



FOOD AND AGRICULTURE ORGANIZATION OF THE UNITED NATIONS
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JOINT FAO/WHO FOOD STANDARDS PROGRAM
CODEX ALIMENTARIUS COMMISSION

CODEX COMMITTEE ON METHODS OF ANALYSIS AND SAMPLING
REPORT OF SECOND SESSION - BERLIN, 20-23 SEPTEMBER 1966

1. The Codex Committee on Methods of Analysis and Sampling held its second session from September 20th to 23rd, 1966, at Berlin under the chairmanship of Professor Dr. R. Franck. There were 30 delegates and observers present, representing 18 countries and 4 international organizations. Messrs. Gosselé and Mollenhauer were elected as rapporteurs. The Provisional Agenda was adopted with the change that Points 5: General Principles for sampling in the field of foods, and 14: Methods for the analysis and sampling of processed fruits and vegetables would be discussed together. The list of participants appears as Appendix 1 and the list of documents which the Committee had before it, as Appendix 2 to this Report.
2. After a full discussion of the problem of copyright and of reproducing existing methods of analysis, the Committee was of the opinion that methods of international organizations can only be considered in the chapter "Methods of Analysis" of the Codex Alimentarius, if the copyright could be waived. The Committee decided to ask the Commission to consider the matter.
3. The Committee was unanimously of the opinion that the "Standard Layout for a Standard Method of Chemical Analysis" published on page 10 of ISO Recommendation R 78 "Guide on the form for Standards for Chemical Products and for Methods of Chemical Analysis", First Edition, December 1958, should be taken as the basis for the format for methods of analysis. In order to meet the requirements of methods of analysis for foods, the scheme should be referred to as "Standard Layout for a Standard Method of Food Analysis" and should be slightly altered as shown in Appendix 3 to this Report. The Committee recommended that this format should be used as far as possible by all Committees in presenting their methods of analysis. It was agreed that this amended scheme be submitted at Step 3 of the Procedure for the Elaboration of Standards to Member Governments for comments.

WHL/51171

4. The Committee noted with interest the document "General Statement on Sampling in the field of foods" of ISO TC/34 (ALINORM 65/25(1), October 1965) and is looking forward to a revised edition from this technical committee of ISO on this subject. The Committee considered whether a more suitable name for the document could be found, expressing the fact that the document deals with the statistical principles for the sampling of commercial quantities, that is the numerical selection of samples. Also under consideration was document SP 10/70-SP, July 1966, "Proposed sampling plans for processed fruits and vegetables including frozen foods" prepared by the US and the same comments as above were made concerning the suitability of the title of this document. The Committee was of the opinion that with regard to sampling one should be aware of the various aspects of sampling including sampling for commercial quality control and for control with the aim of consumer protection. It was decided to submit the US document containing these plans to Member Governments under Step 3 of the Procedure for the Elaboration of Standards. Comments should be made only with respect to the part of the document which is headed "Application", from page 5 to 10. The document will be distributed together with this Report. (Member Governments should note that the same document has also been distributed in connection with the third session of the Codex Committee on Processed Fruit and Vegetables). The Committee recommended that the heading on page 7 "Application" should read "Method".

5. The delegation of the Federal Republic of Germany agreed to prepare for the next session of the Committee draft guidelines on techniques for taking, preserving, transporting and storage of samples along the lines laid down in the Code of Principles concerning Milk and Milk Products, 5th Edition (1966) "Standard Methods of Sampling Milk and Milk Products", Standard No. B.1 (1962).

6. In accordance with the allocation of work during the first session of the Committee the Swiss delegation had suggested using the methods of the Office Internationale du Cacao et Chocolate (OICC) as standards for the analysis of cocoa and chocolate products. The Committee considered it necessary to compare these methods with the methods of AOAC, NMKL and ISO for these products, and therefore asked the Swiss delegation to prepare a comparative synopsis of methods of analysis on a broad scale for the next session of the Committee. The Swiss delegation was asked to forward this synopsis to the Secretariat in Rome with a copy to the Chairman of the Committee not later than 31st January 1967. The synopsis will be sent out by the Secretariat in Rome to Codex Contact Points and participants of this meeting for comments with a datelimit for replies to be fixed at the time of despatch. Governments are requested to either comment on the OICC method alone or to comment on the basis of the synopsis if they feel that other matters than those included in the OICC methods should also be contained in the standard methods of analysis for cocoa and chocolate products. Those Governments which did not already have the OICC methods and need them for the preparation of their comments should request them direct from Dr. O. Schetty, Office International du Cacao et du Chocolat, 2003 Neuchâtel, Switzerland.

7. Regarding fruit juices the Committee accepted a proposal by the delegation of the Federal Republic of Germany to prepare a comparative synopsis of the methods of analysis of the International Fruit Juice Union and of the International Wine Office (OIV). Also considered in the synopsis should be the methods of analysis of AOAC, ISO and NMKL and the synopsis should refer to all juices. The German Secretariat would send the synopsis before 31st January 1967 to the Secretariat in Rome which in turn will distribute it to Codex Contact Points and participants at the session for comments. Comments should be made either on the methods of analysis of the International Fruit Juice Union alone or on the basis of the synopsis.

8. The Committee considered the document Codex/ANALYS/66-12 containing methods of analysis for honey compiled by the UK. The revised methods of analysis are attached to this Report as Appendix 4 and will be incorporated in the draft provisional honey standard to be considered by the Fourth Session of the Codex Alimentarius Commission which will decide under what step in the Procedure for the Elaboration of Standards the standard and the methods of analysis should be sent out for comments.

9. The Committee thanked the Netherlands delegation for their report on preservatives. In view of the many methods listed it was considered desirable to prepare a tabular summary, indicating as far as possible which methods are applicable to which kinds of foods. The Netherlands delegation was asked to supply this synopsis to the Secretariat of the Committee with a copy to the Secretariat in Rome not later than 31st January 1967. The Committee also considered it necessary to put this material at the disposal of the other Codex Committees, so that they can choose a method suitable for determining preservatives in their commodities. For this purpose the Secretariat in Rome should supply Codex Commodity Committees with a copy of the Netherlands synopsis and report on the methods of analysis for the preservatives which are of concern to these Committees.

10. The Committee expressed its gratitude to the delegation of the Netherlands for their report on antioxidants. In view of the fact that the subject is not as extensive as the analysis of preservatives, the Committee asked the Netherlands delegation to propose suitable methods for high fat and low fat foods and to supply these methods to the Committee for further discussion at the next meeting, stating also why such methods are considered as being especially suitable. The delegation of the Netherlands agreed to supply this to the Secretariat of the Committee, with a copy to the Secretariat in Rome, not later than 31st January 1967. This material will also be made available to the Committee on Fats and Oils.

11. The Committee noted that the Codex Committee for Sugars had considered the question of methods for the analysis of sugar and these methods are at present subject to discussions with ICUMSA. The delegation of the United Kingdom hopes to be able to present these methods to the Committee in time for the next session.

12. The Committee expressed its thanks to the delegation of the United Kingdom for their proposal regarding the examination of the purity of colouring matters. The representative of the Secretariat in Rome pointed out that methods of analysis regarding purity of food colours already exist

within the frame of FAO/WHO; therefore it does not seem to be necessary to elaborate these methods within this Committee. The Committee asked the delegation of the United Kingdom to propose methods for the detection and determination of colouring matters in foods taking into consideration existing methods.

13. Methods for the analysis of margarine are being elaborated at present by the Committee on Fats and Oils. The delegation of the United Kingdom will present a proposal for the next session.

14. The Committee had before it the Standard Methods of Sampling and Analysis for Milk Products from the Code of Principles on Milk and Milk Products referred to earlier. The Committee agreed that they would look at these methods of analysis next year and make, if necessary, suggestions for possible incorporation in the next revision of this Code.

15. In connection with a verbal report on the standardization of olive oil jointly carried out by the International Olive Oil Council and the Codex Committee on Fats and Oils, it was noted that methods of analysis for this commodity will eventually be placed before the Committee.

16. Following a discussion of the papers on papain, diastase, alpha-amylase and rennet it was agreed that the first three should come before the Committee again next year, whereas the work on rennet should be postponed and inquiries should be made of the status of the work on this topic in the International Dairy Federation. The delegation of the United States of America agreed to cooperate with the delegation of the Federal Republic of Germany in providing information on methods of analysis for enzymes.

17. The Committee considered both the Table of Contents of the general part of the chapter "Methods of Analysis" of the Codex and the "Working program for the unification of methods to be applied for sensoric (organoleptic) analysis" prepared by the Polish delegation. The members of the Committee to whom the Polish document was distributed were requested to submit comments on these two subjects of the document to the Codex Contact Point of the Codex Committee of Poland not later than 31st January 1967. The Polish delegation agreed to prepare a resumé of the comments for consideration at the next meeting of the Committee.

18. The Committee considered the list of organizations and Bibliography prepared by the Committee Secretariat. Members of the Committee were requested to send in any corrections or amendments to the Secretariat of the Committee not later than 31st October 1966. The Committee recommended that in view of the valuable information contained in this document and its utility in the development of methods of analysis, the corrected version should be sent by the Secretariat in Rome to Governments for immediate use. The Committee decided to recommend to the Commission that this document should not go through the normal procedure for standards in view of its completeness. It is intended to bring the document up to date every year with a supplement.

19. The Committee thanked the Nordisk Metodik-Komite for Levnedsmidler for its list of methods and for the offer to supply copies of these methods to all delegates.

20. The decision of the first session of the Committee that the methods of analysis should appear as a separate chapter of the Codex Alimentarius was confirmed.

21. The Committee decided to recommend to the Codex Alimentarius Commission the following terms of reference for its work in respect of sampling and analysis for the purpose of determining the composition of food:

- (a) to specify standard methods which are generally applicable to a number of foods
- (b) to consider, amend if necessary and ratify draft methods prepared or proposed by Codex Commodity Committees in the drafting of commodity standards, or
- (c) to develop in collaboration with other Committees such methods for subsequent ratification by this Committee
- (d) to revise as necessary such methods, and
- (e) to consider specific sampling and analytical problems assigned to it by the Commission.

22. The Polish delegation informed the Committee that their country provided the Secretariat for Sub-Committee 3: Fruits, Vegetables and their derived products, of the Technical Committee 34 of ISO: Agricultural Food Products (ISO/TC 34/SC 3). The Polish delegation emphasized that this Sub-Committee would make available to the Committee any unpublished information they may have on methods of analysis for fruits and vegetables.

23. The Chairman suggested as a date and place for the next session of the Committee the 11th to the 15th September, 1967, in Berlin.

JOINT FAO/WHO FOOD STANDARDS PROGRAM
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CODEX COMMITTEE ON METHODS OF ANALYSIS AND SAMPLING
Second Session - Berlin, 20-23 September 1966

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List of documents placed before the Second Session
of the Codex Committee on Methods of Analysis and
Sampling

- Codex/ANALYS/66-4 Bibliography: List of some already existing collections of analytical methods, and of Organizations occupying themselves with analytical methods, prepared by the German Secretariat
- Codex/ANALYS/66-5 Note by the Polish delegation: Table of contents of the general part of the chapter "Methods of Analysis" of the Codex Alimentarius and "Working Program for the Unification of Methods to be applied for sensoric (organoleptic) Analysis"
- Codex/ANALYS/66-7 Note prepared by the German delegation on Methods of Analysis for Fruit Juices
- Note prepared by the German delegation on Methods of Analysis for Enzyme Preparations:
- Codex/ANALYS/66-8,1 Part 1: Papain
Codex/ANALYS/66-8,2 Part 2: Determination of Diastatic Activity
Codex/ANALYS/66-8,3 Part 3: Determination of alpha-amylase and of rennet activity
- Codex/ANALYS/66-9 Note prepared by the delegation of the Netherlands: Study of and proposed methods for identification and determination of preservatives in foods
- Part I: Sulphur dioxide
Part II: Sorbic acid
Part III: Nitrate and Nitrite
Part IV: Benzoic acid
- Codex/ANALYS/66-10 Note prepared by the delegation of the Netherlands. Antioxidants
- Codex/ANALYS/66-11 Methods of analysis on colouring matters. Documentation submitted by the United Kingdom delegation
- Codex/ANALYS/66-12 Joint FAO/WHO Draft Provisional Standard for Honey. Honey - Methods of analysis. Note by the United Kingdom delegation.
- Codex/ANALYS/66-13 List of methods of analysis published by the Nordisk Metodik-Komite for Levnedsmidler
- ALINORM 65/25(1) General Statement on Sampling in the field of foods, prepared by the Secretariat of ISO/TC 34, October 1965

SP 10/70-SP
July 1966

Proposed Sampling Plans for Processed Fruits and
Vegetables including Frozen Foods. Author Country: USA

SP 10/110

Code of Principles concerning Milk and Milk Products
and Associated Standards, Joint FAO/WHO Food Standards
Program (Fifth Edition, January 1966)

ISO Recommendation R 78

Guide on the form for standards for Chemical Products
and for Methods of Chemical Analysis, First Edition,
December 1958

SP 10/101-USSR

Index from USSR Books, State Standards:

- a) Canned and Preserved Fish - 1963
- b) Dairy Products and Canned Milk and Dairy Products - 1962
- c) Meat and Canned Meat - 1963

Standard Layout for a Standard Method of Food Analysis

1. Title
2. Scope
3. Definition
4. Principle of Method, Reactions
5. Reagents
6. Apparatus
7. Sample or Sampling
 - 7.1 Sampling Plan
 - 7.2 Procedure for taking samples
8. Procedure
 - 8.1 Preparation of test sample
 - 8.2 Blank test
 - 8.3 Determination(s)
9. Expression of Results
 - 9.1 Method of calculation and formulae
 - 9.2 Accuracy of determination
 - 9.2.1 Repeatability
 - 9.2.2 Reproducibility
10. Special cases
11. Notes on Procedure
12. Test Report
13. Schematic Representation of Procedure

Joint FAO/WHO Draft Provisional Standard for Honey

Honey - Methods of Analysis

a) Reducing substances calculated as invert sugar:

Using a modification of the Lane and Eynon (1923) procedure.

Sampling

Before sampling, the honey should be melted over hot (60°C) water in a closed container for not more than 30 minutes or should be melted over warm (40°C) water in a closed container (no time limit specified). After melting and cooling the honey should be well mixed and agitated to ensure that any condensate on other parts of the container is re-incorporated.

From the homogeneous honey a sample of around one gm. is accurately weighed out and dissolved in distilled water. The sample is then made up to 200 ml. with distilled water in a graduated flask.

Reagents

1. Soxhlet's modification of Fehling's solution
 - A. 69.28 gm. of $\text{CuSO}_4 \cdot 5\text{H}_2\text{O}$ per litre
 - B. 346 gm. Rochelle salt
 - 100 gm. Sodium hydroxide} per litre

Equal volumes of the two solutions combined just before use.

2. Standard Invert sugar solution 1%
3. Methylene blue solution 1%

Procedure

The Fehling's solution A is standardised so that exactly 5ml. when mixed with approximately 5 ml. of solution B, will react completely with 50 mg. invert sugar in an added volume of 20 ml.

Honey solution is diluted fifty to one hundred and titrated against the mixed Fehling's solution using the following method.

About 10 ml. of diluted honey solution and 5 ml. of distilled water are added to 10 ml. of mixed Fehlings solution in a 250 ml. conical flask. The mixture is brought to the boil and boiled for 2 minutes, when a few drops of methylene blue solution are added. The titration is completed within the next minute until the colour of the methylene blue is completely discharged. Other and more accurate titrations follow where the difference between the titration volume and 20 ml. is added at the beginning as distilled water, and all but 1 ml. of honey solution is added at the same time.

The result is expressed as grams of invert sugar per 100 gm. of honey.

b) Apparent sucrose content:

Sampling

Use the same procedure as for (a)

Reagents

1. As used for (a)
2. Hydrochloric acid 6.34 Normal
3. Sodium hydroxide 5 Normal

Procedure

Using the Walker (1917) inversion method.

50 ml. of honey solution are added to a 100 ml. graduated flask together with 25 ml. distilled water. The solution is heated over a water bath to 65°C. The flask is removed from the water bath and 10 ml. of 6.34 N Hydrochloric acid added. The solution is allowed to cool spontaneously for fifteen minutes or as much longer as may be convenient. It is then cooled and neutralised to litmus with 5N sodium hydroxide, cooled again and made up to volume.

The solution is titrated against Fehling's solution as for (a) and the sucrose content found in terms of invert sugar by subtracting the percentage of invert sugar before inversion from the percentage of invert sugar after inversion. The percentage of sucrose as invert sugar times 0.95 gives the true sucrose figure.

A quantitative method for actual sucrose content when the apparent sucrose content is above 5% should be evaluated preferably using chromatographic techniques. If such a method becomes available, it is recommended that the Coordinating Committee for Europe consider changing the heading in the standard for honey from "apparent sucrose content" to "sucrose content".

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c) Moisture content

Sampling

The sample to be prepared as in (a) and used without dilution.

Procedure

The refractive index to be determined at 20°C using a refractometer, and the reading converted to % moisture by use of the Wedmore (1955) table, see Appendix A.

d) Water insoluble solids content

Sampling

The sample to be prepared as in (a) and used without dilution.

Procedure

Weigh a suitable quantity of honey out to the nearest centigram, i.e. 20 gm. Dissolve in distilled water at 80°C,

mix well and filter through a previously dried and weighed fine glass sintered crucible (degree of fineness to be supplied later). Wash thoroughly with hot (80°C) water until free from sugars (Mohr test). Dry crucible for one hour at 135°C, cool and weigh to 0.1 mg. Express as grams solids per 100 gm. honey.

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e) Ash content

Sampling

The sample to be prepared as in (a) and used without dilution.

Procedure

Weigh 5-10 gm. honey into an ignited and pre-weighed Pt or silica dish. Place in muffle and heat gently until the sample is black and dry, and there is no danger of loss by foaming. Heat in the muffle at 600°C to constant weight. Cool and weigh. Express as percentage ash.

f) Acidity

Sampling (AOAC procedure)

The sample to be prepared as in (a), 10.0 gm. weighed out and dissolved in 75 ml. of CO₂ free distilled water.

Procedure

Titrate the sample with 0.1 N Sodium hydroxide, carbonate free, with 4-5 drops neutralised phenolphthalein indicator. The end-point colour should persist for 10 seconds. For darkly coloured samples a smaller weight should be taken. As an alternative a pH meter may be used and the sample titrated to

a pH of 8.3.

Express as milliequivalents normal sodium hydroxide per 100 gm. honey.

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g) Diastase Number

Using the method of Schade et al (1958) containing modifications by White et al (1959) and Hadorn (1961).

Sampling

The sample should not be heated in any way, but well mixed until homogeneous and the sample for the determination weighed out.

Reagents

1. Iodine stock solution:- Dissolve 8.8 gm. of AR iodine in 30-40 ml. water containing 22 gm. AR potassium iodide, and dilute to one litre with water.
2. Iodine solution 0.0007N:- Dissolve 20 gm. AR potassium iodide in 30-40 ml. water in a 500 ml. volumetric flask. Add 5.0 ml. iodine stock solution and make up to volume. Make up a fresh solution every second day.
3. Acetate buffer - pH 5.3 (1.59M):- dissolve 87 gm. sodium acetate - $3H_2O$ in 400 mls. water, add about 10.5 ml. glacial acetic acid in a little water and make up to 500 ml. Adjust the pH to 5.3 with sodium acetate or acetic acid as necessary, using a pH meter.
4. Sodium chloride 0.5M:- dissolve 14.5 gm. sodium chloride AR in boiled-out distilled water and make up to 500 ml. Keeping time limited by mould growth.

5. Starch solution:- Use a standard starch (of a quality equivalent to Lintner starch as supplied by Pfanstiehl Laboratories, Inc. Washington, Ill.). If a comparable starch is not available, the following method for determining the blue value of starch should be used.

Weigh out that amount of starch which is equivalent to 2.0 gm. anhydrous starch. Mix with 90 ml. of water in a 250 ml. conical flask. Bring rapidly to the boil, swirling the solution as much as possible, heating over a thick wire gauze preferably with an asbestos centre. Boil gently for three minutes, cover and allow to cool spontaneously to room temperature. Transfer to a 100 ml. volumetric flask, place in a water bath at 40°C to attain this temperature and make up to volume at 40°C.

Method for determining blue value of starch

The amount of starch equivalent to 1 gm. anhydrous starch is dissolved by the above method, cooled and 2.5 ml. acetate buffer added before making up to 100 ml. in a volumetric flask.

To a 100 ml. volumetric flask add 75 ml. water, 1 ml. normal hydrochloric acid and 1.5 ml. of 0.02N iodine solution. Then add 0.5 ml. of the starch solution and make up to volume with water. Stand for one hour in the dark and then read off on a spectrophotometer at 575 m μ using a blank containing everything except the starch using 2 cm. cells.

Reading on optical density scale = Blue value

Apparatus

1. Water bath - at 40°C \pm 0.2°C
2. Spectrophotometer - to read at 660 m μ .

Procedure

1. Honey solution:- Weigh a 10.0 gm. sample into a 50 ml. beaker and add 5.0 ml. acetate buffer solution, and 20 ml. water to dissolve the sample. Completely dissolve the sample by stirring the cold solution. Add 3.0 ml. sodium chloride to a 50 ml. volumetric flask and transfer the dissolved honey sample to this. Make up to 50 ml.

N.B. It is essential that the honey should be buffered before coming into contact with the sodium chloride.

2. Standardisation of the starch solution:-
Warm the starch solution to 40°C and pipette 5 ml. into 10 ml. water at 40°C and mix well. Pipette 1 ml. of this solution into 10 ml. iodine solution diluted with 35 ml. of water. Mix well and read the colour at 660 mμ against a water blank.

The optical density should be 0.760 ± 0.020 .

If necessary adjust the volume of added water to obtain the correct optical density.

3. Pipette 10 ml. of honey solution into a 50 ml. conical flask. Place this flask and the flask of starch solution in a water bath at 40°C to warm up. After at least 15 minutes, pipette 5 ml. starch solution into the honey solution, shaking vigorously and starting a stopwatch at the same time. Take 1 ml aliquots at five minute intervals and add to 10 ml. iodine solution diluted with the standard volume of water. Determine the optical density immediately, and continue taking aliquots until an optical density of less than 0.235 is reached.

4. Calculation:- Plot the optical density against time on rectilinear paper. Draw a straight line through at least the last three points on the graph to determine the time when the reaction mixture reaches an optical density of 0,235.

Divide 300 by this time in minutes to obtain the diastase number (DN). This number expresses the diastase activity as mls. 1% starch hydrolysed by the enzyme in 1 gm. of honey in one hour at 40°C.

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Recommendation: Insert method for invertase and use diastase/invertase ratio as measure of the condition of the honey (see Kiermier, F., Köberlein W. (1954): Z. Unters. Lebensmitt 98.329).

h) Hydroxymethylfurfuraldehyde content:

Using the O. Winkler (1955) method

Sampling

As for (g) above

Reagents

1. Barbituric acid solution:- Dry barbituric acid at 105°C and weigh out 500 mg. Transfer to 100 ml. graduated flask using 70 ml. water. Place in a hot water bath until dissolved, cool and make up to volume.
2. p-toluidine solution:- Weight out 10.0 gm. AR p-toluidine and dissolve in about 50 ml. isopropanol by gentle warming on a water bath. Transfer to a 100 ml. graduated flask with isopropanol, and add 10 ml. glacial acetic acid. Cool and make up to volume with isopropanol. Keep the solution in the dark. The solution darkens gradually and eventually has to be renewed.

Apparatus

1. Spectrophotometer to read at 550 m μ .
2. Test tubes

Procedure

Weigh out 10 gm. of honey sample and dissolve in 20 ml. distilled water without heating. Transfer to a 50 ml. graduated flask and make up to volume.

Pipette 2.0 ml. of honey solution into each of two test tubes and add 5.0 ml. p-toluidine solution to each. Into one test tube pipette 1 ml. water, and into the other 1 ml. barbituric acid solution. Shake both mixtures, the one with added water being the water blank. The addition of the reagents should be done without pause and should be finished in about 1-2 minutes.

Wait for 3 minutes after adding the barbituric acid and read the extinction of the sample against the blank at 550 m μ using a 1 cm. cell.

Calculation

The method may be calibrated by using a standard solution of hydroxymethylfurfuraldehyde (HMF) standardised by dissolving commercial or laboratory prepared HMF and assaying spectrophotometrically where $\epsilon = 16,830$ (J.H. Turner 1954) at 284 m μ ; using 0-300 μ g. standards.

An equation is given by which results may be roughly worked out

$$\text{mg/100 g HMF} = \frac{\text{Extinction}}{\text{thickness of layer}} \times 19.2$$

- (i) Fermentation test (to be drawn up)
- (j) Dirty honey: AOAC method 36.068 (Tenth edition) (Tolerance needed)
- (k) Pollen identification: needed to determine origin of honey (to be supplied).

Appendix A

Refractive Index (20°C)	Moisture Content (%)	Refractive Index (20°C)	Moisture Content (%)	Refractive Index (20°C)	Moisture Content (%)
1.5044	13.0	1.4961	16.2	1.4880	19.4
1.5038	13.2	1.4956	16.4	1.4875	19.6
1.5033	13.4	1.4951	16.6	1.4870	19.8
1.5028	13.6	1.4946	16.8	1.4865	20.0
1.5023	13.8	1.4940	17.0	1.4860	20.2
1.5018	14.0	1.4935	17.2	1.4855	20.4
1.5012	14.2	1.4930	17.4	1.4850	20.6
1.5007	14.4	1.4925	17.6	1.4845	20.8
1.5002	14.6	1.4920	17.8	1.4840	21.0
1.4997	14.8	1.4915	18.0	1.4835	21.2
1.4992	15.0	1.4910	18.2	1.4830	21.4
1.4987	15.2	1.4905	18.4	1.4825	21.6
1.4982	15.4	1.4900	18.6	1.4820	21.8
1.4976	15.6	1.4895	18.8	1.4815	22.0
1.4971	15.8	1.4890	19.0		
1.4966	16.0	1.4885	19.2		

Wedmore E.B. Bee World 36.197 1955Temperature corrections

Refractive Index:-

Temperatures above 20°C - Add 0.00023 per °C

Temperatures below 20°C - Subtract 0.00023 per °C

Appendix B (not part of standard)

Comments on the method for diastase determination

By diastase the material α amylase is understood. All the methods developed for the determination of α amylase are the same in principal. Honey is allowed to act under certain experimental conditions on a given quantity of starch solution. The breakdown of the starch is followed by the gradual decrease in the blue colour of the starch-iodine reaction.

The first quantitative procedure for the determination of diastase was that of Gothe (1914). This was a visual method which has since been improved upon by Kiermier and Köberlein (1954).

Instrumental colourimetric methods introduced in later years have all attempted to relate their results to those obtained by the Gothe method for the purpose of comparison. This has led to the Schade, Marsh and Eckert (1958) modification of Schwimmer's colorimetric procedure.

When White and Pairent (1959) tested Schade's method they found poor agreement with figures obtained by the Gothe method, although they found the method well suited for routine use. White adjusted the procedure in order to make the results comparable with the older one, and at the same time improved the graphical method of obtaining the result, making it universally usable for any type of spectrophotometer.

Hadorn (1961) has suggested that White's modification was unnecessary and that the non-agreement of results was attributable to the type of starch used. He altered the wavelength at which readings were made, and introduced a one hour delay before reading the absorbance values. In addition he returned to the Schade method of determining the result.

Our own tests have confirmed that Hadorn was right about the effects of different qualities of starch on the results, and have decided to adopt his suggested blue value. On the other hand, we are not sure of the value of his other suggestions, particularly the one hour delay in taking readings, which results in an impossible situation when dealing with a number of honeys of unknown diastase values. White's procedure has the value of plotting the results as the experiment proceeds.

White (1964) tested Hadorn's method and reported that it gave low results.

We consider that the suggested procedure will probably not produce results in very close agreement with the Gothe method. On the other hand, we think that the method will produce the same results on a given honey when analysed by several collaborators, and have evidence to support this.

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