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GUIDELINE TO FACILITATE INTRA-REGIONAL TRADE IN THE CARIBBEAN

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REGIONAL GUIDELINES FOR
SANITARY MEASURES

GUIDELINE TO FACILITATE INTRA-REGIONAL TRADE IN HONEY

Produced by the Caribbean Agricultural
Health and Food Safety Agency (RPPO)
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This is not an official part of the guideline
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ADOPTION

This guideline was adopted by the Council of Trade and Economic Development (COTED) in June 2022.

APPLICATION:

These SPS compliant guidelines may be used in providing the conditions necessary to allow for trade of honey within the Region.

BACKGROUND/INTRODUCTION:

To ensure SPS compliancy, conditions from the farm to the table must be taken into consideration. This includes conditions in which the bees are housed and raised; the conditions in which the honey is collected and stored; the conditions in which the honey is transported.

Product testing will also be considered, testing assesses the performance of an export registered establishment's food safety management system (approved arrangement) in producing SPS compliant honey. A regular program of product testing provides the importing Governments with a level of assurance, which allows issue of export health certification.

SCOPE

This guideline applies to the transboundary trade in honey from the honey bees of the genus *Apis* (Tribe: Apini). It includes honey traded under an alternate label, such as "sweetner".

The trade in the neotropical stingless bees (Tribe: Meliponini) and their products is excluded from this scope.
These guidelines do not address the importation of live queen, worker and drone honey bees with or without associated brood combs; honey bee semen; honey bee venom, propolis, beeswax, royal jelly or used apicultural equipment.

These guidelines are in compliance with the Agreement on Sanitary and Phytosanitary Measures (SPS). The World Organisation for Animal Health (OIE) and the Codex Alimentarius are considered the standard setting bodies.

The importation of animal products involves a certain level of disease risk to the importing country. This risk may be represented by one or several diseases, infections or infestations. Therefore, importing countries may wish to conduct risk analyses. According to OIE, the
The principal aim of an import risk analysis is to provide importing countries with an objective and defensible method of assessing the disease risks associated with the importation of animal products.

The risk analysis should be transparent. Transparency means the comprehensive documentation and communication of all data, information, assumptions, methods, results, discussion and conclusions used in the risk analysis. This is necessary so that the exporting country and all interested parties are provided with clear reasons for the imposition of import conditions or refusal

to import. The components of risk analysis are hazard identification, risk assessment, risk management and risk communication.

An importing country may decide to permit the importation using the appropriate sanitary standards recommended in the OIE Terrestrial Animal Health Code, therefore, eliminating the need for a risk assessment.

These guidelines provide details as to the appropriate standards recommended within the OIE Terrestrial Animal Health Code with respect to the production of honey.

PROCESS

1. Application to import honey from Country X by importer

2. Veterinary Authority of Importing Country may carry out a Risk Analysis of Honey Production, Collection, Storage and Transportation within Country X
    a. If the Honey is produced, collected, stored and transported according to OIE Standards, then risk should be low.
    b. If testing for microbial and other contaminants are done at various points of the process regularly and these results easily accessed by the Veterinary Authority in the importing country, then the risk of importing may be considered low.

3. If Risk is low an Import Permit may be Issued

CONTENTS

This section includes details as to the appropriate standards recommended within the OIE Terrestrial Animal Health Code with respect to the production of Honey.

MANAGEMENT OF HONEY FROM FARM TO TABLE

Biosecurity procedures in Honey Production (Chap. 9 OIE-Terrestrial Animal Health Code)
Biosecurity procedures should be implemented with the objective of preventing the introduction and dissemination of infectious agents in the honey production chain. Biosecurity will be enhanced with the adoption and implementation of the principles of Good Agricultural Practices and the Hazard Analysis Critical Control Point (HACCP) system.

General Biosecurity Requirements

- All establishments should have a written biosecurity plan. Personnel working within the establishments should have access to basic training in biosecurity relevant to honey production and understand the implications to animal health, human health and food safety.
- There should be good communication between personnel involved in the honey production chain to ensure that steps are taken to minimise the introduction and dissemination of infectious agents.

- Traceability at all levels of the honey production chain should be possible.
- Records should be maintained on an individual apiary basis and include data on hive health, production, medications, mortality and surveillance. Records should be maintained on cleaning and disinfection of apiary buildings and equipment. Records should be readily available for inspection on site.
- the Veterinary Authority or other Competent Authority with responsibility for reporting and controlling diseases of honey shall have current knowledge of, and authority over, all domesticated apiaries in the country.

- To prevent the introduction of new pests and diseases, the import of used bee equipment shall conform to Chapter 9 OIE Terrestrial Code. Veterinary Authorities of importing countries should require the presentation of an international veterinary certificate
- To avoid the development of antimicrobial resistance, antimicrobial agents should be used in accordance with relevant directions of the Veterinary Services and manufacturer's instructions.
- Establishments should be free from used equipment and debris such as livestock manure that could attract or harbour pests, such as the Greater wax moth *(Galleria mellonella*) and the Lesser wax moth (*Achroia grisella*)
- Procedures for the prevention of entry of feral colonies into hives and apiary buildings, and the control of vermin such as rodents and arthropods should be implemented.
- Access to the establishment should be controlled to ensure only authorised persons and vehicles enter the site.
- All personnel and visitors entering an establishment should follow a biosecurity procedure.
    - All personnel and visitors entering a honey house should wash their hands with soap and water or sanitize them using a disinfectant.
    - Entry of visitors and vehicles should be registered by the establishment.
    - Personnel who have direct contact with honey should maintain a high degree of personal cleanliness and, where appropriate, wear suitable protective clothing, footwear and head covering that is not likely to introduce contamination into beekeeping areas.
        - Personnel should wash their hands before starting work that involves the handling of honey, each time they return to handling areas after a break, immediately after using the toilet, and after handling anything which may contaminate honeys.
- Personnel and visitors should not have had recent contact with other honey, honey waste, or honey processing plant(s). This time period should be based on the level of risk of transmission of infectious agents.

- Any vehicle entering an establishment should be cleaned and disinfected in accordance with a biosecurity plan. Delivery vehicles should be cleaned and disinfected before loading each consignment of honeys or honey.
- The establishment should be surrounded by a security fence to prevent the entry of unwanted animals and people.
- A sign indicating restricted entry should be posted at the entrance to the establishment.

Feeding colonies

Infected honey and pollen can efficiently spread bee diseases when used in the composition of feed for colonies. The importation of honey, pollen and other apiary products used in feeding colonies should conform to OIE Terrestrial Animal Health Code *Recommendations for the importation of honey, honey bee-collected pollen, beeswax, propolis and royal jelly for use in apiculture* (relevant sections of Chapter 9). Veterinary Authorities of importing countries may require the presentation of an international veterinary certificate attesting that the commodities come from apiaries situated in a country or zone free from pests and diseases; or have been processed or treated.

Documentation and Record Keeping **(3. -** Codex Alimentarius Code)

Records should be kept, as necessary and where practicable, to enhance the ability to verify the effectiveness of the control systems. Documentation of procedures can enhance the credibility and effectiveness of the food safety control system.

With respect to food safety, records should be kept on:

- Prevention and control of diseases with an impact on public health;
- Identification and movement of bees and honey;
- Use of agricultural and pest control chemicals;
- Nature and source of feed, feed ingredients and water;
- Use of veterinary drugs/medicines;
- Results of testing where testing is performed.

PESTS & PATHOGENS GENERALLY ASSOCIATED WITH HONEY PRODUCTION

PEST/PATHOGEN	Recommendations for the importation of honey for human consumption
Paenibacillus* larvae (American foulbrood)** ***and **Melissococcus plutonius (European foulbrood)**	The honey shall EITHER 1. come from apiaries situated in a country or zone free from American foulbrood; OR 2. have been processed to ensure the destruction of both bacillary and spore forms of P. larvae by irradiation or any procedure of equivalent efficacy recognised by the Veterinary Authorities of the importing and exporting countries; OR 3. have been found free from spore forms of P. larvae by a test method described in the relevant chapter of the Terrestrial Manual.

PEST/PATHOGEN	Recommendations for the importation of honey for human consumption
***Aethina tumida* (small hive beetle)**	1. the honey: EITHER a. comes from apiaries situated in a country or zone free from A. tumida; OR b. has been strained through a filter of pore size no greater than 0.42 mm; OR c. has been treated to ensure the destruction of A. tumida in accordance with one of the following procedures: i. heating to 50°C core temperature and holding at that temperature for 24 hours; or ii. freezing at core temperature of minus 12°C or less for at least 24 hours; or iii. irradiation with 400 Gray; or iv. by any procedure of equivalent efficacy recognised by the Veterinary Authorities of the importing and exporting countries; AND 2. all precautions have been taken to prevent contamination with A. tumida.
***Varroa* spp. (varroosis)**	1. comes from *apiaries* situated in a country or *zone* free from *Varroa* spp.; or 2. has been strained through a filter of pore size no greater than 0.42 mm; or 3. has been treated to ensure the destruction of *Varroa* spp. in accordance with one of the following procedures: a. heating to 50°C core temperature and holding at that temperature for 20 minutes; or b. freezing at core temperature of minus 12°C or less for at least 24 hours; or c. irradiation with 350 Gray; or d. by any procedure of equivalent efficacy recognised by the *Veterinary Authorities* of the *importing* and *exporting countries*.

Acarapisosis

Acarapiosis has not been shown to be transmitted in honey. When authorising import or transit of honey, Veterinary Authorities should not require any acarapisosis-related conditions, regardless of the acarapisosis status of the honey bee population of the exporting country or zone (OIE Terrestial code Chapter 9.5.1)

Tropilaelaps

Infestation of honey bees with *Tropilaelaps* spp. (OIE Terrestial code Chapter 9.5.) has been excluded from this guideline. These mites have not spread significantly beyond Asia.

REFERENCES

CODEX STANDARD FOR HONEY. CODEX STAN 12-1981 Rev. 1 (1987) (World-wide standard)

https://www.fao.org/3/w0076e/w0076e30.htm

OIE Terrestrial Animal Health Code.. https://www.oie.int/en/what-we-do/standards/codes-and-manuals/terrestrial-code-online-access/?id=169&L=1&htmfile=chapitre_varroa_spp.htm

RECOMMENDATIONS

1. To prevent the introduction of new pests and diseases, the import of used bee equipment shall conform to Chapter 9 OIE Terrestrial Code. Veterinary Authorities of importing countries should require the presentation of an international veterinary certificate
2. In archipelago states, Competent Authorities should seek to provide evidence supporting the pest-free status of zones such as offshore islands.

APPENDIX: Relevant Chapters of the OIE Terrestrial Animal Health Code

Infestation of honey bees with *Tropilaelaps* spp. (OIE Terrestial code Chapter 9.5.) has been excluded. These mites have not spread significantly beyond Asia.

CHAPTER 9.2.

INFECTION OF HONEY BEES WITH *PAENIBACILLUS LARVAE* (AMERICAN FOULBROOD)

Article 9.2.1.

General provisions

For the purposes of the *Terrestrial Code*, American foulbrood is a disease of the larval and pupal stages of honey bees (species of the genus *Apis*) caused by *Paenibacillus larvae* (*P.larvae*), which is widely distributed. *P.larvae* is a bacterium that can produce over one billion spores in each infected larva. The spores are very long-living and extremely resistant to heat and chemical agents, and only the spores are capable of inducing the disease.

Combs with American foulbrood infected pre-imago of honey bees show distinctive clinical signs which can allow the disease to be diagnosed in the field. However, subclinical *infections* are common and require *laboratory* diagnosis.

When authorising import or transit of honey, *Veterinary Authorities* should require the conditions prescribed in this chapter relevant to the American foulbrood status of the honey bee population of the *exporting country* or *zone*.

Article 9.2.3.

Determination of the American foulbrood status of a country or zone

The American foulbrood status of a country or *zone* can only be determined after considering the following criteria:

1. a *risk assessment* has been conducted, identifying all potential factors for American foulbrood occurrence and their historic perspective;
2. American foulbrood is notifiable in the whole country or *zone*, and all clinical signs suggestive of American foulbrood are subjected to field and *laboratory* investigations;
3. an ongoing awareness programme is in place to encourage reporting of all cases suggestive of American foulbrood;
4. the *Veterinary Authority* or other *Competent Authority* with responsibility for reporting and control of diseases of honey bees has current knowledge of, and authority over, all domesticated *apiaries* in the country.

Article 9.2.4.

Country or zone free from American foulbrood

1. Historically free status

   A country or *zone* may be considered free from the disease after conducting a *risk assessment* as referred to in Article 9.2.3. but without formally applying a specific *surveillance* programme if the country or *zone* complies with Chapter 1.4.

2. Free status as a result of an eradication programme

A country or *zone* which does not meet the conditions of point 1 above may be considered free from American foulbrood after conducting a *risk assessment* as referred to in Article 9.2.3. and when:

a. the *Veterinary Authority* or other *Competent Authority* with responsibility for reporting and control of diseases of honey bees has current knowledge of, and authority over, all domesticated *apiaries* existing in the country or *zone*;
b. American foulbrood or foulbrood is notifiable in the whole country or *zone*, and any clinical cases suggestive of American foulbrood foulbrood are subjected to field and *laboratory* investigations;
c. for the five years following the last reported isolation of the American foulbrood agent, annual surveys supervised by the *Veterinary Authority* or other *Competent Authority*, with no positive results, have been carried out on a representative sample of *apiaries* in the country or *zone* to provide a confidence level of at least 95% of detecting American foulbrood if at least 1% of the *apiaries* were infected at a within-*apiary* prevalence rate of at least 5% of the hives; such surveys may be targeted towards areas with the last reported isolation of the American foulbrood agent;
d. to maintain free status, an annual survey supervised by the *Veterinary Authority* or other *Competent Authority*, with no positive results, is carried out on a representative sample of hives in the country or *zone* to indicate that there has been no new isolations; such surveys may be targeted towards areas with a higher likelihood of isolation;
e. either there is no *wild* or self-sustaining *feral* population of species of the genus *Apis* in the country or *zone*, or there is an ongoing *surveillance* programme of the *wild* or self-sustaining *feral* population of species of the genus *Apis* which demonstrates no evidence of the presence of the disease in the country or *zone*;

f. all equipment associated with previously infected *apiaries* has been sterilised or destroyed;
g. the importation of the *commodities* listed in this chapter into the country or *zone* is carried out in accordance with the recommendations of this chapter.

Article 9.2.8.

Recommendations for the importation of honey, honey bee-collected pollen, beeswax, propolis and royal jelly for use in apiculture

Veterinary Authorities of *importing countries* should require the presentation of an *international veterinary certificate* attesting that the *commodities*:

1. come from *apiaries* situated in a country or *zone* free from American foulbrood; or
2. have been processed to ensure the destruction of both bacillary and spore forms of *P. larvae* by irradiation with ten kilogray or any procedure of equivalent efficacy recognised by the *Veterinary Authorities* of the *importing* and *exporting countries*; or
3. have been found free from spore forms of *P. larvae* by a test method described in the relevant chapter of the *Terrestrial Manual*.

Article 9.2.9.

Recommendations for the importation of honey, honey bee-collected pollen, beeswax, propolis and royal jelly for human consumption

Veterinary Authorities of *importing countries* free from American foulbrood should require the presentation of an *international veterinary certificate* attesting that the products:

1. come from *apiaries* situated in a country or *zone* free from American foulbrood; or
2. have been processed to ensure the destruction of both bacillary and spore forms of *P. larvae* by irradiation with ten kilogray or any procedure of equivalent efficacy recognised by the *Veterinary Authorities* of the *importing* and *exporting countries*; or
3. have been found free from spore forms of *P. larvae* by a test method described in the relevant chapter of the *Terrestrial Manual*.

CHAPTER 9.3.

INFECTION OF HONEY BEES WITH *MELISSOCOCCUS PLUTONIUS* (EUROPEAN FOULBROOD)

Article 9.3.1.

General provisions

For the purposes of the *Terrestrial Code*, European foulbrood is a disease of the larval and pupal stages of honey bees (species of the genus *Apis*), caused by *Melissococcusplutonius* (*M.plutonius*), a non-sporulating bacterium, which is widely distributed. Subclinical *infections* are common and require *laboratory* diagnosis. *Infection* remains enzootic because of mechanical contamination of the honeycombs. Recurrences of disease can therefore be expected in subsequent years.

When authorising import or transit of the honey covered in the chapter, *Veterinary Authorities* should require the conditions prescribed in this chapter relevant to the European foulbrood status of the honey bee population of the *exporting country* or *zone*.

Standards for diagnostic tests are described in the *Terrestrial Manual*.

Article 9.3.2.

Safe commodities

When authorising import or transit of the following *commodities*, *Veterinary Authorities* should not require any European foulbrood-related conditions, regardless of the European foulbrood status of the honey bee population of the *exporting country* or *zone*:

1. honey bee semen;
2. honey bee venom.

Article 9.3.3.

Determination of the European foulbrood status of a country or zone

The European foulbrood status of a country or *zone* can only be determined after considering the following criteria:

1. a *risk assessment* has been conducted, identifying all potential factors for European foulbrood occurrence and their historic perspective;
2. European foulbrood is notifiable in the whole country or *zone*, and all clinical signs suggestive of European foulbrood are subjected to field and *laboratory* investigations;
3. an ongoing awareness programme is in place to encourage reporting of all cases suggestive of European foulbrood;
4. the *Veterinary Authority* or other *Competent Authority* with responsibility for reporting and control of diseases of honey bees has current knowledge of, and authority over, all *apiaries* in the whole country.

Article 9.3.4.

Country or zone free from European foulbrood

1. Historically free status

   A country or *zone* may be considered free from the disease after conducting a *risk assessment* as referred to in Article 9.3.3. but without formally applying a specific *surveillance* programme if the country or *zone* complies with Chapter 1.4.

2. Free status as a result of an eradication programme

   A country or *zone* which does not meet the conditions of point 1 above may be considered free from European foulbrood after conducting a *risk assessment* as referred to in Article 9.3.3. and when:

   a. the *Veterinary Authority* or other *Competent Authority* with responsibility for reporting and control of diseases of honey bees has current knowledge of, and authority over, all domesticated *apiaries* existing in the country or *zone*;
   b. European foulbrood is notifiable in the whole country or *zone*, and any clinical cases suggestive of European foulbrood are subjected to field and *laboratory* investigations;
   c. for the three years following the last reported isolation of the European foulbrood agent, an annual survey supervised by the *Veterinary Authority* or other *Competent Authority*, with no positive results, have been carried out on a representative sample of *apiaries* in the country or *zone* to provide a confidence level of at least 95% of detecting European foulbrood if at least 1% of the *apiaries* were infected at a within-*apiary* prevalence rate of at

least 5% of the hives; such surveys may be targeted towards areas with the last reported isolation of the European foulbrood agent;

d. to maintain free status, an annual survey supervised by the *Veterinary Authority* or other *Competent Authority*, with no positive results, is carried out on a representative sample of hives in the country or *zone* to indicate that there has been no new isolations; such surveys may be targeted towards areas with a higher likelihood of isolation;
e. either there is no *wild* or self-sustaining *feral* population of species of the genus *Apis* in the country or *zone*, or there is an ongoing *surveillance* programme of the *wild* or self-sustaining *feral* population of species of the genus *Apis* which demonstrates no evidence of the presence of the disease in the country or *zone*;
f. the importation of the *commodities* listed in this chapter into the country or *zone* is carried out in accordance with the recommendations of this chapter.

CHAPTER 9.4.

INFESTATION WITH *AETHINA TUMIDA* (SMALL HIVE BEETLE)

Article 9.4.1.

General provisions

For the purposes of the *Terrestrial Code*, *infestation* with *Aethina tumida* (also known as small hive beetle) is an *infestation* of bee colonies (species of the genera *Apis* and *Bombus* and also stingless bees) by the beetle *A.tumida*, which is a free-living predator and scavenger affecting bee populations.

The adult beetle is attracted to bee colonies to reproduce, although it can potentially survive and reproduce independently in other natural environments, using other food sources, including certain types of fruit. Hence once it is established within a localised environment, it is extremely difficult to eradicate.

The life span of an adult beetle depends on environmental conditions such as temperature and humidity but, in practice, adult female beetles can live for at least six months and, in favourable reproductive conditions, the female is capable of producing up to a thousand eggs over a lifespan of four to six months. The beetle is able to survive at least two weeks without food.

Early signs of *infestation* and reproduction may go unnoticed. When the bees cannot prevent beetle mass reproduction on the combs, this leads to abandonment or collapse of the colony. Because *A. tumida* can be found and can thrive within the natural environment, and can fly up to 6-13 km from its nest site, it is capable of dispersing rapidly and directly invading new hives. Spread of *infestation* does not require contact between adult bees. The movement of adult bees, honeycomb and other apiculture products and used apicultural equipment may all cause *infestations* to spread to previously unaffected colonies.

When authorising import or transit of the *commodities* covered in the chapter, with the exception of those listed in Article 9.4.2., *Veterinary Authorities* should require the conditions prescribed in this chapter relevant to the *A. tumida* status of the honey bee and bumble bee population of the *exporting country* or *zone*.

Standards for diagnostic tests are described in the *Terrestrial Manual*.

Article 9.4.2.

Safe commodities

When authorising import or transit of the following *commodities*, *Veterinary Authorities* should not require any *A. tumida*-related conditions, regardless of the *A. tumida* status of the *exporting country* or *zone*:

1. honey bee semen;
2. honey bee venom.

Article 9.4.3.

Determination of the *A. tumida* status of a country or zone

The *A. tumida* status of a country or *zone* can only be determined after considering the following criteria:

1. a *risk assessment* has been conducted, identifying all potential factors for*A. tumida* occurrence and their historic perspective;
2. the presence of *A. tumida* is notifiable in the whole country, and all signs suggestive of *A. tumida* *infestation* are subjected to field and *laboratory* investigations;
3. ongoing awareness and training programmes are in place to encourage reporting of all cases suggestive of *A. tumida* *infestation*;
4. the *Veterinary Authority* or other *Competent Authority* with responsibility for reporting and control of diseases of honey bees has current knowledge of, and authority over, all domesticated *apiaries* in the country.

Article 9.4.4.

Country or zone free from *A. tumida*

1. Historically free status

A country or *zone* may be considered free from *A. tumida* after conducting a *risk assessment* as referred to in Article 9.4.3. but without formally applying a specific *surveillance* programme if the country or *zone* complies with Chapter 1.4.

2. Free status as a result of an eradication programme

A country or *zone* which does not meet the conditions of point 1 above may be considered free from *A. tumida* after conducting a *risk assessment* as referred to in Article 9.4.3. and when:

a. the *Veterinary Authority* or other *Competent Authority* with responsibility for reporting and control of diseases of honey bees has current knowledge of, and authority over, all domesticated *apiaries* existing in the country or *zone*;
b. the presence of *A. tumida* is notifiable in the whole country or *zone*, and any clinical cases suggestive of *A. tumida infestation* are subjected to field and *laboratory* investigations; a contingency plan is in place describing controls and inspection activities;
c. for the five years following the last report of the presence of *A. tumida*, an annual survey supervised by the *Veterinary Authority* or other *Competent Authority*, with no positive results, has been carried out on a representative sample of *apiaries* in the country or *zone* to provide a confidence level of at least 95% of detecting *A. tumida* if at least 1% of the *apiaries* were infested at a within-*apiary* prevalence rate of at least 5% of the hives; such surveys may be targeted towards areas with a higher likelihood of *infestation*;
d. to maintain free status, an annual survey supervised by the *Veterinary Authority* or other *Competent Authority*, with no positive results, is carried out on a representative sample of *apiaries* to indicate that there have been no presence of *A.*

tumida; such surveys may be targeted towards areas with a higher likelihood of *infestation*;

e. all equipment associated with previously infested *apiaries* has been destroyed, or cleaned and sterilised to ensure the destruction of *A. tumida* in accordance with one of the following procedures:
    i. heating to 50°C core temperature and holding at that temperature for 24 hours; or
    ii. freezing at core temperature of minus 12°C or less for at least 24 hours; or
    iii. irradiation with 400 Gray; or
    iv. by any procedure of equivalent efficacy recognised by the *Veterinary Authority* of the *importing* and *exporting countries*;
f. the soil and undergrowth in the immediate vicinity of all infested *apiaries* has been treated with a soil drench or similar suitable treatment that is efficacious in destroying incubating *A. tumida* larvae and pupae;
g. the importation of the *commodities* listed in this chapter into the country or *zone* is carried out in accordance with the recommendations of this chapter.

Article 9.4.9.

Recommendations for the importation of honey

Veterinary Authorities of *importing countries* should require the presentation of an *international veterinary certificate* attesting that:

1. the honey:

    EITHER

a. comes from *apiaries* situated in a country or *zone* free from *A. tumida*;

OR

b. has been strained through a filter of pore size no greater than 0.42 mm;

OR

c. has been treated to ensure the destruction of *A. tumida* in accordance with one of the following procedures:
    i. heating to 50°C core temperature and holding at that temperature for 24 hours; or
    ii. freezing at core temperature of minus 12°C or less for at least 24 hours; or
    iii. irradiation with 400 Gray; or
    iv. by any procedure of equivalent efficacy recognised by the *Veterinary Authorities* of the *importing* and *exporting countries*;

AND

2. all precautions have been taken to prevent contamination with *A. tumida*.

Article 9.4.10.

2. to prevent contamination with *A. tumida*.

CHAPTER 9.6.

INFESTATION OF HONEY BEES WITH *VARROA* SPP. (VARROOSIS)

Article 9.6.1.

General provisions

For the purposes of the *Terrestrial Code*, varroosis is a disease of honey bees (species of the genus *Apis*) caused by mites in the genus *Varroa*, primarily *Varroadestructor*. The mite is an ectoparasite of adults and brood of honey bees and spreads by direct contact from adult honey bee to adult honey bee, and by the movement of infested honey bees, bee brood, bee products and used apicultural equipment.

The number of mites steadily increases with increasing brood production and the growth of the honey bee population, especially late in the season when clinical signs of *infestation* can first be recognised. The lifespan of an individual mite depends on temperature and humidity but, in practice, it can be said to last from some days to a few months.

Honey bee colonies are often carriers of viruses. The mite acts as a *vector* for viruses (particularly deformed wing virus) facilitating their penetration and the *infection* of the honey bees. Most of the symptoms of varroosis are therefore the results of the combined action of *Varroa* spp. mites and viruses. The viral load within the colony increases with the mite *infestation*. Insufficient or late treatments lead to the killing of mites but the virus load remains high for several weeks with deleterious effects on the honey bee population. The control of the varroosis is mainly performed by the control of *Varroa* spp. and the diagnosis of varroosis is also performed by measuring the parasitic load.

When authorising import or transit of the *commodities* covered in the chapter, with the exception of those listed in Article 9.6.2., *Veterinary Authorities* should require the

conditions prescribed in this chapter relevant to the varroosis status of the honey bee population of the *exporting country* or *zone*.

Standards for diagnostic tests are described in the *Terrestrial Manual*.

Article 9.6.2.

Safe commodities

When authorising import or transit of the following *commodities*, *Veterinary Authorities* should not require any *Varroa* spp.-related conditions, regardless of the *Varroa* spp. status of the honey bee population of the *exporting country* or *zone*:

1. honey bee semen;
2. honey bee venom;
3. honey bee eggs;
4. royal jelly.

Article 9.6.3.

Determination of *Varroa* spp. status of a country or zone

The *Varroa* spp. status of a country or *zone* can only be determined after considering the following criteria:

1. a *risk assessment* has been conducted, identifying all potential factors for *Varroa* spp. occurrence and their historic perspective;
2. the presence of *Varroa* spp. is notifiable in the whole country or *zone*, and all clinical signs suggestive of varroosis are subjected to field and *laboratory* investigations;

3. an ongoing awareness programme is in place to encourage reporting of all cases suggestive of varroosis;
4. the *Veterinary Authority* or other *Competent Authority* with responsibility for reporting and control of diseases of honey bees has current knowledge of, and authority over, all domesticated *apiaries* in the country.

Article 9.6.4.

Country or zone free from *Varroa* spp.

1. Historically free status

   A country or *zone* may be considered free from *Varroa* spp. after conducting a *risk assessment* as referred to in Article 9.6.3. but without formally applying a specific *surveillance* programme (historical freedom) if the country or *zone* complies with Chapter 1.4.

2. Free status as a result of an eradication programme

   A country or *zone* which does not meet the conditions of point 1 above may be considered free from *Varroa* spp. after conducting a *risk assessment* as referred to in Article 9.6.3. and when:

   a. the *Veterinary Authority* or other *Competent Authority* with responsibility for reporting and control of diseases of honey bees has current knowledge of, and authority over, all domesticated *apiaries* existing in the country or *zone*;
   b. the presence of *Varroa* spp. is notifiable in the whole country or *zone*, and any clinical cases suggestive of varroosis are subjected to field and *laboratory* investigations;
   c. for the three years following the last report of the presence of *Varroa* spp., an annual survey supervised by the *Veterinary Authority* or other *Competent Authority*, with no positive results,

have been carried out on a representative sample of *apiaries* in the country or *zone* to provide a confidence level of at least 95% of detecting *Varroa* spp. if at least 1% of the *apiaries* were infested at a within-*apiary* prevalence rate of at least 5% of the hives; such surveys may be targeted towards areas with a higher likelihood of *infestation*;

d. to maintain free status, an annual survey supervised by the *Veterinary Authority* or other *Competent Authority*, with no positive results, is carried out on a representative sample of *apiaries* in the country or *zone* to indicate there has been no new *cases*; such surveys may be targeted towards areas with a higher likelihood of *infestation*;
e. either there is no *wild* or self-sustaining *feral* population of species of the genus *Apis* in the country or *zone*, or there is an ongoing *surveillance* programme of the *wild* or self-sustaining *feral* population of species of the genus *Apis* which demonstrates no evidence of the presence of the mite in the country or *zone*;
f. the importation of the *commodities* listed in this chapter into the country or *zone* is carried out in accordance with the recommendations of this chapter.

Recommendations for the importation of honey

Veterinary Authorities of *importing countries* should require the presentation of an *international veterinary certificate* attesting that the honey:

4. comes from *apiaries* situated in a country or *zone* free from *Varroa* spp.; or
5. has been strained through a filter of pore size no greater than 0.42 mm; or
6. has been treated to ensure the destruction of *Varroa* spp. in accordance with one of the following procedures:

a. heating to 50°C core temperature and holding at that temperature for 20 minutes; or
b. freezing at core temperature of minus 12°C or less for at least 24 hours; or
c. irradiation with 350 Gray; or
d. by any procedure of equivalent efficacy recognised by the *Veterinary Authorities* of the *importing* and *exporting countries*.

CODEX STAN
12-1981 Rev. 1 (1987)

CODEX STANDARD FOR HONEY

(World-wide standard)[5]

1. SCOPE

1.1 This standard applies to all honeys produced by honeybees and covers all styles of honey presentation which are offered for direct consumption.

1.2 The standard also covers honey which is packed in non-retail (bulk) containers and is intended for re-packing into retail packs.

2. DEFINITION

2.1 Definition of Honey

Honey is the natural sweet substance produced by honeybees from the nectar of blossoms or from secretions of living parts of plants or excretions of plant sucking insects on the living parts of plants, which honeybees collect, transform and combine with specific substances of their own, store and leave in the honey comb to ripen and mature.

2.2 Description

Honey consists essentially of different sugars predominantly glucose and fructose. The colour of honey varies from nearly colourless to dark brown. The consistency can be fluid, viscous or partly to entirely crystallized. The flavour and aroma vary, but usually derive from the plant origin.

2.3 Subsidiary Definitions and Designations

2.3.1 Origin

2.3.1.1 Blossom Honev or Nectar Honey is the honey which comes from nectaries of flowers.

2.3.1.2 Honeydew Honey is the honey which comes mainly from secretions of living parts of plants or excretions of plant sucking insects on the living parts of plants. Its colour various from very light brown or greenish to dark brown.

2.3.2 Methods of Processing

2.3.2.1 Extracted Honey is honey only obtained by centrifuging decapped broodless combs.

2.3.2.2 Pressed Honey is honey obtained by pressing broodless combs with or without the application of moderate heat.

2.3.2.3 Drained Honey is honey obtained by draining decapped broodless combs.

2.3.3 Styles - Honey which meets all the compositional and quality criteria of Section 3 of this standard may be presented as follows:

(a) Honey which is honey in liquid or crystalline state or a mixture of the two;

(b) Comb Honey which is honey stored by bees in the cells of freshly built broodless combs and which is sold in sealed whole combs or sections of such combs

(c) Chunk Honey which is honey containing one or more pieces of comb honey;

(d) Crystallized or Granulated Honey which is honey that has undergone a natural process of solidification as a result of glucose crystallization;

(e) Creamed (or creamy or set) Honey is honey which has a fine crystalline structure and which may have undergone a physical process to give it that structure and to make it easy to spread.

3. ESSENTIAL COMPOSITION AND OUALITY FACTORS

3.1 Honey shall not have any objectionable flavour, aroma, or taint absorbed from foreign matter during its processing and storage. The honey shall not have begun to ferment or effervesce.

3.2 Honey shall not be heated to such an extent that its essential composition and quality is impaired.

3.3 Apparent reducing sugar content, calculated as invert sugar:

(a)	Honey not listed below	-	Not less than 65%
(b)	Honeydew honey	-	Not less than 60%
(c)	Blackboy (Xanthorrhoea preissii)	-	Not less than 53%

3.4 Moisture Content

(a)	Honeys not listed below	-	Not more than 21%
(b)	Heather honey (Calluna)	-	Not more than 23%
(c)	Clover honey (Trifolium)	-	Not more than 23%

3.5 Apparent Sucrose Content

(a)	Honeys not listed below	-	Not more than 5%
(b)	Honeydew honey, blends of honeydew honey and blossom honey, Robinia, Lavender, Citrus, Alfalfa, Sweet-clover, Red Gum (Eucalyptus Camaldulensis), Acacia, leatherwood (Eucryphia Lucinda), Menzies Banksia (Banksia menziesii)	-	Not more than 10%
(c)	Red Bell (Calothamnus sanguineus), White stringy bark (Eucalyptus scabra), Grand Banksia (Banksia grandis), Blackboy (Xanthorrhoea preissi)	-	Not more than 15%

3.6 Water Insoluble Solids Contents

(a)	For honeys other than pressed honey	-	Not more than 0.1%
(b)	Pressed honey	-	Not more than 0.5%

3.7 Mineral Content (ash)

(a)	Honeys not listed below	-	Not more than 0.6%
(b)	Honeydew honey or a mixture of honeydew honey and blossom honey	-	Not more than 1.0%

3.8 Acidity	-	Not more than 40 milliequivalents acid per 1000 grammes
3.9 Diastase Activity		
Determined after processing and blendig in accordance with Section 7.7	-	Not more than 3
3.10 Hydroxymethylfurfural Content	-	Not more than 80 mg/kg

4. FOOD ADDITIVES

4.1 None permitted.

5. HYGIENE

5.1 It is recommended that the product covered by the provisions of this standard be prepared in accordance with the appropriate sections of the General Principles of Food Hygiene recommended by the Codex Alimentarius Commission (Ref. No. CACIRCP 1-1969, Rev. 2 (1985)).

5.2 Honey should be free from visible mould and, as far as practicable, be free from inorganic or organic matters foreign to its composition, such as, insects, insect debris, brood or grains of sand, when the honey appears in retail trade or is used in any product for human consumption.

5.3 Honey shall not contain toxic substances arising from microorganisms or plants in an amount which may constitute a hazard to health.

6. LABELLING

In addition to Sections 2, 3, 7 and 8 of the General Standard for Labelling or Prepackaged Foods (CODEX STAN 1~1985)6 the following specific provisions apply:

6.1 The Name of the Food

6.1.1 Subject to the provisions of 6.1.4 products conforming to the standard shall be designated "honey".

6.1.2 No honey may be designated by any of the designations in Section 2.3 unless it conforms to the appropriate description contained therein. The Styles in 2.3.3 (a), (c), (d) and (e) shall be declared.

6.1.3 Honey may be designated by the name of the geographical or topographical region if the honey was produced exclusively within the area referred to in the designation.

6.1.4 Honey may be designated according to floral or plant source if it comes wholly or mainly from that particular source and has the organoleptic, physicochemical and microscopic properties corresponding with that origin.

6.1.5 Honey complying with Sections 3.3(b) and (c), 3.4(b) and 3.5(b) and (c) shall have in close proximity to the word ʏʏ~~~~ʏl the common name or the botanical name of the floral source or sources.

6.2 Labelling of Non-Retail Containers

In addition to Sections 2, 3 and 8.1.3 of the General Standard the following specific provisions applies:

6.2.1 Information on labelling as specified in this Section shall be given either on the container or in accompanying documents, except that the name of the product, lot identification, and the name and address of the manufacturer or packer shall appear on the container.

6.2.2 Lot identification, and the name and address of the manufacturer or packer may be replaced by an identification mark provided that such a mark is clearly identifiable with the accompanying documents.

6.2.3 Outer containers holding prepackaged foods in small units (see Section 6 of the General Standard) shall be fully labelled.

7. METHODS OF ANALYSIS AND SAMPLING

7.1 Determination of reducing sugar content (Type I Method)

7.1.1 Principle of method

The method is a modification of the Lane and Bynon (1923) procedure involving the reduction of Soxhlet's modification of Fehling's solution by titration at boiling point against a solution of reducing sugars in honey using methylene blue as an internal indicator.

The maximum accuracy for this type of determination is attained by ensuring that the reduction of the Fehling' 5 solution during the standardization step and in the determination of the reducing sugars in the honey solution are carried out at constant volume. A preliminary titration is, therefore, essential to determine the volume of water to be added before the determinations are carried out to satisfy this requirement.

7.1.2 Reagents

7.1.2.1 Soxhlet's Modification of Fehling's Solution

Solution A: Dissolve 69.28 g copper sulphate pentahydrate ($CuSO_4.5H_20$; MW + 249.71) with distilled water to 1 litre. Keep one day before titration.

Solution B: Dissolve 346 g sodium potassium tartrate ($C_4H_4K\ NaO_6.4H_20$; MW + 282.23) and 100 g sodium hydroxide (NaOH) with distilled water to 1 litre. Filter through prepared asbestos.

7.1.2.2 Standard Invert Sugar Solution (10 gIL)

Weigh accurately 9.5 g pure sucrose, add 5 mL hydrochloric acid ca. 36.5 percent wiw pure HC1) and dilute with water to about 100 mL, store this acidified solution for several days at room temperature (ca. 7 days at 120 to 15^0C, or 3 days at 200 to 25°C), and then dilute to 1 litre. (N.B. Acidified 1.0 percent invert sugar remains stable for several months). Neutralize a suitable volume of this solution with iM sodium hydroxide solution (40 gIL) immediately before use and dilute to the required concentration (2 gIL) for the standardization.

7.1.2.3 Methylene Blue Solution

Dissolve 2 g in distilled water and dilute to 1 litre.

7.1.2.4 Alumina Cream

Prepare cold satured solution of alum ($K_250_4A1_2\ (50_4)_3.24H_20$) in water. Add ammonium hydroxide with constant stirring until solution is alkaline to litmus, let precipitate settle and wash by decantation with water until wash-water gives only slight test for sulphate with barium chloride solution. Pour off excess water and store residual cream in stoppered bottle.

7.1.3 Sampling

7.1.3.1 Liquid or Strained Honey

If sample is free from granulation, mix thoroughly by stirring or shaking; if granulated, place closed container in water-bath without submerging, and heat 30 mm. at 60^0C; then if necessary heat at 65 ^{0}C until liquefied. Occasional shaking is essential. Mix thoroughly and cool rapidly as soon as sample liquefies. Do not heat honey intended for hydroxymethylfurfural or diastatic determination. If foreign matter, such as wax, sticks, bees, particles of comb, etc., is present, heat sample to 40^0C in water-bath and strain through cheesecloth in hot-water-funnel before sampling.

7.1.3.2 Comb Honey

Cut top of comb, if sealed, and separate completely from comb by straining through a sieve the meshes of which are made by so weaving wire as to form square opening of 0.500 mm by 0.500 mm[7] when portions of comb or wax pass through sieve, heat sample as in 7.1.3.1 and strain through cheesecloth. If honey is granulated in comb, heat until wax is liquefied; stir, cool and remove wax.

7.1.4 Procedure

7.1.4.1 Preparation of Test Sam~le - First Procedure (applicable to honeys which may contain sediment)

(a) Transfer an accurately weighed sample of approximately 25 g (W_1) from the homogenized honey to 100 mL volumetric flask, add 5 mL alumina cream (7.1.2.4) dilute to volume with water at 20^0C and filter.

(b) Dilute 10 mL of this solution to 500 mL with distilled water (diluted honey solution).

OR

7.1.4.2 Pre aration of Test Sam le - Second Procedure

(a) Weight accurately a representative quantity of about 2 g (W_2) of the homogeneous honey sample, dissolve in distilled water and dilute to 200 mL in a calibrated flask (honey solution).

(b) Dilute 50 ml of the honey solution to 100 mL using distilled water (diluted honey solution).

7.1.4.3 Standardization of the Modified Fehlin~'s Solution

Standardize the modified Fehling' 5 solution A so that exactly 5 mL (pipette), when mixed with approximately 5 mL of Fehling's solution B, will react completely with 0.050 g invert sugar added as 25 mL dilute invert sugar solution (2 gIL).

7.1.4.4 Preliminary Titration

The total volume of the added reactants at the completion of the reduction titration must be 35 mL. This is made up by the addition of a suitable volume of water before the titration commences. Since the compositional criteria of the honey standard specify that there should be more than 60 percent reducing sugars (calculated as invert sugar) a preliminary titration is necessary to establish the volume of water to be added to a given sample to ensure the reduction is carried out at constant volume. This volume of water to be added is calculated by subtracting the volume of diluted honey solution consumed in the preliminary titration (c mL) from 25 mL.

Pipette 5 mL Fehling's solution A into a 250 mL Erlenmeyer flask and add approximately 5 mL Fehling's solution B. Add 7 mL distilled water, a little powdered pumice or other suitable antibumping agent, followed by about 15 mL diluted honey solution from a burette. Heat the cold mixture by boiling over a wire gauze, and maintain moderate ebullition for 2 mm. Add 1 mL 0.2 percent aqueous methylene blue solution whilst still boiling and complete the titration within a total boiling time of 3 minutes, by repeated small additions of diluted honey solution until the indicator is decolorized. It is the colour of the supernatant liquid that must be observed. Note the total volume of diluted honey solution used (x mL).

7.1.4.5 Determination

Calculate the amount of added water necessary to bring the total volume of the reactants at the completion of the titration to 35 mL by subtracting the preliminary titration (x mL) from 25 mL.

Pipette *5* mL Fehling's solution A into a 250 mL Erlenmeyer flask and add approximately 5 mL Fehling's solution B.

Add (25-x) mL distilled water, a little powdered pumice or other suitable antibumping agent and, from a burette, all but 1.5 mL of the diluted honey solution volume determined in the preliminary titration. Heat the cold mixture to boiling over a wire gauze and maintain moderate ebullition for 2 mm. Add 1.0 mL 0.2 percent methylene blue solution whilst still boiling and complete the titration within a total boiling time of 3 mm. by repeated small additions of diluted honey solution until the indicator is

decolorized. Note the total volume of diluted honey solution (y mL). Duplicate titrations should agree within 0.1 mL.

7.1.5 Calculation and Expression of Results

7.1.5 Calculation and Expression of Results

Where the First Procedure (7.1.4.1) has been used:

C = ---- X ----

Wher the Second Procedure (7.1.4.2) has been used:

C = ---- X ----

Where C = g invert sugar per 100 g honey

W_1 = weight (g) of honey sample taken according to sub-section

W_2 = weight (g) of honey sample taken according to sub-section 7.1.4.2

Y_1 = volume (mL) of diluted honey solution consumed in the determination carried out according to the First Procedure (7.1.4.1)

Y_2 = volume (mL of diluted honey solution consumed in the determination carried out according to the Second Procedure (7.1.4.2)

7.1.6 Notes on the Procedure

It is essential to the accuracy and repeatability of the determination that the volume of water necessary to bring the reactant mixture to a total volume of 35 mL be determined for each individual sample; the following table gives typical volumes which may be encountered at the preliminary titration stage for the incremental contents of invert sugar shown, assuming the test sample (7.1.4.1) weighs about 25 g or test sample (7.1.4.2) weighs about 2 g.

Invert Sugar content %	**Volume of Distilled Water to be Added mL**
60	8.3
65	9.6
70	10.7

75 11.6

7.2 Determination of Apparent Sucrose Content (Type I Method)

7.2.1 Principle of the Method

Based on the Walker (1917) inversion method.

7.2.2 Reagents

7.2.2.1 Soxhlet modification of Fehling's solution (7.1.2.1)

7.2.2.2 Standard invert sugar solution (7.1.2.2)

7.2.2.3 Hydrochloric acid (6.34 M aqueous)

7.2.2.4 Sodium hydroxide solution 2 gIl litre (7.1.2.3)

7.2.2.5 Methylene blue solution 2 gIl litre (7.1.2.3)

7.2.3 Sampling

The honey is prepared for sampling as in 7.1.3

7.2.4 Procedure

7.2.4.1 Preparation of test sample

Prepare the honey sample as in 7.1.4.1(a). Dilute 10 mL of this solution to 250 mL with distilled water: honey solution (for sucrose determination) OR prepare the honey solution as in 7.1.4.2(a).

7.2.4.2 Hydrolysis of the test samnle

The honey solution (50 mL) is placed in a 100 mL graduated flask, together with 25 mL distilled water; heat the test sample to 65 ^{0}C over a boiling water-flask. The flask is then removed from the water-bath and 10 ml of 6.34 M hydrochloric acid added. The solution is allowed to cool naturally for 15 minutes, and then brought to 20^{0}C and neutralizing with *5* M sodium hydroxide, using litmus paper as indicator, cooled again, and the volume adjusted to 100 mL (diluted honey solution).

7.2.4.3 Titration

As in 7.1.4.4 and 7.1.4.5.

7.2.5 Calculation and expression of results

Calculate percent invert sugar (g invert sugar per 100 g honey) after inversion using the appropriate formula as percent invert sugar before inversion in 7.1.5.

Apparent sucrose content = (invert sugar content after inversion minus invert sugar content before inversion) X 0.95

The result is expressed as g apparent sucrosell00 g honey.

7.3 Determination of Moisture Content (Type I Method)

7.3.1 Principle of Method

Based on the refractometric method of Chataway (1932), revised by Wedmore (1955).

7.3.2 Apparatus

Refractometer

7.3.3 Sampling

The honey is prepared for sampling as in 7.1.3.

7.3.4 Procedure

7.3.4.1 Determination of the Refractive Index

Determine the refractive index of the test sample using a refractometer at a constant temperature near 20^0C. Convert the reading to moisture content (percent mim) using the table given below. If the determination is made at a temperature other than 20^0C, convert the reading to standard temperature of 20^0C, according to ~he temperature corrections quoted. The method used is to be noted in the test report.

TABLE FOR THE ESTIMATION OF MOISTURE CONTENT

Refractive Index (20°C)	Moisture Content (percent)	Refractive Index (20°)	Moisture Content (percent)	Refractive Index (20°C)	Moisture Content (percent)

1.5044	13.0	1.4935	17.2	1.4830	21.4
1.5038	13.2	1.4930	17.4	1.4825	21.6
1.5033	13.4	1.4925	17.6	1.4820	21.8
1.5028	13.6	1.4920	17.8	1.4815	22.0
1.5023	13.8	1.4915	18.0	1.4810	22.2
1.5018	14.0	1.4910	18.2	1.4805	22.4
1.5012	14.2	1.4905	18.4	1.4800	22.6
1.5007	14.4	1.4900	18.6	1.4795	22.8
1.5002	14.6	1.4895	18.8	1.4790	23.0
1.4997	14.8	1.4890	19.0	1.4785	23.2
1.4992	15.0	1.4885	19.2	1.4780	23.4
1.4987	15.2	1.4880	19.4	1.4775	23.6
1.4982	15.4	1.4875	19.6	1.4770	23.8
1.4976	15.6	1.4870	19.8	1.4765	24.0
1.4971	15.8	1.4865	20.0	1.4760	24.2
1.4966	16.0	1.4860	20.2	1.4755	24.4
1.4961	16.2	1.4855	20.4	1.4750	24.6
1.4956	16.4	1.4850	20.6	1.4745	24.8
1.4951	16.6	1.4845	20.8	1.4740	25.0
1.4946	16.8	1.4840	21.0		
1.4940	17.0	1.4835	21.2		

7.3.4.2 Temperature Corrections - Refractive Index:

Temperatures above 20°C - Add 0.00023 per °C
Temperatures below 20°C - Subtract 0.00023 per °C

7.4 Gravimetric Determination of Water-insoluble Solids Content (Type II Method)

7.4.1 Sampling

The honey is prepared for sampling as in 7.1.3.

7.4.2 Procedure

7.4.2.1 Preparation of Test Sample

Honey (20 g) is weighed to the nearest centigram (10 mg) and dissolved in a suitable quantity of distilled water at 80^0C and mixed well.

7.4.2.2 Gravimetric Determination

The test sample is filtered through a previously dried and weighed fine sintered glass crucible (pore size 15.40 ~m) and washed thoroughly with hot water (80^0C) until free from sugars (Mohr test). The crucible is dried for one hour at 135 ^{0}C, cooled and weighed to 0.1 mg.

7.4.3 Expression of Results

The result is expressed as percent water-insoluble solids (m/m).

7.5 D~ination of Mineral Content ash (Type I Method)

7.5.1 Sampling

Honey is prepared for sampling as in 7.1.3.

7.5.2 Procedure

7.5.2.1 I~mtionofthe Honev

Honey (5010 g) is weighed accurately into an ignited and pre-weighed platinum or silica dish and gently heated in a muffle furnace until the sample is black and dry and there is no danger of loss by foaming and overflowing. An infra-red lamp can also be used to char the sample before inserting into the furnace. If necessary, a few drops of olive oil may be added to prevent frothing. The sample is then ignited at 600^0C to constant weight. The sample is cooled before weighing.

7.5.3 Expression of Results

The result is expressed as percent ash *(mim).*

7.6 Determination of Acidity (Type II Method)

7.6.1 Sampling

The honey is prepared for sampling as in 7.1.3.

7.6.2 Reagents

7.6.2.1 Sodiumhdroxide0.1N (carbonate-free)

7.6.2.2 Phenolnhthalein indicator 1 percent *(mlv)* in ethanol, neutralized.

7.6.2.3 Distilled Water made carbon dioxide free by boiling and subsequent cooling.

7.6.3 Procedure

7.6.3.1 Preparation of Test Sample

Honey (10.0 g) is weighed accurately and dissolved in 75 mL distilled water

(7.6.2.3).

7.6.3.2 Titration

The test sample is titrated against carbonate-free 0.1 M sodium hydroxide solution using 4-5 drops of neutralized 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 pH 8.3.

7.6.4 Calculation and Expression of Results

The result is expressed as millival (milli-equivalents acid/kg honey and is calculated as follows:

Acidity = 10 v

where v = the number of mL 0.1 M NaOH used in the neutralization of 10 g honey.

7.7 Determination of Diastase Activit (Type I Method)

7.7.1 Principle of the Method

Based on the method of Schade et al., (1985) modified by White et al., (1959) and Hadorn (1961).

7.7.2 Reagents

7.7.2.1 Iodine Stock Solution:

Dissolve 8.8 g of iodine analytical grade, in 30-40 mL water containing' 22 g potassium iodine, analytical grade, and dilute to 1 litre with water.

7.7.2.2 Iodine solution 0.0007 N:

Dissolve 20 g potassium iodine, analytical grade, 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.

7.7.2.3 Acetate Buffer - pH 5.3 (1.59M):

Dissolve 87 g sodium acetate.$3H_20$ in 400 mL 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.

7.7.2.4 Sodium Chloride Solution 0.5M:

Dissolve 14.5 g sodium chloride, analytical grade, in ;boiled-out distilled water and make up to 500 mL. The keeping time is limited by mould growth.

7.7.2.5 Starch Solution:

(a) Preparation of soluble starch

In a conical flask immersed in a water-bath and fitted with a reflux condenser, boil 20 g of potato starch for one hour in the presence of a mixture of 100 mL of 95 percent ethanol and 7 mL of 1 M hydrochloric acid. Cool, filter through a filtering crucible (pore size 90 - 150 ~m) and wash with water until the wash/water ceases to give any chloride reaction. Drain thoroughly and dry the starch in air at 35 ^{0}C. The soluble starch must be stored in a well stoppered flask.

(b) Determination of moisture content of soluble starch

Accurately weigh a quantity of approximately 2 g of soluble starch and spread in a thin layer over the bottom of a weighing bottle (diameter *5* cm). Dry for one and a half hours at 130^0C. Allow to cool in a dessicator and re-weigh. The weight loss with respect to 100 g represents the moisture content. The moisture content of such starch should be 7-8% mlm depending on the humidity of the air in which the sample has been dried.

(c) Preparation of starch solution

Use a starch with a blue value between *0.5-0.55* using a 1 cm cell, as determined by the method below. Weigh out the amount of starch which is equivalent to 2.0 g 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 3 mm., cover and allow to cool spontaneously to room temperature. Transfer to a 100 mL volumetric flask, place in a water bath at 40^0C 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 g 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 M hydrochloric acid and 1.5 mL of 0.02 N iodine solution. Then add 0.5 mL of the starch solution and make up to volume with water. Allow to stand for oneJIour in the dark and read in 1 cm cell using a spectrophotometer at 660 nm against a blank containing all the ingredients except the starch solution. Reading on the absorbance scale = Blue value.

7.7.3 Apparatus

7.7.3.1 Water-bath at 40 ± 0.2^0C.
7.7.3.2 Spectrophotometer to read at 660 rim.

7.7.4 Sampling

The honey sample is prepared as in 7.1.3 without any heating.

7.7.5 Procedure

7.7.5.1 Preparation of test sam les

Honey solution: 10.0 g honey is weighed into a 50 mL beaker and 5.0 mL acetate buffer solution is added, together with 20 mL water to dissolve the sample. The sample is completely dissolved by stirring the cold solution. 3.0 mL sodium chloride solution is added to a 50 mL volumetric flask and the dissolved honey sample is transferred to this and the volume adjusted to 50 mL.

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

Standardization of the starch solution

The starch solution is warmed to 40^0C and *5* mL pipetted into 10 mL of water at 40^0C and mixed well. I mL of this solution is pipetted into 10 mL 0.0007 N iodine solution, diluted with 35 mL of water and mixed well. The colour is read at 660 nm against a water blank using a 1 cm cell.

The absorbance should be 0.760 $\pm$ 0.020. If necessary the volume of added water is adjusted to obtain the correct absorbance.

7.7.5.2 Absorbance determination

Pipette 10 mL honey solution into 50 mL graduated cylinder and place in $_{400} \pm 2^0$C water-bath with flask containing starch solution. After 15 minutes, pipette *5* starch solution into the honey solution, mix, and start stop-watch. At *5* minutes intervals remove 1 mL aliquots and add to 10.00 mL 0.0007 N iodine solution. Mix and dilute to standard volume (see 6.7.5.1). Determine absorbance at 660 nm in spectrophotometer immediately using I cm cell. Continue taking 1 mL aliquots at intervals until absorbance of less than 0.235 is reached.

7.7.6 Calculation and expression of results

The absorbance is plotted against time (mm) on a rectilinear paper. A straight line is drawn through at least the last three points on the graph to determine the time when the reaction mixture reaches an absorbance of o.235. Divide 300 by the time in minutes to obtain the diastase number (DN). This number expresses the diastase activity as ml 1 percent starch solution hydrolysed by the enzyme in 1 g of honey in 1 h at 40^0C. This diastase number corresponds with the Gothe-scale number.

Diastase activity = DN = ml starch solution 1 percent)/g honey/h at 40^0C.

7.8 Spectrophotometric determination of hydroxymethylfurfural (HMF) content (Type II Method)[8]

According to the AOAC method (AOAC, 14th Ed., 1984, Hydroxymethylfurfural in Honey, Spectrophotometric Method, 31.153).

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[5] Supersedes the Codex European Regional Standard for Honey (CODEX STAN 12-1981).

[6] Hereafter referred to as "The General Standard"

[7] Ref. ISO 565-1983. Such sieve could be replaced by U.S. sieve with No.40 Standard screen (size of opening 0.420 mm).

[8] Adopted by the 17 Session of the Commission.