ISO maintain registers of currently valid International Standards.

ISO 1042:1983, *Laboratory glassware — One-mark volumetric flasks*.

ISO 3509:1989, *Coffee and its products — Vocabulary*.

ISO 3726:1983, *Instant coffee — Determination of loss in mass at 70 °C under reduced pressure*.

3 Definitions

For the purposes of this International Standard, the definitions given in ISO 3509 and the following definitions apply.

3.1 free carbohydrate content: Content of each individual monosaccharide (arabinose, fructose, galactose, glucose, mannose), and the sucrose and mannitol contents, determined under the conditions described (method A). Content is expressed as a percentage by mass on a dry basis.

3.2 total carbohydrate content: Content of each individual monosaccharide (arabinose, galactose, glucose, mannose, xylose) and the mannitol content, determined under the conditions described, which includes a strong hydrolysis step (method B). Content is expressed as a percentage by mass on a dry basis.

4 Principle

4.1 Method A

Dissolution of a test portion in water. Separation of the carbohydrates present in the filtered extract by ion chromatography on a high-performance anion-exchange column (HPAEC) using pure water as eluent. Electrochemical detection of the eluted compounds by means of a pulsed amperometric detector (PAD) and quantification by comparison with peak areas given by standard solutions.

4.2 Method B

Hydrolysis of a test portion with aqueous hydrochloric acid. Analysis of the carbohydrates present in the filtered hydrolysed solution as described in method A.

5 Reagents

Use only reagents of recognized analytical grade, unless otherwise specified, and distilled or demineralized water or water of equivalent purity.

5.1 Sodium hydroxide (NaOH), 50 % (m/m) aqueous solution.

The reagent should contain the minimum amount of sodium carbonate and mercury. Do not shake or stir the solution before use.

5.2 Hydrochloric acid (HCl), 1,00 mol/l standard volumetric solution.

5.3 Eluent 1 (S1), demineralized water (18 M Ω -cm).

Filter the demineralized water through 0,2 μ m membrane filters. Degas by sparging with helium for between 20 min and 30 min.

5.4 Eluent 2 (S2), sodium hydroxide (NaOH), 300 mmol/l solution.

To 985 ml of degassed water (5.3), pipette 15,6 ml of the sodium hydroxide solution (5.1).

CAUTION — It is extremely important to remove dissolved carbon dioxide from the eluents prior to use. Carbonate will act as a strong "pusher" on the column, resulting in a drastic reduction in resolution and efficiency. Prepare the solution the day before the analysis.

5.5 Carbohydrate standard solutions.

Prepare fresh solutions of arabinose, fructose, galactose, glucose, mannose, sucrose and mannitol.

Weigh, to the nearest 0,1 mg, approximately 100 mg of each carbohydrate into separate 100 ml volumetric flasks (6.2) and dilute to the mark with water (stock standard solutions of 1 000 mg/l).

Mixed standard solutions can also be prepared from separate stock solutions once the retention time of each carbohydrate is known under the prevailing chromatographic conditions.

Further dilute the standard solutions to reach carbohydrate concentrations similar to those found in the non-hydrolysed or hydrolysed instant coffee sample solutions.

The resolution of rhamnose from arabinose is difficult to achieve. If these two monosaccharides coelute, do not add rhamnose in a mixed standard solution.

6 Apparatus

Usual laboratory apparatus and, in particular, the following.

6.1 Analytical balance, capable of weighing to an accuracy of $\pm 0,1$ mg.

6.2 One-mark volumetric flasks, of capacity 100 ml (in accordance with class A of ISO 1042).

6.3 Graduated cylinders, of capacities 1 000 ml and 50 ml, tall form.

6.4 Vacuum filtration system.

6.5 Folded filter papers, medium fast, qualitative.

6.6 Disposable C18 filter cartridges¹⁾, to be used according to the manufacturer's recommendations.

6.7 Disposable membrane filters, 0,2 μ m pore size.

6.8 Water bath, capable of being maintained at 100 °C \pm 5 °C.

1) Sep-Pack C18 (Waters) and Supelclean LC-18 (Supelco) are examples of suitable products available commercially. This information is given for the convenience of users of this International Standard and does not constitute an endorsement by ISO of these products.

6.9 Metal-free liquid chromatograph²⁾, with a high-performance anion-exchange column³⁾ filled with pellicular polystyrene-divinylbenzene resin and pre-column (guard column)⁴⁾ and postcolumn delivery system.

6.10 Pulsed amperometric detector (PAD) with gold electrode⁵⁾.

6.11 Integrator chromatography data station⁶⁾.

6.12 Disposable cartridges⁷⁾, to be used according to the manufacturer's recommendations.

7 Sampling

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

Sampling is not part of the method specified in this International Standard. A recommended sampling method is given in ISO 6670:1983, *Instant coffee in cases with liners — Sampling*.

When the instant coffee is not in cases with liners, take a well-mixed representative sample from single packed units.

8 Procedure

8.1 Determination of dry matter

Calculate the dry matter determined on a portion of the laboratory sample in accordance with ISO 3726.

8.2 Preparation of sample for analysis — Method A

Weigh, to the nearest 0,1 mg, approximately 300 mg of the laboratory sample directly into a 100 ml volumetric flask (6.2). Add, using a graduated cylinder (6.3), 70 ml of water and shake until dissolution is

complete. Dilute to the mark with water. Filter 5 ml to 10 ml of this solution through a cartridge (6.6). Discard the first few millilitres.

8.3 Preparation of sample for analysis — Method B

Weigh, to the nearest 0,1 mg, approximately 300 mg of the laboratory sample directly into a 100 ml volumetric flask (6.2). Add 50 ml of the hydrochloric acid (5.2) and swirl. Place the flask in a boiling water bath (6.8) for 150 min.

Keep the level of the sample solution always below that of the water in the bath. Swirl the solution by hand every 30 min. Cool to room temperature by passing the flask under tap water. Dilute to the mark with water and filter the solution through a folded filter paper (6.5). Pass 3 ml of the filtrate through a disposable cartridge (6.12). Discard the first millilitre.

8.4 Chromatographic analysis

Set up the chromatograph (6.9), detector (6.10) and integrator (6.11).

Allow the chromatograph to equilibrate.

Filter the standard solutions (5.5) and the test solutions (8.2 or 8.3) through 0,2 µm membrane filters (6.7).

Inject the same volume of filtered standard and test solutions into the chromatograph and separate carbohydrates under the conditions given in tables 1 and 2.

Identify and quantify carbohydrates in the sample solution by comparison with retention times and areas of corresponding peaks obtained using the standard solution.

Inject a standard solution every four injections, in order to account for any changes in retention times or peak integrations.

2) The BioLC system (Dionex) consisting of a model GPM-II quarternary gradient pump (Dionex) with a model SP8875 autosampler (Spectra - Physics) filled with a 20 µl loop, model EDM-II eluent degas module (Dionex) and reagent reservoir for NaOH postcolumn addition (Dionex) are examples of suitable equipment available commercially.

3) CarboPac PA1 (10 µm, 250 mm × 4 mm) (Dionex) is an example of a suitable analytical column available commercially.

4) CarboPac PA (Dionex) is an example of a suitable precolumn available commercially.

5) Model PAD-II (Dionex) is an example of suitable equipment available commercially.

6) Model Autolon AI-450 is an example of suitable equipment available commercially.

7) On Guard-AG (Dionex) is an example of a suitable cartridge commercially available.

This information is given for the convenience of users of this International Standard and does not constitute an endorsement by ISO of these products.

Table 1 — Preparation of column

Eluent	Time min	Eluent S1 ml	Eluent S2 ml	Procedure
Isocratic	0	100	0	Start data acquisition
	50,0	100	0	Stop data acquisition
	50,1	0	100	Start clean-up
	65,0	0	100	Stop clean-up
	65,1	100	0	Start re-equilibrium
	80,0	100	0	Stop re-equilibrium

NOTES

1) Retention times tend to vary from one column to another. Start clean-up only when the last monosaccharide (ribose) has been eluted.

2) It may be necessary to perform two or three injections of standard solution or to increase the re-equilibrium time in order to achieve a good separation of sucrose and xylose.

Table 2 — Conditions for analysis

Injection	20 µl
Flowrate	1,0 ml/min
Postcolumn addition	Eluent S2 (5.4) at a flowrate of 0,6 ml/min
Temperature	Ambient
Detector	Fill up the reference cell with Eluent S2 (5.4). Use the optimum conditions given by the manufacturer.

9 Calculation

The carbohydrate content, ω , expressed as a percentage by mass, is equal to

$$\omega = \frac{A \cdot m_0 \cdot V}{A_0 \cdot m \cdot V_0} \times 100$$

where

- A is the peak area of the individual carbohydrate in the test solution (8.4);
- A_0 is the peak area of the individual carbohydrate in the standard solution (8.4);

m is the mass, in grams, of the test portion in the test solution (8.2 or 8.3), expressed on a dry basis;

m_0 is the mass, in grams, of the carbohydrate in the standard solution (5.5);

V is the volume, in millilitres, of the test solution (8.2 or 8.3);

V_0 is the volume, in millilitres, of the standard solution (5.5).

Take as the result the arithmetic mean of the two determinations. Express the result either as free (method A) or total (method B) carbohydrate content to the nearest 0,01 % (m/m) for each carbohydrate of interest, or the total of all carbohydrates detected.

10 Precision

Details of an interlaboratory test on the precision of the method are summarized in annex A. The values derived from this interlaboratory test may not be applicable to concentration ranges and matrices other than those given.

The values of repeatability and reproducibility for each carbohydrate show a marked relationship with the wide percentage concentration of each carbohydrate, which in many cases in the samples examined was very low. This is reflected especially in some poor reproducibility figures, as might be expected. However, with contents at higher concentrations, both precision values are acceptable, as is also indicated in annex A.

10.1 Repeatability

Repeatability is defined as the absolute difference between two independent single test results obtained on identical test material in the same laboratory by the same operator using the same equipment within a short interval of time.

It is not possible therefore to give an exact repeatability figure for each and every carbohydrate, both free and total, for a full range of possible percentage contents.

Provided, however, that the content of a given carbohydrate is greater than 0,3 % (*m/m*), the data show that the relative standard deviation averages 4,5 %.

10.2 Reproducibility

Reproducibility is defined as the absolute difference between two single test results, obtained using the same method on identical test material in different

laboratories with different operators using different equipment.

Again it is not possible to give an exact reproducibility figure for each and every carbohydrate, both free and total, for a full range of possible carbohydrate contents.

Provided, however, that the content of a given carbohydrate is greater than 0,3 % (*m/m*), the data show that the relative standard deviation averages 14,3 % (except for fructose data). At a content of less than 0,3 % (*m/m*), the coefficient of variation increases very sharply.

For each carbohydrate, the reproducibility and repeatability ranges as found in the interlaboratory test and the applied mean content range are given in table 3.

11 Test report

The test report shall specify

- the method in accordance with which sampling was carried out, if known,
- the method used,
- the result obtained for each carbohydrate of interest,
- if the repeatability has been checked, the final quoted result obtained.

It shall also mention 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.

The test report shall include all information necessary for the complete identification of the sample.

Table 3 — Coefficient of variation of reproducibility and repeatability

Carbohydrate	Mean content range applied in test % (m/m) d.b.	Range of coefficient of variation of reproducibility, %	Range of coefficient of variation of repeatability, %
Free mannitol	0,02 to 1,6	59,5 to 9,9	0,9 to 8,2
Free arabinose	0,46 to 1,3	13,8 to 5,1	1,6 to 7,3
Total arabinose	3,5 to 4,8	21,1 to 4,9	6,6 to 3,0
Free galactose	0,19 to 0,56	13,0 to 4,1	9,8 to 3,0
Total galactose	8,1 to 18,5	7,5 to 12,9	8,1 to 1,7
Free glucose	0,04 to 2,0	23,8 to 6,1	10,2 to 2,5
Total glucose	0,68 to 16,6	12,5 to 24,3	8,7 to 3,8
Free mannose	0,16 to 1,0	40,0 to 16,9	8,2 to 3,8
Total mannose	2,6 to 19,1	10,6 to 21,7	2,0 to 5,8
Total xylose	0,1 to 1,9	37,7 to 20,2	22,9 to 3,7
Free fructose	0,05 to 3,6	45,2 to 15,5	21,0 to 0,2
Sucrose	0,15 to 1,3	41,6 to 10,0	15,1 to 1,8

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Annex A

(informative)

Results of an interlaboratory test

An interlaboratory test, carried out at the international level in 1991 under the auspices of ISO/TC 34, *Agricultural food products*, SC 15, *Coffee*, and executed by the technical committee of AFCASOLE, in which 11 laboratories participated, each of which carried out 2 determinations on each sample of 6 different commercial coffees, gave the statistical results (evaluated in accordance with ISO 5725⁸⁾) shown in tables A.1 to A.8.

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8) ISO 5725:1986, *Precision of test methods — Determination of repeatability and reproducibility for a standard test method by interlaboratory tests*.

Table A.1 — Determination of mannitol content for instant coffees

Sample	1		2		3		4		5		6	
	Free	Total	Free	Total	Free	Total	Free	Total	Free	Total	Free	Total
Number of laboratories retained after eliminating outliers	7	11	10	11	10	10	11	11	9	11	8	10
Mannitol content	Free	Total	Free	Total	Free	Total	Free	Total	Free	Total	Free	Total
Mean content, % (<i>m/m</i>)	0,024	0,179	0,060	0,151	1,582	1,854	0,619	0,782	0,192	0,300	0,059	0,179
Standard deviation of repeatability, <i>s_r</i>	0,000	0,013	0,001	0,011	0,044	0,041	0,022	0,036	0,016	0,032	0,004	0,018
Repeatability, 2,83 <i>s_r</i>	0,001	0,035	0,002	0,032	0,124	0,115	0,063	0,102	0,045	0,091	0,012	0,050
Coefficient of variation of repeatability, %	1,547	6,986	0,914	7,609	2,777	2,198	3,594	4,594	8,245	10,776	7,071	9,801
Standard deviation of reproducibility, <i>s_R</i>	0,015	0,075	0,034	0,069	0,157	0,331	0,150	0,161	0,065	0,112	0,029	0,090
Reproducibility, 2,83 <i>s_R</i>	0,041	0,213	0,098	0,196	0,444	0,938	0,424	0,454	0,183	0,316	0,083	0,254
Coefficient of variation of reproducibility, %	59,454	41,996	57,581	45,961	9,925	17,866	24,162	20,535	33,789	37,260	49,132	50,084

Table A.2 — Determination of arabinose content for instant coffees

Sample	1		2		3		4		5		6	
	Free	Total	Free	Total	Free	Total	Free	Total	Free	Total	Free	Total
Number of laboratories retained after eliminating outliers	11	11	9	11	11	11	11	11	10	9	11	11
Arabinose content	Free	Total	Free	Total	Free	Total	Free	Total	Free	Total	Free	Total
Mean content, % (<i>m/m</i>)	0,891	3,544	1,325	4,833	0,464	4,759	0,747	4,539	0,505	4,081	0,629	3,786
Standard deviation of repeatability, s_r	0,033	0,234	0,021	0,160	0,017	0,147	0,054	0,209	0,022	0,122	0,026	0,216
Repeatability, 2,83 s_r	0,092	0,662	0,060	0,453	0,049	0,417	0,154	0,590	0,063	0,344	0,073	0,612
Coefficient of variation of repeatability, %	3,664	6,597	1,603	3,311	3,755	3,098	7,297	4,594	4,419	2,979	4,092	5,709
Standard deviation of reproducibility, s_R	0,123	0,749	0,068	0,836	0,049	0,598	0,087	0,831	0,039	0,199	0,056	0,771
Reproducibility, 2,83 s_R	0,349	2,120	0,191	2,365	0,139	1,691	0,247	2,353	0,110	0,562	0,158	2,182
Coefficient of variation of reproducibility, %	13,832	21,141	5,105	17,289	10,556	12,559	11,707	18,319	7,729	4,868	8,895	20,366

Table A.3 — Determination of galactose content for instant coffees

Sample	1		2		3		4		5		6	
	Free	Total	Free	Total	Free	Total	Free	Total	Free	Total	Free	Total
Number of laboratories retained after eliminating outliers	11	10	11	10	11	11	11	11	9	9	11	10
Galactose content	Free	Total	Free	Total	Free	Total	Free	Total	Free	Total	Free	Total
Mean content, % (m/m)	0,562	17,779	0,339	18,481	0,191	8,085	0,438	16,269	0,475	18,444	0,362	17,687
Standard deviation of repeatability, s_r	0,017	1,441	0,014	0,430	0,019	0,219	0,026	0,632	0,015	0,306	0,018	0,760
Repeatability, 2,83 s_r	0,047	4,078	0,039	1,216	0,053	0,619	0,074	1,789	0,041	0,867	0,051	2,152
Coefficient of variation of repeatability, %	2,964	8,105	4,070	2,325	9,810	2,704	5,948	3,886	3,085	1,661	5,006	4,300
Standard deviation of reproducibility, s_R	0,030	1,582	0,027	2,181	0,025	0,647	0,036	2,097	0,019	1,377	0,045	1,503
Reproducibility, 2,83 s_R	0,084	4,478	0,077	6,171	0,070	1,831	0,103	5,935	0,055	3,898	0,127	4,255
Coefficient of variation of reproducibility, %	5,261	8,909	8,045	11,800	13,032	8,003	8,317	12,891	4,075	7,468	12,417	8,500

Table A.4 — Determination of glucose content for instant coffees

Sample	1		2		3		4		5		6	
	Free	Total	Free	Total	Free	Total	Free	Total	Free	Total	Free	Total
Number of laboratories retained after eliminating outliers	11	11	9	10	10	11	10	11	10	11	11	11
Glucose content	Free	Total	Free	Total	Free	Total	Free	Total	Free	Total	Free	Total
Mean content, % (<i>m/m</i>)	0,105	0,684	0,042	0,826	2,041	16,649	1,657	4,385	0,186	1,951	0,186	1,025
Standard deviation of repeatability, <i>s_r</i>	0,010	0,059	0,004	0,062	0,050	0,990	0,068	0,166	0,019	0,111	0,018	0,081
Repeatability, 2,83 <i>s_r</i>	0,029	0,168	0,012	0,174	0,143	2,803	0,192	0,469	0,053	0,313	0,052	0,230
Coefficient of variation of repeatability, %	9,911	8,661	10,151	7,449	2,470	5,949	4,092	3,780	10,006	5,674	9,853	7,937
Standard deviation of reproducibility, <i>s_R</i>	0,022	0,114	0,009	0,178	0,128	4,053	0,101	1,038	0,040	0,245	0,044	0,140
Reproducibility, 2,83 <i>s_R</i>	0,062	0,323	0,024	0,504	0,361	11,471	0,286	2,938	0,113	0,693	0,125	0,397
Coefficient of variation of reproducibility, %	21,057	16,667	20,419	21,551	6,249	24,345	6,091	23,672	21,370	12,541	23,808	13,702

Table A.5 — Determination of mannose content for instant coffees

Sample	1		2		3		4		5		6	
	Free	Total										
Number of laboratories retained after eliminating outliers	11	10	10	11	11	11	11	11	10	11	11	10
Mannose content	Free	Total										
Mean content, % (<i>m/m</i>)	0,583	17,913	0,155	14,365	0,470	2,601	0,329	5,598	0,277	7,653	0,991	19,067
Standard deviation of repeatability, s_r	0,028	1,038	0,013	0,380	0,020	0,052	0,023	0,171	0,012	0,212	0,038	0,426
Repeatability, 2,83 s_r	0,080	2,938	0,036	1,075	0,056	0,147	0,065	0,483	0,033	0,600	0,107	1,206
Coefficient of variation of repeatability, %	4,875	5,796	8,155	2,645	4,176	2,003	6,960	3,047	4,166	2,772	3,801	2,234
Standard deviation of reproducibility, s_R	0,142	2,029	0,056	2,180	0,082	0,352	0,061	0,859	0,111	0,808	0,168	4,137
Reproducibility, 2,83 s_R	0,402	5,741	0,158	6,168	0,233	0,997	0,174	2,431	0,313	2,288	0,474	11,708
Coefficient of variation of reproducibility, %	24,373	11,324	36,065	15,173	17,514	13,546	18,647	15,346	39,969	10,563	16,918	21,698

Table A.6 — Determination of fructose content for instant coffees

Sample	1		2		3		4		5		6	
	9	6	8	—	9	9	10	7	10	7	8	6
Number of laboratories retained after eliminating outliers	9	6	8	—	9	9	10	7	10	7	8	6
Fructose content	Free	Total	Free	Total	Free	Total	Free	Total	Free	Total	Free	Total
Mean content, % (<i>m/m</i>)	0,171	0,189	0,054	—	3,622	2,010	3,124	1,368	0,282	0,244	0,460	0,363
Standard deviation of repeatability, <i>s_r</i>	0,029	0,046	0,011	—	0,105	0,114	0,091	0,076	0,026	0,049	0,024	0,027
Repeatability, 2,83 <i>s_r</i>	0,082	0,130	0,032	—	0,297	0,323	0,258	0,214	0,072	0,140	0,067	0,075
Coefficient of variation of repeatability, %	16,882	24,416	21,035	—	2,900	5,683	0,219	5,527	9,045	20,261	5,167	7,310
Standard deviation of reproducibility, <i>s_R</i>	0,054	0,129	0,018	—	0,667	1,440	0,569	0,958	0,128	0,144	0,072	0,247
Reproducibility, 2,83 <i>s_R</i>	0,152	0,366	0,052	—	1,886	4,076	1,611	2,713	0,361	0,406	0,203	0,700
Coefficient of variation of reproducibility, %	31,370	68,482	33,846	—	18,403	71,660	18,217	70,068	45,292	58,874	15,549	68,236

Table A.7 — Determination of xylose content for instant coffees

Sample	1		2		3		4		5		6	
	Free	Total										
Number of laboratories retained after eliminating outliers	—	g	—	9	—	11	—	11	—	11	—	9
Xylose content	Free	Total										
Mean content, % (m/m)	—	0,097	—	0,146	—	1,855	—	0,736	—	0,029	—	0,133
Standard deviation of repeatability, s_r	—	0,022	—	0,014	—	0,085	—	0,027	—	0,007	—	0,019
Repeatability, 2,83 s_r	—	0,063	—	0,040	—	0,239	—	0,077	—	0,020	—	0,053
Coefficient of variation of repeatability, %	—	22,929	—	9,750	—	4,555	—	3,717	—	24,731	—	14,175
Standard deviation of reproducibility, s_R	—	0,037	—	0,030	—	0,424	—	0,204	—	0,008	—	0,032
Reproducibility, 2,83 s_R	—	0,103	—	0,084	—	1,200	—	0,578	—	0,023	—	0,090
Coefficient of variation of reproducibility, %	—	37,730	—	20,226	—	22,844	—	27,759	—	28,215	—	24,006

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