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Methods for assessing quality characteristics of non-grain starch staples. (Part 4. Advanced Methods.)

Methods for Assessing Quality Characteristics of Non-Grain Starch Staples

PART 4.
ADVANCED METHODS

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Methods for Assessing Quality Characteristics of Non-Grain Starch Staples

Part 4. Advanced Methods

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Natural Resources Institute

Overseas Development Administration

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Cover illustration. Clockwise from top left. Banana (*Musa* spp.), cassava (*Manihot esculenta*) and sweet potato (*Ipomoea batatas*). *Drawings reproduced by kind permission of the Royal Botanic Gardens, Kew.*

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Section 4.1 Gas chromatographic and high performance liquid chromatographic analytical techniques

INTRODUCTION

The gas chromatographic (GC) and high performance liquid chromatographic (HPLC) analytical methods given in this manual are taken from published scientific literature. They have been selected for their applicability for the analysis of non-grain starch staples (NGSS).

The purpose of these methods is primarily for research and development work, for example, in determining factors influencing insect resistance, and changes during storage and food preparation. These methods have not been validated by NRI experts and further work may be necessary for reliable results to be obtained. Some of the methods have been developed for a single commodity only; it is suggested that the researcher considers suitable modifications for his own application and available facilities.

The sections containing methods for extending HPLC and GC columns and consumables may be of use to those who are working to a limited resource budget or experiencing difficulty in obtaining consumables. These methods include the recycling and recovery of organic solvents, the preparation of HPLC grade water, mobile phase filtration, solvent degassing and prolonging the life of HPLC and GC columns.

GAS CHROMATOGRAPHIC METHODS FOR SUGARS, ORGANIC ACIDS AND VOLATILE COMPOUNDS

Soluble sugars in yam tubers, roots, leaves and stems

Introduction

Soluble sugars in yam tubers, stems and roots include fructose, glucose, sucrose and maltose. Fructose, glucose and polyols (2-deoxysorbitol, 6-deoxysorbitol, glycerol) are found in the leaves. The method described enables the quantitative determination of each of the above sugars in fresh tubers, stems, roots and leaves.

Rationale

Soluble sugars in yam tubers influence the flavour and textural characteristics of the cooked product.

Suitability

This method accurately determines the presence of soluble sugars in fresh yam tubers (including leaves, stems and roots). Although other commodities such as banana, plantain, sweet potato, cassava and cocoyam have not been included, it should be possible to use this method, with suitable modifications to the extraction method determined by the researcher.

Limitations

Limitations have not been reported. It is recommended that the accuracy, precision and detection limits are determined by the researcher for his particular analytical system.

Principles

This method describes the quantification of volatile trimethyl esters of soluble sugars by means of gas chromatographic separation and mass spectrophotometric detection and identification after prior extraction of the dried flour in ethanol.

Requirements

Equipment

- Laboratory oven
- Mill
- Analytical balance accurate to four decimal places
- Freezer (–20 °C)
- Rotary evaporator
- Vacuum pump
- Gas chromatograph equipped with a flame ionization detector
- Mass spectrometer
- Freeze drier

Consumables

All reagents are analytical grade unless otherwise stated.

- Distilled water
- Meso-inositol
- Ethanol
- Pyridine
- Hexamethyldisilazane
- Trimethylchlorosilane
- Heptane
- Gases (nitrogen, air, hydrogen)
- Capillary column (30 m × 0.32 mm i.d.; silicone OV-101)
- Liquid nitrogen

Procedure

Storage of tubers

The tubers should be stored at 25 °C and 80% relative humidity.

Extraction of soluble sugars

Dry the leaves, stem, roots and sliced tubers in an oven at 80 °C. Mill each sample to form a flour.

Hydrate 3 mg of flour with 0.5 ml distilled water followed by the addition of 0.5 ml aqueous meso-inositol (100 µg/ml) and 5 ml ethanol. Leave mixture at –20 °C for 72 h, then filter. Evaporate the filtrate to dryness at 30 °C.

Silylation of the sugars

Redissolve the extract in 2 ml distilled water. Freeze dry to remove the water. Add 400 µl pyridine, 200 µl hexamethyldisilazane and 100 µl trimethylchlorosilane and leave for 30 min at room temperature. Remove excess reagents by evaporation under nitrogen gas. Dissolve the silyated sugars in heptane and separate by GC.

Gas chromatography

Use a GC equipped with a flame ionization detector (FID) and the following:

- capillary column, silicone OV-101 (30 m × 0.32 mm i.d.);
- injector temperature of 220 °C;
- FID temperature of 240 °C;

- column oven, programmed from 100–240 °C at either 2 or 4 °C min.

The concentration of the sugars can be determined by the external standard method in which:

$$\text{concentration (g/100 g)} = \frac{\text{R sample}}{\text{R standard}} \times \frac{\text{standard concentration (g/100 ml)}}{\text{sample weight (g)}} \times \frac{\text{injection volume standard (µl)}}{\text{injection volume sample (µl)}}$$

where: R = peak area or height.

Mass spectrometry

When suitable standards are not available or the identity of a component is unknown, mass spectrometry can be used to determine its nature.

The following conditions are recommended:

- electron energy, 70 eV;
- ionizing current, 0.2 mA.

References

KOUASSI, B., DIOPOH, J. and FOURNET, B. (1990) Soluble sugars from yam and changes during their storage. *Phytochemistry,* **29**: 1069–1072.

Soluble sugars and organic acids in sweet potato

Introduction

Soluble sugars and organic acids in sweet potatoes consist of fructose, glucose and sucrose and malic, citric and quinic acids. The method described enables the quantitative determination of each of the above sugars and organic acids in fresh sweet potato tubers.

Rationale

The sensory quality of cooked sweet potato depends partially on the sugar and organic acid content of the raw root. In the case of the sugars, those present in raw roots (fructose, glucose and sucrose) are sweeter on a weight basis than maltose; maltose, although not present in raw roots, is formed during cooking to produce the most abundant sugar in the cooked product. The presence of sugars and organic acids can also be used to monitor changes during storage of tubers.

Suitability

This method determines accurately the presence of soluble sugars (fructose, glucose, sucrose) and organic acids (malic, citric, quinic) in sweet potato tubers. Although other commodities such as banana, plantain, yam, cassava and cocoyam have not been included, it should be possible to use this method, with suitable modifications to the extraction procedure as determined by the researcher.

Limitations

Limitations for this method have not been reported. It is recommended that the accuracy, precision and detection limits be determined by the researcher for his particular analytical system.

Principles

This method allows the quantification of volatile trimethyl esters of soluble sugars and organic acids by means of GC separation and detection after previous extraction in methanol of the fresh tuber.

Requirements

Equipment

- Tissue homogenizer
- Analytical balance accurate to four decimal places
- Sonicator

- Water-bath
- Freezer (–20 °C)
- Gas chromatograph equipped with a flame ionization detector

Consumables

All reagents are analytical grade unless stated otherwise.

- Distilled water
- Methanol
- Dimethylformide (DMF)
- N,O-bis (trimethyl silyl)-trifluoroacetamide (BSTFA)
- Malic acid
- Citric acid
- Quinic acid
- Chlorogenic acid
- Fructose
- Sucrose
- Glucose
- Inositol
- Phenyl-β-D-glucopyranoside
- Gases (nitrogen, air, hydrogen)
- Capillary column (12 m × 0.5 mm i.d.; SE-54)

Procedure

Storage of tubers

The tubers should be stored at 13 °C and 85% relative humidity.

Extraction of soluble sugars and organic acids

Weigh 530–570 mg of tissue into a 20 ml scintillation vial and add 15 ml methanol. Homogenize for 3 min, then seal the vial and ultrasonicate for 30 min. Filter and store at –18 °C until required for analysis.

Silylation of sugars and organic acids

Warm samples to room temperature. To a portion of the methanol extract (equivalent to 15–20 µl fresh tissue) add 15 µl internal standard (phenyl-β-D-glucopyranoside, 8.89 µg/µl). Remove the solvent under N_2 at 40 °C. Add 75 µl BSTFA:DMF (1:1) and seal the vial. Sonicate for 30 min followed by heating at 76 °C for 50 min.

Gas chromatography of sugars and acids

Use a GC equipped with a flame ionization detector (FID) and the following:

- capillary column, SE-54 (12 m × 0.5 mm i.d.);
- injector temperature of 250 °C;
- FID temperature of 325 °C;
- column oven, programmed at 100 °C for 1 min then increased at 7 °C/min to 320 °C and held for 25 min;
- injection volume of 1 µl (splitless using a 1 min purge activation time).

The concentration of the sugars and organic acids can be determined by the internal standard method in which:

$$\text{concentration of unknown} = \frac{\text{CR sample}}{\text{CR ISTD}} \times \frac{\text{standard concentration}}{\text{sample weight}} \times \frac{\text{injection volume standard (µl)}}{\text{injection volume sample (µl)}}$$

where: CR = corrected response of sample and internal standard peaks after correction by their calibration curves;
ISTD = internal standard.

References

SON, K., SEVERSON, R. F., SNOOK, M. E. and KAYS, S. J. (1991) Root carbohydrate, organic acids and phenolic chemistry in relation to sweet potato weevil resistance. *Hortscience,* **26**: 1305–1308.

Volatile compounds of ripening bananas

Introduction

The changing pattern of the volatile compounds during fruit ripening can be used to monitor the biochemical events that take place in the live tissue. Volatile compounds reported to be present in ripening bananas include acetate esters, butyrate esters, alcohols, 2-butanol and 2,3-butylene glycol. The production of these compounds has been reported to increase steadily to a maximum, reach a plateau, and thereafter begin to decline, or follow a cyclic pattern, depending on the volatile sampling method used. The method described here minimizes sample alteration and maximizes analytical efficiency.

Rationale

The profile or pattern of volatiles alters during fruit ripening and can be used to monitor biochemical processes that occur in the live tissue.

Suitability

The method described below determines the presence of volatile compounds (acetate esters, butyrate esters, alcohols, 2-butanol and 2,3-butylene glycol) in ripening bananas. Although other commodities such as yam, plantain, sweet potato, cassava and cocoyam have not been included, it is possible to use this method for them with suitable modifications determined by the researcher.

Limitations

Limitations to this method are not known to have been reported. It is recommended that the accuracy, precision and detection limits are determined by the researcher for his particular analytical system.

Principles

This method allows the quantification of volatile compounds of banana (fruit) by GC separation after previous extraction in pure air in a glass chamber housed within a thermostatically controlled water-bath.

Requirements

Equipment

- Thermostatically controlled water-bath (± 0.5 °C)
- Glass chamber (10 l)
- Gas chromatograph equipped with a flame ionization detector (FID)
- Mass spectrometer

Consumables

All reagents are analytical grade unless stated otherwise.

- Air (99.99% pure)
- Ethyl acetate
- Isobutyl alcohol
- Butanol
- 2-pentanone
- 2-pentanol

- Propyl acetate
- Isopentyl alcohol
- Isobutyl acetate
- Ethyl butyrate
- Butyl acetate
- 2-pentyl acetate
- Isoamyl acetate
- Isobutyl butyrate
- Butyl butyrate
- 2-pentyl butyrate
- Isoamyl butyrate
- Isoamyl isovalerate
- Gases (nitrogen, air, hydrogen)
- Capillary column (28 m × 0.25 mm i.d.; DB-1, 0.25 μm film thickness)

Procedure

Extraction of volatiles

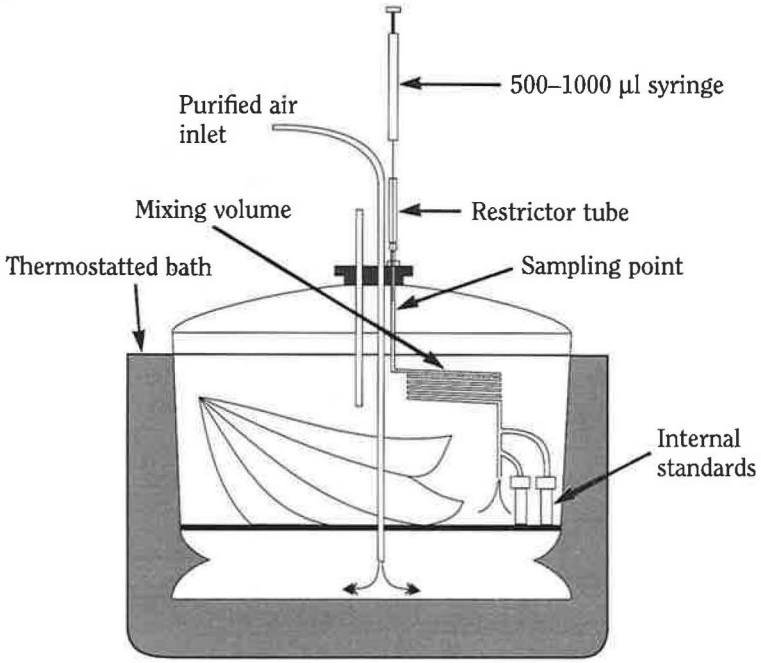

Figure 4.1 Equipment for volatile compound sampling

Place 1 kg of bananas in a 10 l glass chamber housed within a thermostatically controlled water-bath (20.5 ± 0.5 °C) as shown in Figure 4.1. Allow air (99.99% purity) to pass into the chamber at a flow rate of 300 ml/min (equivalent to sweeping the chamber volume every 30 min). Methyl valerate and isopropyl propionate are used as internal standards. The sample volatiles and internal standards are sampled with a syringe fitted with a fused silica capillary needle (500 μl). Sample over 50 s at a rate of 100 μl/s. Samples can be taken over a period of 10 days.

Gas chromatography

Use a GC equipped with an FID and the following:

- capillary column, 0.25 μm film thickness of DB-1 (28 m × 0.25 mm i.d.);
- injector temperature of 220 °C;

- FID temperature of 230 °C;
- column oven, programmed at 30 °C for the first 5 min and then increased at 3 °C/min until the end of the separation.

The concentration of the volatiles (when appropriate standards are available) can be determined by the internal standard method, in which:

$$\text{concentration of unknown} = \frac{\text{CR sample}}{\text{CR ISTD}} \times \frac{\text{standard concentration}}{\text{sample weight}} \times \frac{\text{injection volume standard (µl)}}{\text{injection volume sample (µl)}}$$

where: CR = corrected response of sample and internal standard peaks after correction by their calibration curves;
ISTD = Internal standard.

Table 4.1 Typical peak numbers and retention times for volatiles from ripening bananas

Compound	Peak no.	Retention time (min)
Ethyl acetate	7	1.9
Isobutyl alcohol	8	2.1
Butanol	10	2.4
2-pentanone	11	2.6
2-pentanol	12	2.9
Propyl acetate	13	3.2
Isopentyl alcohol	14	3.8
Isopropyl propionate	*ISTD*	*4.3*
Isobutyl acetate	16	4.8
Ethyl butyrate	18	6.1
Butyl acetate	19	6.7
Methyl valerate	*ISTD*	*7.4*
2-pentyl acetate	20	8.4
Isoamyl acetate	25	9.9
Isobutyl butyrate	33	14.3
Butyl butyrate	34	16.5
2-pentyl butyrate	41	18.2
Isoamyl butyrate	46	19.8
Isoamyl isovalerate	49	22.5

Note: ISTD = internal standard

Mass spectrometry

When suitable standards are not available, or the identity of a component is unknown, mass spectrometry can be used to determine the nature of the component.

References

MACKU, C. and JENNING, W. G. (1987) Production of volatiles by ripening bananas, *Journal of Agriculture and Food Chemistry,* **35**: 845–848.

HIGH PERFORMANCE LIQUID CHROMATOGRAPHIC METHODS FOR SUGARS, PHENOLICS, ORGANIC ACIDS AND ETHANOL

Sugar in banana pulp, cassava starch hydrolysate and sweet potatoes

Introduction

Soluble sugars found in sweet potato and bananas include glucose, fructose and sucrose, and, in cassava, the starch hydrolysates glucose, maltose and trisaccharides. The method described here enables the quantitative determination of these sugars in fresh tubers, stems, roots and leaves.

Rationale

Sweet potato. The sensory quality of cooked sweet potato partially depends on the sugar content of the raw root, because the sugars (fructose, glucose and sucrose) present in raw roots are sweeter on a weight basis than maltose; maltose, although not present in raw roots, is formed during cooking to produce the most abundant sugar in the cooked product.

Cassava starch hydrolysate. Cassava starch can be used for the production of alcohol and has an impact on the sensory quality of the cooked root. The starch hydrolysate contains glucose, maltose and trisaccharides.

Banana. The determination of sugars in banana is important in determining the maturity and sensory properties of the raw product. Sugars found in banana include glucose, fructose and sucrose.

Suitability

The analytical methods described allow the accurate determination of soluble sugars in sweet potato (tissue and root), banana and cassava starch hydrolysate. Although other commodities such as cassava tuber, yam and cocoyam have not been included, it should be possible to use the method with suitable modifications, as determined by the researcher, to the extraction procedures.

Limitations

Limitations to these methods have not been reported. It is recommended that the accuracy, precision and detection limits are determined by the researcher for his particular analytical system.

Principles

These methods allow the quantification of soluble sugars (glucose, fructose, sucrose, trisaccharides) by HPLC separation and refractive index (RI) detection after previous extraction.

Requirements

Equipment

- Laboratory oven
- Analytical balance accurate to four decimal places
- Freezer (–10 °C)
- Centrifuge
- Homogenizer
- Waring blender
- Isocratic HPLC system equipped with a refractive index (RI) detector
- Food grater

Consumables

All reagents are analytical grade unless stated otherwise.

- Distilled/deionized water
- Water (HPLC grade)
- Ethanol
- Acetonitrile (HPLC grade)
- Glucose
- Fructose
- Sucrose
- Maltose
- Mixed ion-exchange resin
- 0.2 μm disposable filter
- HPLC column A—Aminex HPX-85 carbohydrate analysis column (Biorad Labs, UK)
- HPLC column B—Bondapak TM carbohydrate column
- HPLC column C—Amino 5S column (Biorad Labs, UK)
- Cheesecloth

Procedure

Extraction of soluble sugars

Sweet potatoes *Cell sap.* Place 25 g of grated sweet potato tuber into a cheesecloth and manually compress the cheesecloth and its contents. Centrifuge 1 ml. Mix 0.2 ml of the supernatant with 0.8 ml ethanol and store at –10 °C until analysis by HPLC.

Whole tissue. To 5 g of grated whole tissue add ethanol (less than 100 ml) and homogenize for 1 min. Transfer mixture to a 100 ml volumetric flask and make up to the mark such that the final concentration of ethanol is 80% (assume the sweet potato has a moisture content of 75%). Hold the mixture for 7 days to equilibrate.

Banana pulp Using a Waring blender, blend 50 g of whole banana with 300 ml deionized water and 0.84 g potassium alum ($Al_2(SO_3)_3.K_2SO_4.24H_2O$). Adjust the pH of the resulting slurry to 6.2 ± 0.1 with 10% (w/v) NaOH. Centrifuge for 20 min at 34 000 *g* and transfer 7 ml of the supernatant to a 50 ml tube containing 7 g mixed ion-exchange resin. Shake the mixture for 10 min, then filter a portion through a 0.2 µm filter. The clear and deionized solution can be stored for up to 20 days at –20 °C before HPLC analysis.

Cassava starch hydrolysate Use commercially available material prepared by acid hydrolysis.

HPLC

Use an isocratic HPLC system equipped with an RI detector and the following:

for banana pulp:

- HPLC column A—Aminex HPX-85 carbohydrate analysis column (Biorad Labs, UK)
- HPLC column temperature of 85 °C
- mobile phase of deionized water
- mobile phase flow rate of 1.0 ml/min
- RI detector temperature of 45 °C.

The concentration of the sugars can be determined by the external standard method in which:

$$\text{concentration (g/100 ml)} = \frac{\text{R sample}}{\text{R standard}} \times \frac{\text{standard concentration (g/100 ml)}}{\text{sample weight (g)}} \times \frac{\text{injection volume standard (µl)}}{\text{injection volume sample (µl)}}$$

where: R = peak area or height.

for cassava starch hydrolysate:

- HPLC column B—Bondapak TM carbohydrate column
- mobile phase of acetonitrile:water (85:15)
- mobile phase flow rate of 2.0 ml/min.

The concentration of the sugars can be determined by the external standard method in which:

$$\text{concentration (g/100 ml)} = \frac{\text{R sample}}{\text{R standard}} \times \frac{\text{standard concentration (g/100 ml)}}{\text{sample weight (g)}} \times \frac{\text{injection volume standard (µl)}}{\text{injection volume sample (µl)}}$$

where: R = peak area or height.

for sweet potato:

- HPLC column C—Amino 5S column (Biorad Labs, UK) (4 mm × 25 cm)
- mobile phase of acetonitrile:water (70:30)
- mobile phase flow rate of 1.0 ml/min

The concentration of the sugars can be determined by the external standard method in which:

$$\text{concentration (g/100 ml)} = \frac{\text{R sample}}{\text{R standard}} \times \frac{\text{standard concentration (g/100 ml)}}{\text{sample weight (g)}} \times \frac{\text{injection volume standard (µl)}}{\text{injection volume sample (µl)}}$$

where: R = peak area or height.

References

AKER, K. C. and ROBINSON, C. W. (1987) Growth of *Candida utilis* on single- and multicomponent-sugars substrates and on waste banana pulp liquors for single cell protein production. *MIRCEN Journal,* **3**: 255–274.

PREMA, P., RAMAKRISHNA, S. V. and MADHUSUDHANA, R. (1986) Influence of composition of sugars in cassava starch hydrolysate on alcohol production. *Biotechnology Letters,* **8**: 449–450.

TOMLINS, K. I., BAKER, D. M. and McDOWELL, I. (1990) HPLC method for the analysis of organic acids, sugars and alcohol in extracts of fermenting cocoa beans. *Chromatographia,* **29**: 557–561.

WALTER, W. M. (1992) Use of refractive index to monitor changes in sugar content of stored sweet potatoes. *Hortscience,* **27**: 333–335.

Phenolic compounds in sweet potato

Introduction

Phenolics in sweet potato determined by the method described here include caffeic acid, caffeoylquinic acids and rutin. The method enables the quantitative determination of each of the above phenolics in sweet potato tubers.

Rationale

Phenolics in sweet potato tubers can influence the flavour of the cooked product and might play a role in insect resistance.

Suitability

This analytical method allows the accurate determination of phenolics (caffeic acid, caffeoylquinic acids and rutin) in sweet potato tubers. Although other commodities such as banana, plantain, yam, cassava and cocoyam have not been included, it should be possible to use this method, with suitable modifications to the extraction method as determined by the researcher.

Limitations

Limitations to this method have not been reported. It is recommended that the accuracy, precision and detection limits are determined by the researcher for his particular analytical system.

Principles

This method allows the quantification of phenolic acids by means of HPLC separation and UV detection after previous extraction of the fresh tuber in methanol.

Requirements

Equipment

- Tissue homogenizer
- Analytical balance accurate to four decimal places
- Sonicator
- Water-bath
- Freezer (–20 °C)
- HPLC with binary gradient capability and UV or diode-array detector

Consumables

All reagents are analytical grade unless otherwise stated.

- Distilled water
- Methanol
- Caffeic acid
- Caffeoylquinic acid
- Rutin

- Sampler filter, 0.45 µl
- Guard column (CO:PELL ODS, Whatman)
- Analytical column (30 cm × 4.6 mm i.d.; Waters µBondapak C18)
- Potassium hydrogen phosphate (KH_2PO_4)
- Phosphoric acid (H_3PO_4)

Procedure

Storage of tubers

The tubers should be stored at 13 °C and 85% relative humidity.

Extraction of phenolic compounds

Weigh 530–570 mg of tissue into a 20 ml scintillation vial and add 15 ml methanol. Homogenize for 3 min, then seal the vial and ultrasonicate for 30 min. Filter and store at –18 °C until required for analysis.

HPLC of phenolic compounds

Use a binary HPLC system equipped with a UV/diode-array detector and the following:

- guard column—CO:PELL ODS (Whatman);
- analytical column—30 cm × 4.6 mm i.d. (Waters µBondapak C18);
- injection volume of 30 µl (loop injection valve);
- mobile phase of 13% MeOH:H_2O to 50% MeOH:H_2O using a concave gradient. Note that the 13% MeOH:H_2O solvent should contain 0.08 M KH_2PO_4 buffer adjusted to pH 4.45 with dilute H_3PO_4;
- mobile phase flow rate of 1.5 ml/min;
- UV detector at 340 nm.

The concentration of the phenolics can be determined by the external standard method in which:

$$\text{concentration (g/100 ml)} = \frac{\text{R sample}}{\text{R standard}} \times \frac{\text{standard concentration (g/100 ml)}}{\text{sample weight (g)}} \times \frac{\text{injection volume standard (µl)}}{\text{injection volume sample (µl)}}$$

where: R = peak area or height.

Table 4.2 Typical retention times and elution order of some of the phenolic compounds

Compound	Elution order	Retention time (min)
5-caffeoylquinic acid	1	7.5
3-caffeoylquinic acid	2	10.5
4-caffeoylquinic acid	3	13.5
Caffeic acid	4	14.5
Dicaffeoylquinic acid	5	23.5
Rutin	7	27.5

References

SON, K., SEVERSON, R. F., SNOOK, M. E. and KAYS, S. J. (1991) Root carbohydrate, organic acids and phenolic chemistry in relation to sweet potato weevil resistance. *Hortscience,* **26**: 1305–1308.

HARVEY, I. M. and REED, J. D. (1992) Identification of phenolic compounds and their relationships to in-vitro digestibility of sorghum leaves from bird-resistant and non-bird-resistant varieties. *Journal of the Science of Food and Agriculture,* **60**: 179–196.

Organic acids, glucose and ethanol in foofoo (fufu)

Introduction

This analytical method enables the quantitative determination of organic acids (acetic, lactic, *n*-butyric, isobutyric), sugars and ethanol in fresh cassava roots, retted roots and foofoo (or fufu).

Rationale

Foofoo can be prepared by the fermentation of cassava. The progress of the fermentation can be monitored by measuring the concentration of the substrate, glucose, and its breakdown products.

Suitability

The method allows the accurate determination of organic acids (acetic, lactic, *n*-butyric, isobutyric), sugars and ethanol in fresh cassava roots, retted roots and foofoo. Although other commodities such as banana, plantain, yam, sweet potato and cocoyam have not been included, it may be possible to use this method, with suitable modifications to the extraction procedure as determined by the researcher.

Limitations

No limitations to this method have been reported. It is recommended that the accuracy, precision and detection limits are determined by the researcher for his particular analytical system.

Principles

This method allows the quantification of organic acids, sugars and alcohol by liquid chromatographic separation, UV and RI detection after previous extraction in distilled water.

Requirements

Equipment

- Tissue homogenizer
- Analytical balance accurate to four decimal places
- Vacuum filtration equipment
- Isocratic liquid chromatograph with UV and RI detectors

Consumables

All reagents are analytical grade unless otherwise stated.

- Distilled water
- Water (HPLC grade)
- Sulphuric acid
- Filter paper
- Acetic acid
- Lactic acid
- *N*-butyric acid
- Isobutyric acid
- Glucose
- Ethanol
- Sample filter, 0.45 µm (Millex, Millipore, UK)
- Guard column (Aminex HPX-87H, Biorad Laboratories, UK)
- Analytical column (30 cm × 7.8 mm i.d.; Aminex HPX-87H, Biorad Laboratories, UK)

Procedure

Extraction of organic acids, sugars and ethanol

Weigh 10 g of roots, retted roots or foofoo and homogenize in distilled water (8.3% w/v) for 2 min. Filter homogenate under vacuum and further filter the filtrate through a Millex 0.45 µm sample filter.

HPLC determination of the organic acids, sugars and ethanol

Use an isocratic HPLC system equipped with a UV/diode-array and/or an RI detector plus the following:

- guard column—Aminex HPX-87H (Biorad Laboratories, UK);
- analytical column—Aminex HPX-87H (30 cm × 7.8 mm i.d.; Biorad Laboratories, UK);
- injection volume of 20 µl (loop injection valve);
- mobile phase of 0.005 M sulphuric acid;
- mobile phase flow rate of 0.5 ml/min;
- detectors—(1) UV detector 215 nm (2) RI detector.

The concentration of the organic acids, sugars and ethanol can be determined by the external standard method in which:

$$\text{concentration (g/100 ml)} = \frac{\text{R sample}}{\text{R standard}} \times \frac{\text{standard concentration (g/100 ml)}}{\text{sample weight (g)}} \times \frac{\text{injection volume standard (µl)}}{\text{injection volume sample (µl)}}$$

where: R = peak area or height.

Table 4.3 Typical retention times and elution order of the individual compounds

Compound	Elution order	Retention time (min)
Glucose	1	10.0
Lactic acid	2	14.5
Acetic acid	3	17.3
Ethanol	4	21.5
Isobutyric acid	5	23.3
N-butyric acid	6	25.8

References

BLANSHARD, A. (1994) PhD thesis. University of Nottingham, *Quality of Processed Cassava Foods in Sierra Leone.*

TOMLINS, K. I., BAKER, D. M. and McDOWELL, I. (1990) HPLC method for the analysis of organic acids, sugars and alcohol in extracts of fermenting cocoa beans. *Chromatographia,* **29**: 557–561.

METHODS FOR EXTENDING THE LIFE OF HPLC AND GC COLUMNS AND CONSUMABLES

HPLC solvent recovery and recycling

Introduction

HPLC grade solvents should be of high purity and free of components that absorb UV in the low 200 nm range. Commonly used solvents are methanol, acetonitrile and tetrahydrofuran. While it is recommended that commercially available grades of HPLC solvent should be employed when available, the analytical methods described enable the recovery of such solvents for re-use in HPLC or in sample extraction methods. Techniques available include direct recycling, automated solvent recycling, use of lesser purity solvents for some isocratic applications, and recovery by distillation.

Rationale

HPLC solvents are required to be of high purity and free from matter that absorbs in the low 200 nm range of the UV spectrum. The advantages of solvent recycling and recovery are reduced solvent costs, and minimal solvent disposal and environmental effects.

Suitability

These methods allow for HPLC solvent recycling and the recovery of common solvents.

Limitations

Direct recycling, automated recycling and use of lesser purity solvents are restricted to isocratic mobile phases only. Recovery by distillation is restricted to simple mixtures of solvent, for example, a single organic solvent dissolved in an aqueous phase. The above methods may not be suitable for trace analysis.

Requirements

Equipment

Direct recycling

- Magnetic stirrer

Automated recycling

- Automated solvent saver (Shandon, UK)

Distillation

- Heating mantle
- 3000 ml round-bottomed flask
- Quickfit thermometer (0–150 °C)
- Fractionating column
- Condenser
- Gas chromatograph
- Scanning UV spectrophotometer

Consumables

Direct recycling, automated recycling

- None

Distillation

- Anti-bumping granules

Procedure

Direct recycling

In the routine use of isocratic HPLC methods, the mobile phase can be continuously recycled. The waste-line from the detector is directed back into the reservoir. The solvent in the reservoir must be stirred continuously using a magnetic stirrer. In this way, the solutes that elute from the analytical column are evenly mixed and diluted out and hence fed onto the column at a steady rate. Over time, impurities build up on the column and will ultimately cause an uneven baseline. While the method works with small batches of solvent (1000 ml), the larger the batch (for example, 10–20 l) the longer the delay before this occurs.

Another point to consider is that volatile mobile phase additives (acetic acid, triethylamine) may be gradually lost during the recycling phase and they must be replenished to their original levels to ensure reproducible chromatography.

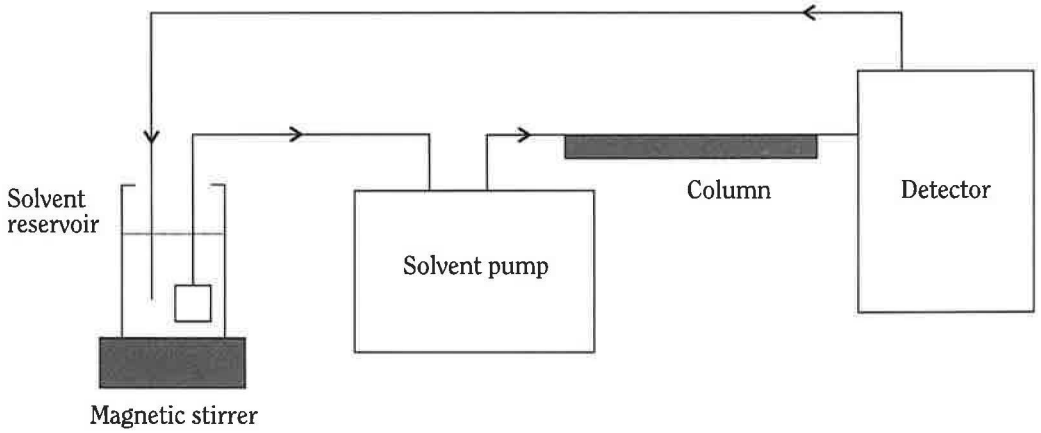

Figure 4.2 Example of direct recycling in HPLC

Automated solvent recycling

A difficulty encountered with direct recycling is that solutes eluting from the sample are returned to the mobile phase reservoir. Automated solvent recycling overcomes this as it recycles only 'clean' solvent and redirects solvent containing sample solutes to waste. The unit achieves this by the monitoring of the HPLC detector signal. When the signal is steady (baseline), the unit recycles the solvent. When a peak is detected, the solvent is directed to waste until no peaks are eluting.

It should be noted that with this unit, it is assumed that all peaks are detected. However, with most single-wavelength UV detectors, this cannot be totally relied upon.

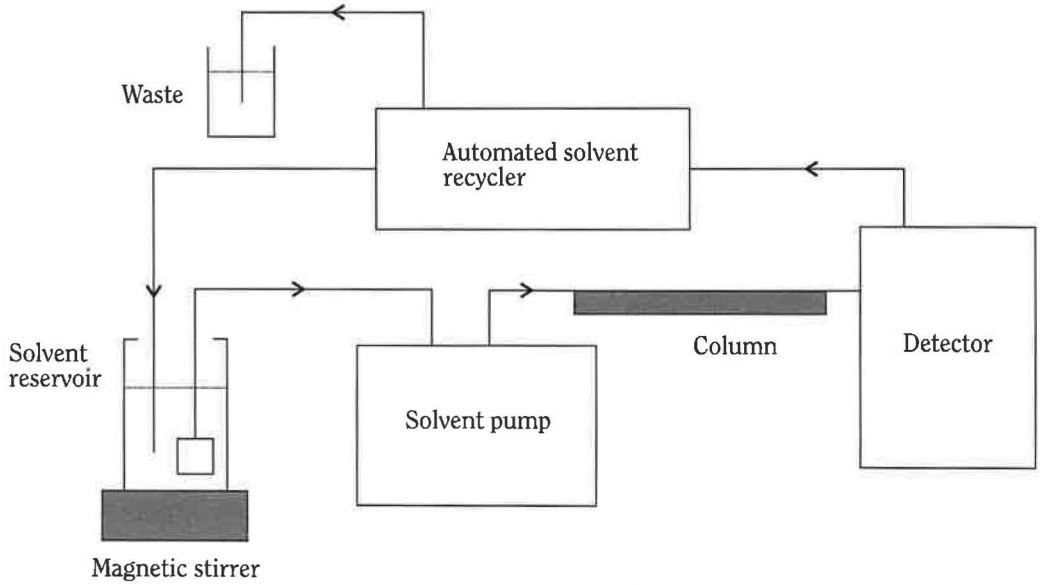

Figure 4.3 Example of automated recycling in HPLC

Lesser purity solvents

A solvent of lower purity and cost can be used when the HPLC method requires UV-absorbance detection above 280 nm. Irregular baselines and drifting retention times are an indication of over-contamination.

Distillation

Distillation with a fractionating column is useful for cleaning-up lower purity solvents when HPLC-grade solvents are not available, and for recovering organic solvents from spent aqueous mobile phases.

Add low purity solvents or spent mobile phase (containing only one organic solvent) to a 3000 ml round-bottomed flask. Attach the fractionating column with a thermometer in the top and a suitable condenser (*see* Figure 4.4). Apply gentle heat and discard the first fraction. When the thermometer records a temperature equal to the boiling point of the solvent, collect the following distillate until the temperature starts to increase.

Redistill by repeating the above step if necessary.

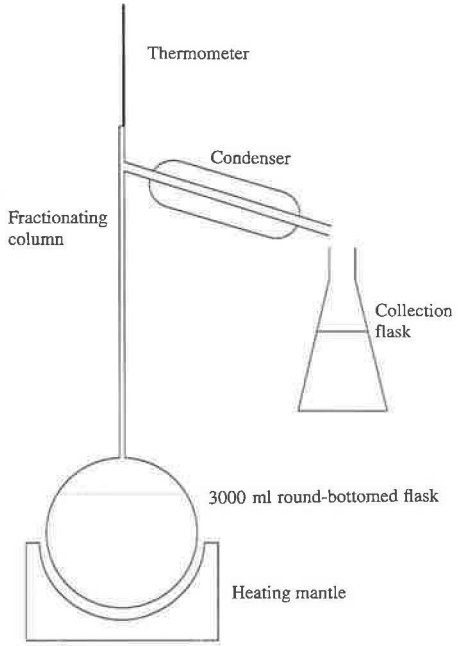

Figure 4.4 Example of solvent recovery/clean-up by distillation with a fractionating column

Determine the recovery of the distillate by GC and compare the peak area/height with authentic HPLC-grade solvent.

Determine the spectral purity by carrying out a UV scan (190–600 nm) of the distillate and compare with authentic HPLC-grade solvent.

References

ANON (1993) *HPLC News Update*. HPLC Technology Ltd, Macclesfield, UK, Summer 1993, 2.

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DOLAN, J. W. (1992) Recycling revisited. *LC-GC International*, **6**: 216–8.

GERTZ, C. (1990) *HPLC Tips and Tricks with Over 1000 Applications*. Oxford: Alden Press.

TOMLINS, K. I. (1992) Methods for extending HPLC and GC consumables in developing countries. Chatham, UK: Natural Resources Institute [unpublished].

Preparation of water for use in HPLC

Introduction

Commercial HPLC grade water is of high purity and does not contain components that absorb UV in the low 200 nm range. While it is recommended that commercially available grades of HPLC water should be employed when available, the method described here is useful in the absence of suitable alternatives. The method involves passing distilled water through a solvated C18 solid phase extraction cartridge.

Rationale

HPLC water is required to be of high purity and free of matter that absorbs in the low 200 nm range of the UV spectrum. This method is recommended when commercial grades of HPLC water are not available.

Suitability

The method allows for low-cost HPLC water preparation in the absence of suitable commercial grades.

Limitations

Water prepared by this method should be checked for suitability by comparing UV scans with authentic commercial HPLC-grade water. Running a gradient with the solvent recommended for the analytical method to check for baseline disturbances is also recommended. This method may not be suitable for trace analysis.

Requirements

Equipment

- Vacuum filtration apparatus (glass)
- Vacuum pump
- Scanning UV spectrophotometer

Consumables

- Methanol
- Solid phase extraction cartridges (C18 500 mg; Millipore, Bondelut, Anachem, etc.)

Procedure

Solvate a C18 solid phase extraction cartridge by passing 2 ml methanol followed by 10 ml distilled water; do not allow to dry. Under vacuum, pass 1000 ml distilled water through the cartridge and collect (*see* Figure 4.5).

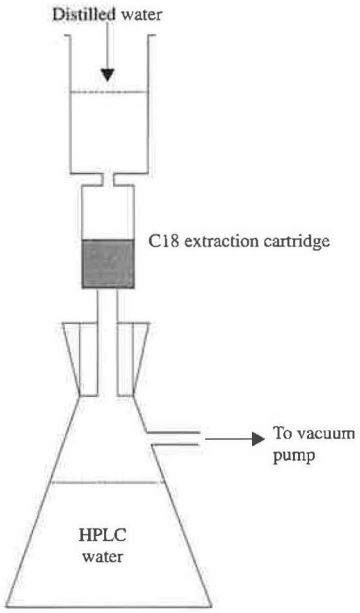


Figure 4.5 Apparatus for preparation of water for HPLC

Validate water purity by gradient elution with the organic solvent recommended in the analytical method and by comparison of the UV spectra with authentic HPLC-grade water.

Note: The C18 cartridge can be re-used after cleaning with 20 ml methanol.

References

GERTZ, C. (1990) *HPLC Tips and Tricks with Over 1000 Applications.* Oxford: Alden Press.

TOMLINS, K. I. (1992) Methods for extending HPLC and GC consumables in developing countries. Chatham, UK: Natural Resources Institute [unpublished].

Mobile phase filtration

Introduction

It is recommended that all HPLC mobile phases be passed through a 0.45 μm filter to prevent damage to the valves and pump seals and avoid shortened column life. An alternative method is described, should 0.45 μm filters be unavailable.

Suitability

This method allows for removal of particulate matter from pure HPLC solvents such as water, methanol, acetonitrile and tetrahydrofuran.

Limitations

Limitations have not been reported, and the use of commercial filters for this purpose is recommended when they are available.

Requirements

Equipment

- Glass still

Consumables

- Water and organic solvents of HPLC grade or as prepared by above methods

Procedure

Ensure that the glass still is clean and free of particulate matter. Distil pure solvents before mixing for HPLC mobile phases.

References

GERTZ, C. (1990) *HPLC Tips and Tricks with Over 1000 Applications*. Oxford: Alden Press.

Extending the life of HPLC reverse phase columns

Introduction

The useful life of a reverse phase HPLC column can be extended, and hence the cost reduced, by taking precautionary steps in normal use. When a column loses its efficiency, these methods of restoration may prove useful.

Rationale

Factors reducing the useful life of an analytical column include the build-up of particulate matter and strongly bound compounds in the sample matrix at the head of the column. The advantages of the methods described are extended column life and reduced cost.

Suitability

The methods are suitable for reverse phase analytical columns.

Limitations

Some of the restoration methods are 'last resort' techniques and should only be attempted if the manufacturer's guarantee has expired and all other clean-up procedures have been tried.

Requirements

Equipment

- HPLC system

Consumables

- Methylene chloride
- Methanol
- Acetonitrile

- 0.45 μm filters
- Ethylenediaminetetra-acetic acid (EDTA)
- Packing material or an old column

Procedure

Precautionary steps in the normal use of reverse phase HPLC columns

- It is recommended that the mobile phase and samples be passed through 0.45 μm filters.
- It is recommended that guard and pre-columns be used to protect and extend the life of analytical columns.
- Store C18 columns in methanol or acetonitrile. C18 columns should be rinsed with several column-volumes of methanol or acetonitrile whenever they are installed or reinstalled.
- Periodic cleaning is recommended after a column has been used for a large number of analyses and if the signal or baseline is no longer stable. Rinse the column with several column-volumes of methanol, acetonitrile or tetrahydrofuran and then return to original operating conditions.

Reverse flow of mobile phase

The life of a column may be extended by reversing the flow after set periods of time (or after analyses). This way, column contamination will be more evenly distributed on the packing material.

Restoration of reverse phase HPLC columns

Symptoms of a deteriorating column are an increase in back-pressure and loss of column efficiency. If this occurs, the first line of action is to try to clean the column (*see* above). If cleaning is unsuccessful, as a last resort, the following restoration methods may recover the column. Note: check whether the manufacturer's warranty has expired.

Organic solvent flush

Occasionally, the strong solvent of the mobile phase is insufficient to remove contaminants from the column. After flushing with these solvents, flush with 10 column-volumes of methylene chloride and then with another 10 volumes of the strong solvent of the mobile phase. Note: it is important to use miscible solvents as methylene chloride is immiscible with aqueous mobile phases. If immiscible solvents are used inadvertently, flush the column with isopropanol or another mutually miscible solvent.

Other solvents recommended are EDTA and guanidine to assist in the removal of metals and proteins, respectively.

'Topping off'

Sometimes during normal use, but usually if excessive pressures are exerted on the column, the packing material may settle, forming a void at the inlet end of the column. This may reduce column efficiency and cause double peaks.

Disconnect the column from the HPLC system. Unscrew the nut at the inlet (pump end) and remove the metal frit. Using a clean spatula, carefully pack packing material of the same type (from the manufacturer, or from the outlet end of an old column) into any apparent void; the packing material should be packed into a shallow cone approximately 1 mm above the column end. Clean the metal frit by sonication in methanol or another suitable solvent. Reassemble the column and return to the original operating conditions. An example of the effect of column restoration on peak shape is show in Figure 4.6.

Replacing the 'head' of the column

Contaminants and particulate matter in the sample (and mobile phase) will, over time, build up at the inlet end of the column. Removal and replacement of the contaminated portion at the head of the column with fresh packing material may restore column efficiency.

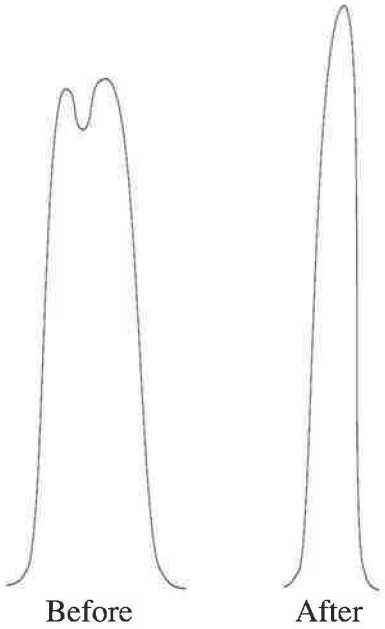


Figure 4.6 Example of column restoration by the 'topping off' method

Disconnect the column. Unscrew the nut at the inlet (pump end) and remove the metal frit. Using a clean spatula, remove the top layers of the column packing material (which may be discoloured) and discard. Carefully repack packing material of the same type (from the manufacturer, or from the outlet end of an old column) into the head of the column. The packing material should be packed into a shallow cone approximately 1 mm above the column end. Clean the metal frit by sonication in methanol or another suitable solvent. Reassemble the column and return to the original operating conditions.

References

DOLAN, J. W. (1991) Solvent recycling. *LC-GC International,* **5**: 14–15.

DOLAN, J. W. (1992) Recycling revisited. *LC-GC International,* **6**: 216–218.

GERTZ, C. (1990) *HPLC Tips and Tricks with Over 1000 Applications*. Oxford: Alden Press.

TOMLINS, K. I. (1992) Methods for extending HPLC and GC consumables in developing countries. Chatham, UK: Natural Resources Institute [unpublished].

HPLC solvent degassing

Introduction

Degassing of HPLC solvents is important for preventing the formation of bubbles in the system which can influence the flow stability of the pump, and signal noise by the occurrence of bubbles in the detector flow cell. Oxygen dissolved in solvents affects UV absorption at wavelengths below 230 nm, causing baseline drift and higher noise levels. Methods used for degassing solvents in HPLC include helium or nitrogen sparging, ultrasonication, vacuum filtration, heating, and electronic degassing.

Suitability

The methods allow for HPLC solvent degassing and vary in effectiveness, complexity and cost.

Electronic degassing

Apart from their high initial capital cost, electronic degassing units are reliable, efficient, operate continuously and have low running costs. They operate by passing the mobile phase through a gas-permeable tube located in a vacuum chamber.

Helium or nitrogen sparging

Helium or nitrogen sparging is effective in replacing oxygen in solvents; proton donators such as water and methanol are more difficult to degas than alkanes. Helium gas has the disadvantage of high cost. Nitrogen is a possible alternative, but while it is more effective than helium at removing oxygen from solvents, it does tend to form bubbles in the system.

Vacuum filtration

Vacuum filtration can be effective for degassing. However, organic and aqueous solvents should be filtered separately before mixing, and solvents may need degassing at regular intervals.

Ultrasonic agitation

Ultrasonic agitation is suitable for mixing, but is ineffective for eliminating dissolved gases.

Heating

Freshly distilled water can be used, as dissolved gases are removed on heating.

References

GERTZ, C. (1990) *HPLC Tips and Tricks with Over 1000 Applications.* Oxford: Alden Press.

Restoration and extending the life of GC capillary columns

Introduction

The working life of a GC capillary column can be extended, and hence the cost reduced, by taking precautionary steps in normal use. When a column loses its efficiency, the methods of restoration below may prove useful.

Rationale

Factors reducing the useful life of an analytical column include the build-up of particulate matter and strongly bound compounds in the sample matrix at the head of the column. The advantages of the methods described are extended column life and reduced cost.

Suitability

The methods are suitable for bonded phase GC capillary columns. The manufacturers' recommendations should be sought where possible.

Limitations

Some of the restoration methods are 'last resort' techniques and should only be attempted if the manufacturer's guarantee has expired and all other clean-up procedures have been tried.

Requirements

Equipment

- GC system

Consumables

- Methylene chloride
- Methanol
- Acetonitrile
- Hexane
- Distilled water
- Silyl-8 (Pierce & Warriner, Chrompack)

Procedure

Precautionary steps in the normal use of capillary columns

- Conditioning of a column (by heating to the maximum operating temperature) is recommended whenever a column (packed or capillary) is installed or reinstalled. A brief periodic conditioning (30 min) will ensure that contaminants do not build up on the column.

- Conditioning is recommended if a column has been used for a period and the signal is no longer stable, if baseline rises are noted on temperature programming, or if peak tailing of active components begins to occur. Heat the column at a rate of 5 °C/min to its maximum continuous operating temperature. The detector signal should increase to a maximum, followed by a gradual decrease, until a flat baseline is achieved. Depending on the level of contamination, a conditioning period of 1–24 h may be required.

Column restoration

Removal of a portion of the capillary column

The first few cm of a capillary column are prone to damage from non-volatile material in the sample, large amounts of solvent etc. If poor peak shape is observed either in the form of tailing or broad peaks, and the capillary material is thought to be the cause of this, remove approximately 50 cm at the injector end.

Solvent washing

If conditioning and removal of a section of the capillary column, cleaning the detector, etc., does not restore the efficiency of the column, bonded and cross-linked stationary phase columns should be washed.

The choice of solvent depends on the nature of the contamination. Initially, it is recommended that a non-polar solvent such as pentane or hexane be used.

Disconnect the column and connect a glass syringe to the detector outlet. Back-wash with about 2 ml hexane. Contamination by polar material can be removed from non-polar columns (BP1, SE-30, CP-Sil 5 CB, CP-Sil 8 CB, etc.) by washing with 2 ml methylene chloride or methanol. Polar columns (BP20, carbowax 20M) should not be washed with methylene chloride or methanol (unless the manufacturers recommend this, as in the case of CP-Wax 52 CB, CP-Wax 57 CB, CP-Wax 58 CB) as some of the stationary phase might bleed from the column. Instead, wash with 2 ml water.

After cleaning, expel the solvent by passing nitrogen gas through the column for 12 h. Connect the column only to the injector with the outlet outside the chromatograph's oven. With carrier gas flowing through the column, heat at 1 °C/min to its maximum operating temperature. Continue conditioning until a stable baseline appears.

Bonded phase capillary conditioners

Non-polar bonded stationary phases such as SE30 can be conditioned by Silyl-8 (Pierce & Warriner; Chrompack). Silyl-8 must not be used on polar stationary phases such as carbowax. Silyl-8 conditions non-polar GC columns by blocking active sites, and the manufacturers recommend regular use.

Inject 10–50 µl Silyl-8 conditioner directly into the column (column temperature 150–205 °C; flow rate 0.5–1.5 ml/min). Conditioning is complete in 2–3 min (175 °C). If necessary, repeat the procedure until the baseline is stabilized.

References

ANON (1990) How to rinse fused silica columns. *Chrompack News,* **17**: 10–11.

ANON (1993) *Chromatography Catalogue and Handbook*. Chester, UK: Pierce & Warriner Ltd.

TOMLINS, K. I. (1992) Methods for extending HPLC and GC consumables in developing countries. Chatham, UK: Natural Resources Institute [unpublished].

Section 4.2 Hazard Analysis Critical Control Point concept

INTRODUCTION

The Hazard Analysis Critical Control Point (HACCP) approach for preventative quality assurance moves away from traditional testing of the final product, and instead emphasizes process and raw material control. Control is taken out of the laboratory and into the processing environment. Until the introduction of HACCP, measurements of physical properties, microbiological testing, chemical analyses and organoleptic evaluations were used as a means of assessing food quality and safety. A number of limitations to this approach were recognized:

- the problems associated with the design and implementation of appropriate sampling plans;
- the time required to obtain results;
- the cost.

HACCP provides a structured and critical approach to the control of identified hazards. It may be used during the production and growing stage of NGSS or during any subsequent handling, processing or packaging of the product(s). To establish the HACCP approach, the following steps will be involved:

- identification, description and assessment of hazards associated with all stages of product handling and processing;
- identification of critical control points (CCPs) at which the identified hazards must be controlled;
- specification of control criteria;
- monitoring of procedures implemented.

A thorough understanding of the whole process is required in order to identify the most appropriate means of monitoring CCPs. On occasion, this will still require chemical or microbiological analysis, while for other stages, inspection or sensory evaluation may be required. Therefore, it is important to assemble a team of specialists who can look at the whole process from the point of view of their own areas of expertise, and who can contribute to the overall plan. The technique was originally adopted for control of microbiological hazards, but it can just as easily be applied to other areas, such as chemicals, foreign bodies, and even economic fraud.

There are some factors outside the handlers' and processors' control that can affect the quality of NGSS and their products. The scale of production may range from large mechanized farms to small family units. Different cultivars have been bred for yield, disease resistance, etc. Hazardous practices, such as the use of raw sewage as fertilizer, or allowing animals carrying bacteria, viruses and parasites to forage amongst the crops, together with inadequate processing and storage facilities, may increase risks associated with the products. All these factors must be considered when drawing up quality systems.

This section is designed to be used as an aid for those responsible for implementing an HACCP system.

DEFINITION OF TERMS

Hazard—a potential to cause harm to the consumer (safety) or the product (spoilage) or to defraud the consumer.

Severity—the seriousness of the hazard.

Risk—the probability of the hazard occurring.

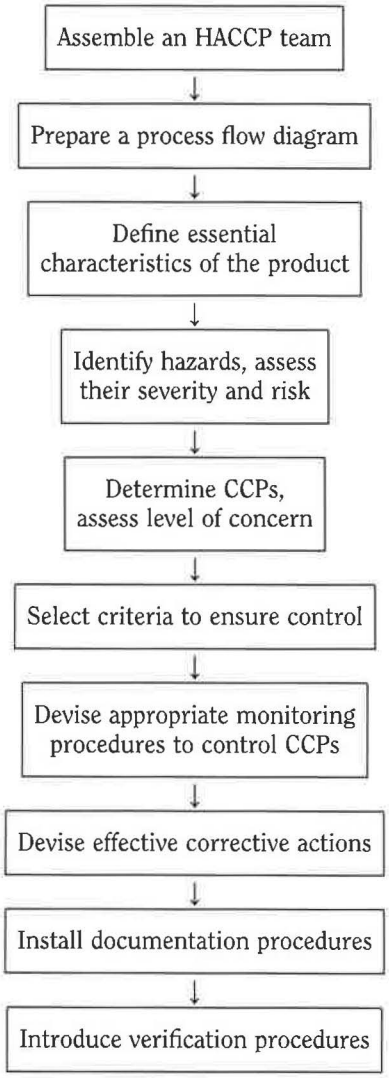
Critical Control Point (CCP)—a location, practice, operation, stage or raw material at which control can be exerted to eliminate, prevent or reduce a hazard or several hazards.

Concern—an expression of the seriousness of a failure to control a critical point, derived from knowledge of a hazard and the risk of it occurring.

Criteria—limits of characteristics of a physical (e.g. time or temperature), chemical (e.g. concentration of salt or acid), biological or sensory nature.

Monitoring—the systematic observation, measurement and/or recording of significant factors for control of the hazard.

STAGES IN DEVELOPING A HAZARD ANALYSIS CRITICAL CONTROL POINT (HACCP) SYSTEM



THE HACCP TEAM

In order to understand the process fully and to be able to identify all likely hazards and CCPs, it is important that the HACCP team be made up of people from a wide range of disciplines. It is essential that the team should have a chairman; he or she will convene the group and direct the work of the team to ensure that the concept is properly applied. Other members of the team should include a production specialist, and technical specialists such as a microbiologist, a chemist, a quality control (QC) manager and a process engineer. Others, such as packaging specialists, raw material buyers or distribution staff, may be brought into the team temporarily to provide the relevant expertise. The team's progress and results of the analysis should be recorded, and, for this purpose, a technical secretary should be employed to allow all members of the team to play a full role in the discussions.

THE PROCESS FLOW DIAGRAM

The first function of the team is to draw up a detailed flow diagram of the process. The expertise of the production specialist is important at this stage. Processes will differ in detail in different plants, and an accurate flow diagram depends on detailed knowledge of the process.

DEFINING PRODUCT CHARACTERISTICS

The team must next examine the product, and identify its characteristics and the ways in which it will be used and handled.

This analysis will help the team to determine the hazards that will threaten the product or consumer. The following headings can be used as a guideline to this process: Storage, Preservation, Packaging, Consumer practices, and Target groups.

IDENTIFICATION OF HAZARDS

Potential hazards can be identified by reviewing epidemiological records for particular products and by gathering technical information on all aspects of production, processing, storage, distribution and use of the product. This should include looking at the hygienic design of equipment, hygiene and sanitation procedures in the field/plant, and health and hygiene of personnel.

HAZARDS

The following is a list of hazards that may be associated with the handling of NGSS. The list is by no means exhaustive and should be used as a guide. The microbiological hazards have been separated according to severity, that is, will they make the consumer ill, or are they life threatening? The same approach can be used for other hazards.

Microbiological

Severe

Bacteria	*Shigella dysenteriae* *Salmonella cholerae-suis* *Salmonella paratyphi* A *Salmonella paratyphi* B *Salmonella paratyphi* C *Salmonella typhi*
Virus	Hepatitis A Poliovirus
Fungi	Mycotoxins

Moderate—potential of extensive spread

Bacteria	Pathogenic *Escherichia coli* Other *Salmonella* species Other *Shigella* species

Moderate—limited or no spread

Bacteria	*Staphylococcus aureus* *Escherichia coli* *Bacillus cereus* *Clostridium perfringens*

Non-microbiological

In raw materials	Heavy metals Pesticide residues
Adventitious contaminants	Fumes/dust Lubricants/hydrocarbons from machinery Refrigerants Pest control agents Chlorophenols Sanitizing agents Water additives Plant/plasticizers
From packaging materials	Plasticizers Printing/code inks Adhesives Lubricants

Foreign bodies	Natural extraneous materials, e.g. stalks, leaves, seeds Foreign material, e.g. infestation, stones, glass, metal, plastic, paper, hair, jewellery, paint, plaster Others, e.g. cross-packing, sabotage
Economic fraud	Underweight packs Spoiled material in packs Mislabelled packs

ANALYSIS OF HAZARDS

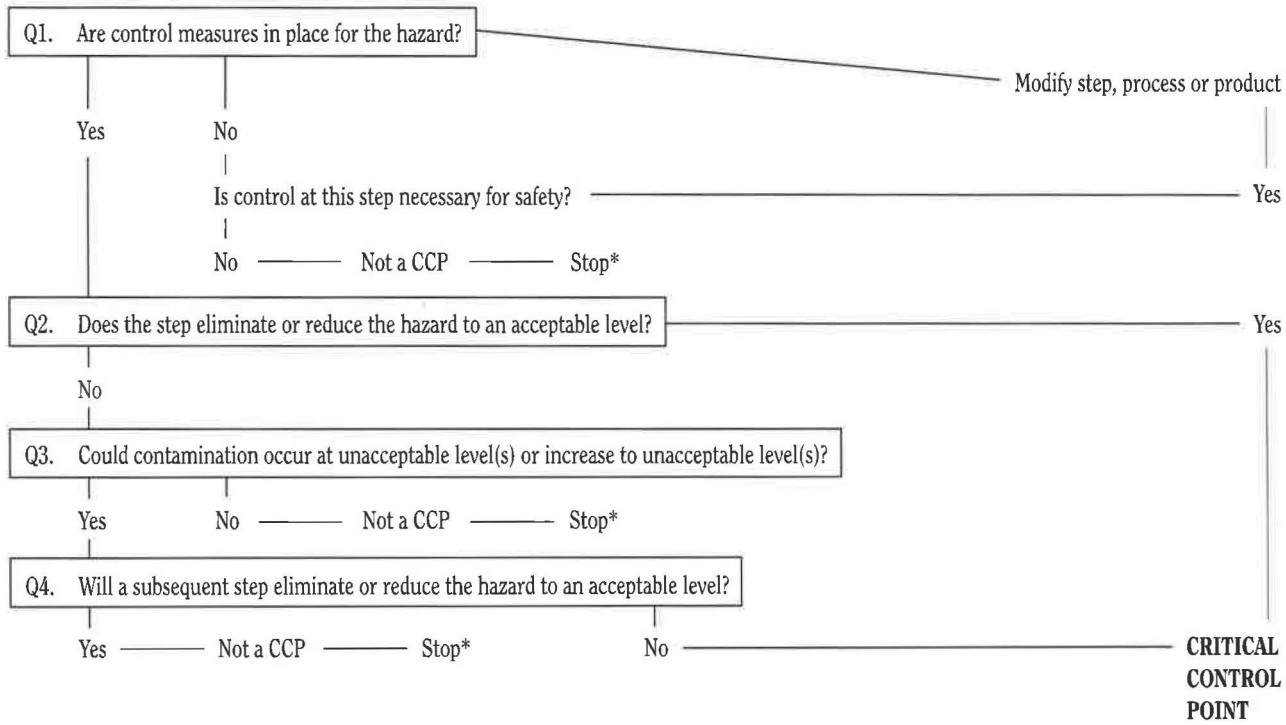
The analysis of hazards has to be quantitative if it is to provide useful information; this involves the assessment of risk and severity. Risk expresses the chance of a hazard occurring and severity relates to the magnitude of the hazard. The resources allocated to controlling the hazard will be dependent on these factors.

DETERMINING CCPs

Each stage of the process is taken in turn and the relevance of each identified hazard is considered. The team must determine whether the hazard can increase at this stage, or whether it can be reduced, prevented or eliminated. If the hazard can be reduced, prevented or eliminated by exerting some form of control at a particular stage, it is a CCP. The HACCP decision tree (Codex Alimentarius Commission, 1991) should be used to identify CCPs.

CCP decision tree

Answer each question in sequence at each step for each identified hazard



*Proceed to next step in the described process

When deciding on the extent to which the CCP will be monitored, a judgement of risk must be made so that a level of concern can be ascribed to it. There are four levels of concern:

- high concern—an expert judgement that without control there is a life-threatening risk;
- medium concern—an expert judgement that there is a threat to the consumer or to the product that must be controlled;

- low concern—an expert judgement that there is little threat to the consumer or the product. It may still be advantageous to control it;
- no concern—an expert judgement that there is no threat to the consumer.

The points where control can be exerted, but which are not critical because of low risk or severity, need less control and monitoring. If a hazard can be controlled at more than one point, then the most effective point for control must be chosen.

SELECTION OF CRITERIA FOR CONTROL

The team must next identify means by which to control the hazard at each CCP. These may include, for example, chlorine levels in wash water; temperatures during storage, etc. These levels must all be documented as statements, or included as specifications in operating manuals. Limits or tolerances should be stated wherever appropriate.

MONITORING PROCEDURES

It is essential that there is a mechanism for checking that processing or handling procedures at each CCP are controlled. Any loss of control must be able to be detected, and information provided, early enough for corrective action to be taken and for any loss of product to be avoided or minimized.

Monitoring can be carried out by observation or by measurement of samples taken in accordance with a statistically-based sampling plan. Monitoring by observation is basic but gives rapid results, and can therefore be acted upon speedily. Visual observations can be carried out on raw materials, worker hygiene, hygiene and sanitation procedures, and processing procedures. The most common measurements taken are time, temperature and pH. For raw materials, however, tests for moisture content, toxins, additives and contaminants, and microbiological tests, may be carried out.

CORRECTIVE ACTION

If monitoring indicates that criteria are not being met, or that the process is out of control, corrective action must be taken as soon as possible. The corrective action should take the worst case possible into account, but it must also be based on the assessment of hazards, risk and severity, and on the final use of the product.

The specific action will depend on the process, and may include the alteration of the chlorine concentration in water, or the re-cleaning of equipment.

DOCUMENTATION

Record keeping is an important part of the HACCP system. It demonstrates that the correct procedures have been followed from the beginning to the end of the process, thus indicating product traceability. It records compliance with the set limits, and can be used to identify problem areas.

There will be documents recording the initiation of the system, such as the selection of critical limits, but most of the documentation will be concerned with the monitoring of CCPs and corrective actions taken.

Record keeping may be carried out in a number of different ways, from simple check-lists, to records and control charts.

VERIFICATION

Once the HACCP system has been drawn up, it must be reviewed before being installed, and reviewed regularly once it is operating. At this stage, the appropriateness of CCPs and control criteria may be determined, and the extent and effectiveness of monitoring verified.

Ways in which the system may be verified include:

- the performing of measurements to check the accuracy of monitoring;

- the collection of samples;
- the interviewing of staff; and
- the observation of operations at CCPs.

It is important to remember that the HACCP system is set up for a particular product formulation that is handled and processed in a given way. If any changes are made to the composition of the product or operational procedures, it may be necessary to alter the CCPs or to change the methods of monitoring.

EDUCATION AND TRAINING

The education and training of operatives on personal hygiene and good manufacturing procedures, relevant to the production of NGSS and their products, should be carried out regularly.

CONCLUSION

HACCP is a powerful and useful tool. Undertaking an HACCP analysis focuses the ideas of everyone involved on the details of the process, and promotes a greater awareness of safety and quality issues. Implementation of an HACCP system is not an end in itself. It requires the commitment of management and the work force. Constant monitoring of the system is required to ensure its success.

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Section 4.3 Mycotoxins in cassava

INTRODUCTION

Mycotoxins are metabolites produced by some strains of certain fungi that cause illness, mycotoxicosis, or death, when ingested by animals and humans. Contamination of foods and feed ingredients by mycotoxins depends largely on the presence of environmental conditions which lead to mould growth and hence to toxin formation. Data on the incidence and levels of contamination are limited by a number of factors, including: the resources to conduct surveys; the availability of laboratory facilities to carry out analyses; the sampling procedure(s); and the reliability and detection limit of the analytical method used. Nevertheless, numerous publications have appeared on the occurrence of mycotoxins in foods and feeds, particularly since the discovery of the aflatoxins, mycotoxins produced by some strains of *Aspergillus flavus, Aspergillus parasiticus* and *Aspergillus nomius*, in the early 1960s. Probably, no food or feed commodity can be regarded as immune to the presence of mycotoxins, since toxin contamination can occur in the field or during harvest, drying, processing, storage and transportation of a particular commodity.

The potential for cassava and processed cassava products to support the growth of mycotoxigenic fungi with concomitant toxin production has not been extensively studied. Although many mycotoxin analyses have been developed, very few have been validated with cassava or other root crops. In this section, the literature concerning mycotoxin contamination is very briefly reviewed, general approaches to analyses are considered, and some analyses that have been validated with cassava are described.

Mycotoxin analyses generally require sophisticated laboratory equipment. Mycotoxins are highly toxic so appropriate training and safety precautions are essential.

FORMATION AND OCCURRENCE OF MYCOTOXINS

Fungal contamination of cassava and cassava products

Inadequate or slow drying, exposure to high humidities or rewetting during storage will make products such as cassava chips and cassava flours susceptible to fungal contamination. The water activity of the products at any given time will determine which species of field and/or storage fungi are able to proliferate.

Field fungi require relatively high moisture contents for growth, usually greater than 19% in cassava, which corresponds to water activities of above 0.9. There is, however, a paucity of information on the occurrence of fungi, particularly toxigenic moulds, on cassava roots pre-harvest, although the isolation of species belonging to the genera *Aspergillus, Penicillium* and *Fusarium*, which are known to include toxin-producing strains, have been reported.

Storage fungi, unlike field fungi, are able to grow at lower water activities (about 0.7), which correspond to moisture contents of approximately 13–14% for a product such as cassava. During traditional sun drying, cassava products are likely to contain a mixture of both field and storage fungi. Initially, field fungi such as *Alternaria, Fusarium* and *Phoma* spp. may grow. As the moisture content decreases, storage fungi such as *Aspergillus* and *Penicillium* spp. are likely to grow.

Occurrence of mycotoxins in cassava

After the discovery of the aflatoxins in the 1960s, a number of reports were published indicating that high levels of aflatoxin had been found in cassava products. However, it was suggested that some of these results may have been erroneous due to the presence in such commodities of a blue fluorescing compound, scopoletin, which is chromatographically similar to aflatoxin B1 (Jones, 1972). Several methods have been developed either for the confirmation of aflatoxin in the presence of scopoletin or for the derivatization of aflatoxin for quantification by HPLC. These methods are discussed in detail below.

High concentrations of zearalenone (3 mg/kg) were found in a sample of cassava meal imported into Italy from Thailand, but lower concentrations of the toxin (90 µg/kg) occurred in a sample from Indonesia; six out of six samples of cassava meal imported into the Netherlands from the same two countries were found to be contaminated with ochratoxin (mean: 3 µg/kg, range: 2–4 µg/kg) and zearalenone (mean: 32 µg/kg, range: 17–86 µg/kg). Ochratoxin A (32 and 65 µg/kg) was also detected in two out of 45 samples of cassava flour in Brazil (Valente Soares and Rodriguez Amaya, 1989). However, in other studies, no aflatoxin, patulin or sterigmatocystin were detected in cassava flour.

ANALYSIS OF MYCOTOXINS IN CASSAVA AND CASSAVA PRODUCTS

Each of the stages involved in mycotoxin analysis is discussed below.

Sampling

Sampling is a critical step in the analytical sequence since, if the sample taken is not representative of the bulk, any analytical results obtained will be meaningless. Because of the heterogeneous distribution of mycotoxins in most naturally contaminated commodities, including cassava and its products, it is essential to follow a statistically valid sampling plan or, where one is not available, to follow a procedure that takes into account both the non-homogeneous nature of the contamination and the particle size of the commodity. Ideally, a large number (at least 100) of small, incremental samples should be taken throughout the batch. These incremental samples are then combined to give a 'bulk sample' of 5–20 kg depending on the particle size of the material. For example, a flour would require a smaller 'bulk-sample' than the dried roots from which it was produced.

Sample preparation

Sample preparation involves the grinding and division of the bulked sample to obtain 'laboratory samples' (usually of 1 kg). Sub-sampling is then carried out in the laboratory to obtain 'analytical samples'. Sub-sampling is normally carried out first by grinding more finely and then either by further physical division or by slurrying. The use of water slurries enables larger, and therefore more representative, analytical samples to be taken. Typically, 1 kg of ground material is blended at high speed with an appropriate amount of water to give a homogeneous slurry (paste) from which 100 g aliquots are taken for analysis.

Defatting

Defatting with a hydrocarbon solvent may be required with some processed products containing a significant amount of oil, but it is not necessary for most cassava and cassava products.

Extraction and clean-up

Cassava and cassava products do not generally present difficulties as far as extraction and clean-up are concerned since, apart from scopoletin referred to earlier, they do not usually contain compounds which interfere in the quantification stage of the analytical sequence. Most studies which have been carried out to determine the mycotoxin contamination of cassava and cassava products have used analytical methods originally developed for other commodities, or for foods and feeds in general (Jones, 1972; Fishbein and Falk, 1970; Scott *et al.*, 1970; Romer, 1975; Pons, 1972; Anon., 1975). These methods utilize such solvents as acetone:water, chloroform:water, methanol:water or ethylacetate:water to extract the toxin from the matrix.

Commonly used clean-up procedures, which specifically remove non-mycotoxin 'interfering' compounds whilst leaving the mycotoxins in the extract, are: column chromatography (Scott, 1990), precipitation/coprecipitation with reagents such as cupric carbonate (Romer, 1975), lead acetate (Pons, 1972), ferric gel (Romer, 1975) and ammonium sulphate (Valente Soares and Rodriguez-Amaya, 1985); and liquid-liquid partitioning (Romer, 1975), sometimes using acid/base extraction (Scott, 1990). The latter technique, often used in conjunction with another clean-up procedure, is useful since it provides additional clean-up, the transfer of toxins from an aqueous phase into an organic phase, and can effect a considerable increase in concentration of the toxin(s). Newer clean-up techniques which have been developed include the use of immuno-affinity columns (Betina, 1993), which utilize antibodies to mycotoxins bound to sepharose gel to retain only the mycotoxin of interest, and bonded phase cartridges (Betina, 1993), which consist of a silica gel substrate bound with a bonded phase such as C18, C8 or phenyl.

'Work-up'

After the clean-up step, the extract must be 'worked-up' in order to concentrate the toxin into a small volume of solvent to enable detection with high sensitivity. This can be achieved by liquid-liquid partitioning (Romer, 1975) into an organic solvent and evaporation of the solvent on a rotary evaporator, concentrator or steam bath.

Quantification

The aflatoxins, and many of the other important mycotoxins apart from the trichothecenes, fluoresce under ultra-violet (UV) light, and this enables them to be detected at very low levels (μg/kg). Those toxins which do not fluoresce may have to be sprayed or made into derivatives to give coloured, fluorescent, or volatile substances (Betina, 1993) that can be quantified by one of the techniques described below.

Qualitative analysis

Extracts possibly containing a toxin which fluoresces under UV light can be applied to 'mini-columns' which, after elution with an appropriate solvent, are viewed under UV light to screen for the possible presence of the toxin (Betina, 1993; Romer, 1975). Extracts which give positive results with a mini-column can be quantified using one of the techniques described below.

Thin-layer chromatography (TLC) has also been used to screen extracts for the possible presence of mycotoxins (Scott *et al.*, 1970; Betina, 1989) using qualitative standards as Rf markers.

Quantitative analysis

Fully quantitative determinations of mycotoxins have been carried out by a variety of techniques including TLC (Betina, 1993), high performance thin-layer chromatography (HPTLC) (Tomlins *et al.*, 1989), HPLC (Betina, 1993), gas-liquid chromatography (GLC) (Betina, 1993) and enzyme linked immunosorbent assay (ELISA) (Betina, 1993).

The quantitative interpretation of developed TLC plates is performed by comparing the fluorescent intensities of standard mycotoxins with the mycotoxin component in the sample (Scott, 1990). TLC has been widely used for mycotoxin analysis, since it is a simple and relatively inexpensive technique provided visual estimation of the developed TLC plates is employed. However, the precision of such determinations is low and the laborious process requires the services of a skilled technician.

The use of automated or semi-automated plate spotters coupled with densitometric evaluation of TLC or, preferably, HPTLC plates can increase the precision, accuracy and sensitivity of such methods significantly (Betina, 1993). Aflatoxin, for example, can be precisely and accurately detected down to picogram levels.

HPLC is now one of the most widely used chromatographic methods in modern analytical laboratories. The advantages of HPLC for the analysis of mycotoxins are its ability to handle a range of chemically-different compounds, its speed, its increased sensitivity, its accuracy, its precision, its high resolving power between chemically-similar toxins and the variety of detection systems now available. The limitations of HPLC are its cost and the wide experience necessary to obtain the best possible benefit from the system. A number of reviews on the HPLC of mycotoxins have been published (Shepherd, 1986; Coker and Jones, 1988). The technique has been used for the analysis of aflatoxin, ochratoxin A and zearalenone in cassava products.

GLC is used as an analytical technique for mycotoxins which can be volatized or which possess a functional group or groups allowing conversion of the toxin into a volatile derivative. The major mycotoxins for which quantification by GLC has been developed include the trichothecenes, zearalenone, patulin, sterigmatocystin and alternariol and related *Alternaria* toxins. GLC has a major advantage over other forms of chromatography, HPLC/mass spectrometry notwithstanding, in that it can be readily coupled to a mass spectrometer to enable more specific detection, quantification and confirmation of mycotoxins. The quantification of zearalenone in cassava meal extracts has been carried out using GLC.

ELISAs, like all immunoassays, are analytical techniques relying on the specific recognition and binding of ligands or analytes by antibodies, which are animal-derived serum proteins. Such assays are now used routinely in many laboratories for mycotoxin analyses with test kits, for a range of mycotoxins. They are commercially available from a large number of manufacturers in different countries. These kits are designed for qualitative, semi-quantitative or fully quantitative analytical procedures. The advantages of ELISA techniques are their sensitivity, their specificity, and the necessity to carry out extensive clean-up procedures which facilitates a high sample through-put. ELISA kits, however, are relatively expensive, have a limited shelf-life and require refrigeration, which may negate or limit their use in less developed countries.

Confirmation

It is essential that confirmatory tests are carried out because sometimes there are compounds which 'interfere' with the quantification of a particular toxin, or can be mistaken for the mycotoxin (as in the case of aflatoxin and scopoletin in cassava extracts). These difficulties can occur despite the use of a clean-up procedure. Confirmatory tests involve the use of two-dimensional TLC, spraying TLC plates, for example, with a reagent to produce a specific coloured spot, or a change in the colour of a fluorescent spot by the formation of a specific derivative, or by mass spectrometry. For example, the colour of the fluorescence of aflatoxin B1 changes from blue to yellow when a TLC plate is sprayed with 50% aqueous sulphuric acid (Jones, 1972). This colour change does not occur with scopoletin. Exposure of a TLC plate spotted with aflatoxin and scopoletin to iodine vapour quenches the fluorescence of scopoletin without affecting that of aflatoxin. The natural fluorescence of sterigmatocystin may be enhanced, to afford a bright yellow spot, by spraying with 24% aluminium chloride in 95% aqueous ethanol and heating at 105 °C for 10 min (Betina, 1993). The non-fluorescent trichothecene, T-2 toxin, appears as a grey-blue fluorescent spot after spraying with 20% sulphuric acid in methanol and heating at 110 °C for 34 min.

The hemiacetal derivative, formed by treatment with trifluoroacetic acid, has been used for the confirmation of aflatoxins B1, G1 and M1 (Scott, 1990). For reverse phase HPLC quantification of the aflatoxins, post-column reaction with iodine converts aflatoxins B1 and G1 to the corresponding hemiacetals (Coker and Jones, 1988) which fluoresce more strongly than the parent toxins, thus increasing the sensitivity of the method. The post-column reaction can also be used for confirmatory purposes by carrying out runs with and without iodine. The identity of sterigmatocystin may be confirmed by the formation of the acetate (Scott, 1990) or hemiacetal (Betina, 1993) derivative and that of ochratoxin A by the formation of the ethyl ester derivative (Scott, 1990).

SPECIFIC METHODS OF ANALYSIS FOR CASSAVA AND CASSAVA PRODUCTS

As stated previously, very few methods have been specifically developed for cassava and cassava products, and most studies have been carried out using methods developed for foods and feed materials in general.

A method for estimating aflatoxin levels in cassava developed by Coker and Tomlins (1986) utilized a modified Romer procedure (Romer, 1975), with TLC quantification and confirmation by spraying with 50% aqueous sulphuric acid, or two-dimensional TLC. The original official method of the European Community for the analysis of aflatoxins in cassava products intended for feed use (Anon., 1982) was based on the so called CB method (Valente Soares and Rodrigues-Amaya, 1989) with the addition of two-dimensional TLC. A more recent method of the European Community (Anon., 1992) uses reverse phase HPLC and post-column reaction with iodine.

Valente Soares and Rodriguez-Amaya (1989) developed a method for screening and quantification of ochratoxin A in cassava flour. The method involved extraction with methanol:aqueous potassium chloride, clean-up with ammonium sulphate, a silica gel/alumina mini-column for screening purposes and quantification by TLC. This method was later re-evaluated and proved appropriate for the simultaneous determination of aflatoxins, ochratoxin A, sterigmatocystin and zearalenone in cassava flour (Valente Soares and Rodriguez-Amaya, 1989).

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Section 4.4 Pesticide residue analysis

INTRODUCTION

The treatment of foodstuffs with pesticides can leave residues (trace quantities) of the material in the food for varying periods of time including, sometimes, up to the point of consumption. This is particularly the case with post-harvest treatments of fungicides or insecticides.

When the pesticides have been applied correctly and as recommended, the residues are normally small and do not give rise to any concern. It is important, however, to analyse for residues of pesticides that are known to be used, to detect cases of excessive application and to protect the health of the consumer.

Pesticide analysis is a broad subject; there are many hundreds of pesticides used singly or in combination. Some methods of analysis detect single pesticides and others are used to screen samples for residues of a wide range of pesticides. The methods in this manual are restricted in their application and of use with only a limited range of products.

A difficulty that arises is the requirement for fairly sophisticated laboratory facilities for even the most simple analyses; very few rapid, minimal-requirement test methods exist. The skills and experience of the person conducting the analysis are also important and, even for the simplest analysis, a clear grasp of the principles and of the use of instrumental techniques are required. Ensuring that the analysis is meaningful is vitally important; some of the key issues are summarized below.

SAMPLING

It is important to take great care in the selection and preservation of the analytical sample. If the sample is not representative of the bulk from which it is taken, or is compromised in any way, the analysis is meaningless and the time and care taken in conducting it is wasted.

Sample selection must be carefully controlled by a defined sampling programme, with the samples being packed or preserved in clean sample bags or wrapped in aluminium foil. The samples must be analysed as soon as possible after collection, or preserved in such a way that deterioration of any pesticide present is minimized.

SAMPLE CONTAMINATION

The possible contamination of samples can present particular difficulties in pesticide residue analysis. It is, therefore, essential that, at all stages of the process from sample collection, transportation to the laboratory through to the final analysis, care is taken to ensure that any external contamination is avoided.

It is particularly important to ensure that all those involved in this process are aware of the potential problem and of how such contamination can arise. If attention is paid to the following, then contamination from pesticides, or other chemicals which may give a similar reaction or interfere with the analysis, can be avoided:

- workers collecting samples should not previously have been handling pesticides;
- all workers involved in the collection or handling of the samples should be wearing clean, uncontaminated clothing and must have clean hands;
- all sample wrapping materials must be new and clean;
- samples being transported to the analytical laboratory must be kept away from other materials;
- laboratory benches must be clean; areas where the samples are sorted/sub-divided must be covered with a suitable, clean material.

SAMPLE ANALYSIS

When samples are analysed in the laboratory, genuine untreated sample must also be analysed in order to identify natural constituents which may give a reaction similar to that of the pesticide. Reagent blanks (aliquots of the analytical reagents put

through the whole extraction and clean-up processes) must also be analysed in order to identify interfering materials from reagent sources. Such blanks may also help highlight laboratory pesticide contamination, if it is present.

VALIDATION

Validation of the analytical method is important to ensure that it is functioning properly; it is a process for analytical quality assurance. Laboratory treated samples must be analysed in parallel with the field samples. Comparison of the quantity of recovered pesticide with the amount known to have been applied gives an estimate of the efficiency of the method. Comparison of the variability of the results for a number of replicates indicates the reproducibility of the method.

ANALYSIS OF THE FUNGICIDE THIABENDAZOLE BY HIGH PRESSURE LIQUID CHROMATOGRAPHY

Introduction

Rationale

The monitoring and determination of residues of any pesticide in food for human or animal consumption is important in terms of human and animal health. The determination of levels of any residue remaining after a post-harvest treatment will also be of benefit in determining the effectiveness of the treatment.

Suitability

This method provides for the quantitative determination of residues of thiabendazole. Full laboratory facilities will, however, be required, with access to an analytical high performance liquid chromatography (HPLC) system.

Limitations

The method is limited by the analytical lower limit of detection for thiabendazole in this medium (approximately 0.1 mg/kg).

Principles

A representative portion of the sample is extracted by maceration with solvent and analysed by HPLC, after solvent transfer, by comparison with reference standard solutions.

Requirements

Equipment

- HPLC system with injector (20 µl sample loop), isocratic pump and fixed wavelength UV detector
- Printer/plotter for HPLC data output
- C18 HPLC column
- 50 µl hypodermic syringe with needle design to suit requirements of HPLC injector system
- Ultrasonic water-bath
- High-speed homogenizer

Consumables

- Methanol, HPLC grade
- Ethyl acetate, glass distilled grade
- Ammonium carbonate, AR
- Sodium hydroxide, AR
- Sodium sulphate, anhydrous, granular

- Celite 545 filter aid
- Whatman No. 41 filter papers *or* Whatman Puradisc 25PP syringe filter, 0.45 μm pore size (or equivalent)
- Hydrochloric acid

Procedure

Sample preparation

Chop the sample into approximately 1 cm cubes with a knife, mix thoroughly and weigh a 50 g sub-sample directly into a maceration jar.

Sample extraction

Add 200 ml of ethyl acetate and macerate the sample for 2 min. Allow the macerate to settle and filter the extract, under vacuum, through a celite filter aid. Return the macerate to the maceration jar and re-extract with a further 100 ml of ethyl acetate. Filter the extract again and combine the extracts. Wash the filter with 25 ml ethyl acetate and combine the rinsings with the primary extracts. Transfer the extract to a round-bottomed flask (500 ml or 1000 ml). Evaporate just to dryness on a rotary vacuum evaporator at 45 °C and redissolve in 25 ml of the HPLC mobile phase (see below). Particulate matter may be visible at this stage and, if so, remove it by filtration using a Whatman No. 41 paper or, preferably, an HPLC filter disc.

Analysis

HPLC conditions

Column:	Chromsphere C18 (or equivalent), 10 cm × 3 mm i.d.
Mobile phase:	methanol, 2% aqueous ammonium carbonate (50:50).
Flow rate:	0.4 ml/min.
Injection volume:	20 μl.
Detector wavelength:	302 nm.

Calibration

Calibrate the HPLC by the sequential injection of three or four reference standards in the range 2–20 μg/ml, depending on instrument sensitivity. Inject the reference standards after every third sample extract injection. Wherever possible, finish the run with a repeat of the initial range of reference solutions.

When the response to the sample extract is high, carefully dilute the sample (with mobile phase) until the response is within the response range for the reference standards.

Validation

Validate the method by the laboratory treatment of otherwise untreated samples with known amounts of thiabendazole and subsequent analysis by the given procedure. Conduct the validation in, at least, duplicate and at a minimum of two treatment levels (e.g. 1 and 5 mg/kg).

Note: If, during the analysis, interference from sample co-extractives is observed, introduce a clean-up stage as described below.

Clean-up

Reduce the volume of the primary ethyl acetate extract (see *Sample extraction* above) to approximately 40 ml on the rotary vacuum evaporator and transfer the extract, with rinsings (10 ml total) to a 250 ml separating funnel. Add 20 ml of 0.1 M hydrochloric acid and shake the funnel for 1 min. Run off the hydrochloric acid into a second funnel and repeat the partition; combine the two hydrochloric acid extracts. Add two drops of phenolphthalein solution and then add sodium hydroxide solution (2 M) dropwise until the extract is just alkaline. Add the 40 ml of ethyl acetate and shake the funnel for 1 min. Allow the layers to separate and run off the lower aqueous layer into a clean beaker. Transfer the ethyl acetate extract to another separating funnel and return the aqueous extract to the separating funnel for a re-extraction with a further 30 ml of ethyl acetate. Combine the ethyl acetate extracts and repeat the separation. Add 20 ml of distilled water and gently shake the separating funnel; allow the layers to separate and discard the aqueous wash. Run off the ethyl acetate layer through a 5 cm column of granular anhydrous sodium sulphate, into a 250 ml round-bottomed flask. Wash the column with about 15 ml of ethyl acetate and collect the rinsings in the same flask. Evaporate the extract just to dryness (rotary vacuum evaporator at 40 °C) and dissolve in the mobile phase as described in *Sample extraction* above.

Analysis of results

Record the detector response (peak height or area) for each calibration standard and determine the mean value for each concentration level. Prepare a calibration curve of mean response against concentration, from which calculate the concentration of residues in the sample extracts.

Calculate residues in the samples, in mg/kg from:

$$\frac{(a) \times (b)}{(c)} = (y)$$

where: (a) is the concentration of thiabendazole in the sample solution calculated from the calibration curve, in µg/ml;
(b) is the sample volume (25 ml × any dilution);
(c) is the sample weight (50 g, normally); and
(y) is the residue in mg/kg.

International standards

Codex Alimentarius Commission Maximum Residue Limits (MRL) exist for thiabendazole on banana and potatoes (April 1994).

Banana: 3 mg/kg
Potato: 5 mg/kg

National MRLs also apply.

References

Codex Alimentarius Commission, Codex Committee on Pesticide Residues; List of MRLs. Available from: FAO, Via delle Terme di Caracalla, 00100, Rome, Italy.

ANALYSIS OF THE FUNGICIDE BENOMYL BY UV SPECTROSCOPY

Introduction

Rationale

The monitoring and determination of residues of any pesticide in food for human or animal consumption is important in terms of human and animal health. The determination of levels of any residue remaining after a post-harvest treatment will also be beneficial in determining the effectiveness of the treatment.

Suitability

This method describes the quantitative determination of residues of benomyl. Full laboratory facilities will be required, with access to a UV spectrophotometer (preferably a recording model). Residues of methyl benzimidazol-2-ylcarbamate (MBC) and thiophanate methyl can also be determined using this procedure.

Limitations

This method is limited by the analytical lower limit of detection for benomyl (approximately 0.1 mg/kg).

Principles

A representative portion of the sample is extracted by maceration with solvent and analysed by UV spectroscopy, after solvent transfer and decomposition to MBC, by comparison with reference standard solutions of known concentration.

Requirements

Equipment

- UV spectrophotometer
- High-speed homogenizer

- Constant temperature water-bath

Consumables

- Ethyl acetate, glass distilled grade
- Sodium hydroxide, AR
- Sodium sulphate, anhydrous, granular
- Celite 545 filter aid
- Hydrochloric acid

Procedure

Sample preparation

Chop the sample with a knife into approximately 1 cm cubes, mix thoroughly and weigh a 50 g sub-sample directly into a maceration jar.

Sample extraction

Add 200 ml of ethyl acetate and macerate the sample for 2 min. Allow the macerate to settle and filter off the extract, under vacuum, through the celite filter aid. Return the macerate to the maceration jar and re-extract with a further 100 ml of ethyl acetate. Filter the extract again and combine the extracts. Wash the filter with 25 ml ethyl acetate, combine the rinsings with the primary extracts, and transfer to a round-bottomed flask (500 ml or 1000 ml). Evaporate the extract to approximately 5 ml on a rotary vacuum evaporator at 45 °C.

Add 0.2 ml of 0.1 M hydrochloric acid to the extract and heat, with occasional swirling, to 75 °C in a constant-temperature water-bath. Allow the extract to cool and transfer, with rinsings of dilute hydrochloric acid (0.1 N), to a 25 ml stoppered measuring cylinder and adjust the acid volume to 10 ml. Shake the cylinder for 1 min and allow the contents to settle.

Analysis

Remove the upper ethyl acetate layer by suction, transfer a portion of the acid layer, by pipette, to a 1 cm silica cell, and measure the absorbance against a reagent blank of hydrochloric acid (0.1 N) saturated with ethyl acetate. Scan the range 250–310 nm and, preferably using a scanning UV spectrophotometer, measure the peaks at 275 and 282 nm against a baseline at 310 nm. Alternatively, obtain the responses manually using the given wavelengths. Note that the peak at 282 nm is very sharp and, with a manual instrument, the location of this peak should be checked.

When the response to the sample extract is high, carefully dilute the sample (with dilute hydrochloric acid saturated with ethyl acetate) until the response is within the response range for the reference standards.

Calibration

Calibrate the UV spectrophotometer using a solution of MBC or benomyl which has been treated with acid.

Standard MBC solution Prepare a solution of MBC (13.2 µg/ml, i.e., equivalent to 20 µg/ml benomyl) in ethyl acetate saturated with hydrochloric acid (0.1 N) by appropriate dilution of a stock solution of pure MBC in ethyl acetate (0.66 mg/ml). If necessary, prepare from a stock solution of pure benomyl in ethyl acetate (1 mg/ml). Treat 2 ml of this solution with hydrochloric acid (0.1 N, 2.0 ml) and ethyl acetate (50 ml) and heat the solution to 75 °C for 15 min with occasional vigorous shaking or swirling. Cool the mixture and make up to 100 ml with ethyl acetate.

Dilute aliquots (2.5, 5.0 and 7.5 ml) of the standard MBC solution to 10 ml with ethyl acetate (saturated with dilute hydrochloric acid) in glass-stoppered test-tubes to give solutions containing benomyl of 5, 10 and 15 µg/ml, respectively. Add 10 ml of hydrochloric acid (saturated with ethyl acetate), shake the stoppered tubes vigorously for 1 min and allow the contents to settle. Remove the upper ethyl acetate layer by suction and transfer portions of the acid layer to 1 cm silica cells for UV measurement.

Validation

Validate the method by the laboratory treatment of otherwise untreated samples with known amounts of benomyl or MBC and subsequent analysis by the given procedure. Conduct the validation in, at least, duplicate, and at a minimum of two treatment levels (e.g. 1 and 5 mg/kg).

Analysis of results

For each calibration standard, measure the absorbance of the peak at 282 nm and prepare a calibration curve of response against concentration from which the concentration of residues in the sample extracts can be calculated.

Calculate residues in the samples, in mg/kg, from:

$$\frac{(a) \times (b)}{(c)} = (y)$$

where: (a) is the concentration of benomyl in the sample solution calculated from the calibration curve, in µg/ml;
(b) is the sample volume (10 ml × any dilution);
(c) is the sample weight (50 g, normally); and
(y) is the residue in mg/kg.

Note: The ratio of the absorbance of the peak at 282 nm divided by that of the 275 nm peak, measured from a baseline at 310 nm, should be about 1.2. Any significant variation from this may indicate the presence of a contaminant.

International standards

Codex Alimentarius Commission Maximum Residue Limits exist for Carbendazim (*) on banana and potatoes (April 1994).

Banana:	1 mg/kg B, C, Th;
Potato:	3 mg/kg B, C;
Sweet potato:	1 mg/kg B;
Taro:	0.1 mg/kg B.

Note: * MRLs cover carbendazim residues occurring as metabolic products of benomyl (B) or thiophanate-methyl (Th), or from direct use of carbendazim (C).

National MRLs also apply.

References

Codex Alimentarius Commission, Codex Committee on Pesticide Residues; List of MRLs. Available from: FAO, Via delle Terme di Caracalla, 00100, Rome, Italy.

COX, J., DONEGAN, L. and PINEGAR, J. A. (1974) The estimation of surface residues of benomyl on treated bananas. *Pesticides Science*, **5**: 134–145.

COX, J. and PINEGAR, J. A. (1976) Benomyl residues in bananas. *Pesticides Science*, **7**: 193–200.

ANALYSIS OF THE FUNGICIDE COPPER OXYCHLORIDE BY ATOMIC ABSORPTION SPECTROSCOPY

Introduction

Rationale

The monitoring and determination of residues of any pesticide in food for human or animal consumption is important in terms of health. The determination of levels of any residue remaining after a post-harvest treatment will also be beneficial when determining the effectiveness of the treatment.

Suitability

The method allows for the quantitative determination of copper. Full laboratory facilities will be required with access to an atomic absorption spectrometer.

Limitations

Care needs to be exercized in the interpretation of results obtained using this method since both free and combined copper are determined. A blank run using starch staples with no known exposure to copper pesticides will be necessary.

Principles

A representative portion of sample is prepared by ashing. Copper is quantitatively determined by atomic absorption spectroscopy and reference with standard solutions.

Requirements

Equipment

- Balance accurate to four figures
- Oven (100 °C)
- Furnace (500 °C)
- Atomic absorption spectroscopy facilities

Consumables

- Deionized water
- Nitric acid (1+1), prepared by mixing equal volumes of HNO_3 and water
- Hydrochloric acid (1+1), prepared by mixing equal volumes of HCl and water
- Perchloric acid (96%)
- Lanthanum solution, prepared by dissolving 58.65 g of La_2O_3 in HCl (250 ml), adding the acid slowly, and diluting to 1l

Procedure

Sample preparation

Thoroughly remove all foreign matter from the root sample, especially adhering soil or sand, but to prevent leaching, avoid excessive washing. Air- or oven-dry as rapidly as possible to prevent decomposition, grind, and store in tightly stoppered bottles. If results are to be expressed on a fresh weight basis, record sample weights before and after drying. The sample should then be ashed by one of the two methods given below.

Note: Care should be taken to avoid contamination of the sample with dust during handling.

Dry ashing

Accurately weigh dried and ground sample (1 g) into a glazed, high-form porcelain crucible. Ash for 2 h at 500 °C and allow to cool. When cool, wet the ash with deionized water (10 drops) and carefully add nitric acid (1+1, 3–4 ml). Evaporate off the excess nitric acid using a hot-plate at 100–120 °C. Return the crucible to the furnace and ash for an additional 1 h at 500 °C. Cool the crucible and dissolve the ash in hydrochloric acid (1+1, 10 ml); transfer quantitatively to a volumetric flask (50 ml).

Wet ashing

Accurately weigh dried and ground sample (1 g) into a Pyrex beaker (150 ml). Add nitric acid (1+1, 10 ml) and allow to soak thoroughly. Add perchloric acid (96%, 3 ml) and heat on a hot-plate, slowly at first, until frothing ceases (caution, perchloric acid explodes at temperatures above 200 °C). Heat to white fumes of $HClO_4$. Cool and add hydrochloric acid (1+1, 10 ml); transfer quantitatively to a volumetric flask (50 ml).

Sample analysis

Add 5% lanthanum solution to the prepared sample and dilute to volume. After allowing the silica to settle, decant the supernatant. Set up the atomic absorption instrument for copper analysis. Less sensitive secondary lines may be used to reduce the dilution required. A minimum of four calibration points should be used to prepare the calibration curve, with recalibration after every 6–12 samples.

Analysis of results

Read the concentration of sample from the plot of absorption against μg/ml

$$\% \text{ element} = (\mu g/ml) \times \frac{F}{\text{sample weight}} \times 10^{-4}$$

where: F = ml original dilution × ml final dilution aliquot, if original 100 ml volume is dilution.

International standards

No known published standards.

References

AOAC Methods, 1984, 3.002a, 3.014, 2.129, 2.130.

ANALYSIS OF THE FUNGICIDE COPPER OXYCHLORIDE BY COLORIMETRY

Introduction

Rationale

The monitoring and determination of residues of any pesticide in food for human or animal consumption is important in terms of health. A knowledge of levels of any residue remaining after post-harvest treatment will also be beneficial in the determination of the effectiveness of the treatment.

Suitability

This method describes the quantitative determination of copper. Full laboratory facilities are required with access to a fume cupboard, centrifuge and colorimeter.

Limitations

Care needs to be exercised in the interpretation of results obtained using this method, since both free and combined copper are determined. A blank run using starch staples with no known exposure to copper pesticides is necessary.

Principles

A representative sample is ashed and extracted using dithizone. The copper content is determined by the formation of a coloured complex with nitrocresol which is measured colorimetrically.

Requirements

Equipment

- Balance accurate to four figures
- Furnace (600 °C)
- Mechanical shaking machine
- Colorimeter
- Centrifuge

Consumables

- Distilled water
- Hydrofluoric acid, 48%
- Hydrochloric acid (1+1), prepared by mixing equal volumes of HCl and distilled water
- Perchloric acid, 60%
- Ammonium citrate solution (40%), prepared by dissolving 800 g of citric acid in distilled water (600 ml) and, while stirring slowly, adding ammonium hydroxide solution (1+1, 900 ml) (Note: this reaction is exothermic and care should be taken to prevent spattering). Adjust the pH of the solution to 8.5, if necessary. Dilute to 2 l and extract with portions (25 ml) of dithizone solution until the aqueous phase stays orange and carbon tetrachloride (CCl_4) remains predominantly green. Then extract with CCl_4 until all orange is removed.
- Phenolphthalein solution (1%)
- Ammonium hydroxide (1+1)
- Dithizone solution, prepared by dissolving 0.5 g dithizone in 600–700 ml of CCl_4, filtering into a separating funnel (5 l) containing ammonium hydroxide (0.02 N, 2.5–3.0 l), shaking well, and discarding the CCl_4 phase. Then shake

with aliquots of redistilled CCl_4 (50 ml) until the CCl_4 phase is green. Add redistilled CCl_4 (1 l) and acidify slightly with HCl (1+1). Shake to transfer the dithizone to the organic layer and discard the aqueous layer. Store in a refrigerator.

- Carbon tetrachloride (distil over calcium oxide, passing the distillate through dry acid wash filter paper before use)
- Hydrochloric acid (0.1 N)
- Hydrochloric acid (0.01 N)
- Sodium diethyldithiocarbamate solution, 0.1%, freshly prepared using distilled water
- Copper standard solutions, 1 µg/ml, prepared by dissolving 0.3929 g $CuSO_4.5H_2O$ in distilled water (5 ml), diluting to 1 l and mixing. Add sulphuric acid (5 ml) to an aliquot (10 l), dilute to 1 l and mix.

Procedure

Preparation of sample

Oven-dry the sample (20 g) for 48 h and prepare for ashing by cutting into small pieces using stainless steel shears.

Ashing of samples

Weigh 6 g of dry sample into a platinum dish. Cover with a watch glass and place in a cool furnace; heat slowly to 500 °C and hold at this temperature overnight. Remove the sample and allow to cool before wetting down the ash with distilled water. Using a dispensing burette, slowly add perchloric acid (60%, 2–5 ml), dropwise at first to prevent spattering. Add hydrofluoric acid (48%, 5 ml) and evaporate using a steam bath. Transfer to a sand bath and maintain at a medium heat until fuming ceases. Cover with a Pyrex watch glass and return to the partially cooled furnace, heat gradually to 600 °C and maintain at this temperature for 1 h. Remove the sample and, when cool, add hydrochloric acid (1+1, 5 ml) and distilled water (approximately 10 ml). Replace the cover glass and warm on the steam bath to dissolve. This will usually give a clear solution free from insoluble material. Transfer the sample to a 50 ml volumetric flask, washing the dish several times with distilled water, dilute to volume and mix thoroughly.

Sample extraction

Transfer a suitable aliquot (2–3 g of dry material) to a separating funnel (120 ml, with petroleum jelly used as stopcock lubricant). Add ammonium citrate solution (40%, 5 ml) and one drop of phenolphthalein before adjusting the pH to 8.5 with ammonium hydroxide solution (1:1). If a precipitate forms, add additional ammonium citrate solution. Add dithizone in carbon tetrachloride (10 ml) and shake for 5 min. Drain the organic phase into a beaker (100 ml). Repeat as many times as necessary, using aliquots (5 ml) of dithizone solution and shaking for 5 min each time. Extraction is complete when the aqueous phase remains orange and the organic phase remains predominantly green. Then add carbon tetrachloride (10 ml), shake for 5 min and combine with the organic extract. The final carbon tetrachloride should be pure green; if not, extraction was incomplete and must be repeated.

Add perchloric acid (60%, 2 ml) to the combined carbon tetrachloride extractions, cover the beaker with a Pyrex watch glass and digest on a hot-plate until colourless. Remove the cover glass and evaporate slowly to dryness.

Note: If the sample is heated for any length of time at high temperatures, losses of copper may occur; heat only enough to evaporate completely to dryness. If free acid remains, it will interfere with the next stage where pH control is important.

Add hydrochloric acid (0.01 N, 5 ml) to the residue. Heat slightly to assure dissolution. Transfer with distilled water to a 25 ml volumetric flask and dilute to volume. Transfer an aliquot (20 ml) to a 60 ml separating funnel and reserve the remainder for copper determination.

Analysis

Transfer an aliquot (0.5–1 g of dry material) from the solution obtained to a 125 ml separating funnel. Add ammonium citrate solution (40%, 2 ml), phenolphthalein (one drop), sodium diethyldithiocarbamate solution (0.1%, 5 ml) and ammonium hydroxide (1+1) until pink. Add carbon tetrachloride (10 ml) and shake for 5 min before draining off the carbon tetrachloride. Centrifuge to ensure complete phase separation and transfer to a cuvette. Determine the absorption at the maximum absorption at about 430 nm. Prepare standard solutions with 0, 1, 5, 10 and 20 µg copper, treated as above, for calibration.

Cleaning of glassware for dithizone extractions

Clean Pyrex separating funnels for dithizone extractions by initially soaking for 30 min in hot nitric acid and rinsing several times with distilled water. Shake with several portions of dithizone in carbon tetrachloride. After use, clean by rinsing with distilled water, and store stoppered.

International standards

No known published standards.

References

AOAC Methods, 1984, 3.020–3.28, 3.033–3.034.

Section 4.5 Sensory evaluation

DEFINITION OF SENSORY EVALUATION

Sensory analysis of food relies upon evaluation through the use of our senses (odour, taste, colour, tactile, temperature, pain, etc.). Only by applying exact scientific testing methods can reproducible results be obtained and analysed statistically (Jellinek, 1985).

The sensory evaluation methods given here are all applicable for NGSS food crops in their cooked and uncooked states. Such foods include cassava, plantain, sweet potatoes, yams and all products made from them.

When we eat food products made from NGSS, we perceive a whole range of different characteristics relating to appearance, flavour and texture. These are called the sensory characteristics and play an important role in attracting people to food and in the regulation of food intake. The sensory characteristics of foods are therefore central to the determination of consumer acceptability.

Variability in the sensory response is inherent in any group of people used for testing and is unalterable. Nonetheless, with training, a group may show consistent individual responses.

The validity of the conclusions drawn from the test results depends on the test method used, how it is carried out and the questions asked in the score sheet.

DEFINING THE SENSORY PROBLEM

In sensory evaluation, for a particular problem, appreciable discussion or thought may be necessary before an appropriate test method is selected.

Preliminary tests may be necessary where many samples are concerned. These can be used to establish:

- appropriate orders of magnitude, such as the concentration of a reference solution to be used;
- the number of samples to be evaluated;
- the importance of the sensory attributes, for example, whether they are representative of the product, whether they are consistently used by the panel, etc.;
- in consumer tests, that there is a real sensory difference between the products in question; in tests which involve a large number of assessors, it is recommended that a smaller panel is used first.

CHOICE OF TEST METHOD

When the type of sensory problem has been determined, the choice of test method will also be related to the degree of confidence in the results required, the nature of the samples and the availability and experience of the assessors.

Because of sensory fatigue and the effects of adaptation, only a limited number of samples can be assessed during a session. The number of samples that can be assessed will depend on the test method and the nature of the product. Whilst control samples are often necessary, their use will limit the number of samples that can be assessed during each session.

RECRUITMENT, SELECTION AND TRAINING OF ASSESSORS

The number of experts, trained assessors and assessors required depends on the test method used. Training will improve precision and will enable the panel leader to select those with the best ability for the particular test method and product. For example, assessors of foofoo should be regular consumers of the product.

It is important to note that assessors are selected for their ability to discriminate between and describe different qualities of food or drink products. In preference tests, however, the assessors selected must be representative of the users of that product in the market place.

When a selection procedure is carried out, the following criteria are important:

- management support within the organization;
- availability of assessors;
- motivation of the assessors;
- good health (absence of specific allergies or medical treatment) and good dental conditions.

It is often useful to ask each new recruit to complete a questionnaire (which can be in confidence) to assist the panel leader in planning the panels and to evaluate each assessor individually. An example of a questionnaire is given below.

Depending on the aim, assessors should be selected and may initially participate in simple screening tests (basic tastes, odour recognition, sensitivity) followed by training by repetition of a test method. Ideally, training should be undertaken using the whole range of test methods likely to be encountered.

Assessor recruitment questionnaire

PRIVATE AND CONFIDENTIAL

TASTE PANEL RECRUITMENT QUESTIONNAIRE

PLEASE USE BLOCK CAPITALS TO FILL IN THIS FORM

Please answer the questions as accurately as possible.

You will not be disregarded because you smoke or are unable to consume specific foods, since that area may be of particular interest in our studies.

1. NAME _____
2. ORGANIZATION _____
3. HOME ADDRESS _____
4. If you are asked to take part in our sensory testing panels, about 15 minutes of your time would be needed on each visit. However, occasionally during training, between 30 and 60 minutes of your time will be required.

   Please circle the days of the week when you will *not* be available.

   MONDAY TUESDAY WEDNESDAY THURSDAY FRIDAY

   Please indicate below other times when you will not be available, i.e., holidays, overseas assignments, etc.
5. Do you suffer from health problems which might affect your ability to test certain products?

   (Examples include: food allergies, diabetes, frequent colds, hayfever, colour blindness, any medication which affects your ability to taste, etc.)

   YES ☐

   NO ☐

   If yes, please outline the nature of this problem below:
6. Are there any types of food or drink which, for any reason, you would not be prepared to test?

   (Examples include: personal dislike, vegetarian, religious belief, etc.)

   YES ☐

   NO ☐

   If yes, please give details below of the foods/drinks.
7. Do you smoke?

   YES ☐

   NO ☐

   If yes, please record below the approximate number of cigarettes you smoke each day.

   _____ cigarettes per day
8. Have you ever taken part in any food testing work before?

   YES ☐

   NO ☐

   If yes, please outline the work, including the duration of the work, when the work took place and for whom the work was carried out.

Thank you for completing this questionnaire.

The same test should be carried out more than once to ensure that every assessor has not given the correct answer by chance.

It is recommended that the number of assessors selected for a given test should normally exceed (e.g. by 50%) that required for the particular panel. This will allow for absences.

It should be noted that a smaller panel of good discriminators may be statistically less efficient than a larger panel that includes poorer discriminators. However, the inclusion of less discriminating assessors simply to increase panel size will not improve the effectiveness of the result. Also, when the recommended number of assessors cannot be obtained, the repeated use of a smaller panel does not give the same result as an equivalently larger panel.

The participation of assessors in a given panel should be on the basis of random selection, but should be balanced to result in equal participation by each person over a number of panels.

References

BS 5929 (1986) *Methods for sensory analysis of food. Part 1—General guide to methodology.*

ISO 6658 (1985) *Sensory analysis. Methodology. General guidance.*

JELLINEK, G. (1985) *Sensory Evaluation of Food—Theory and Practice.* Chichester, UK: Ellis Horwood.

BASIC TASTES

Recognition of the four basic tastes (sweet, salt, sour, bitter) may be useful when selecting and training assessors. These taste qualities are perceived via stimulation of taste receptor cells which occur in the taste buds; these are located primarily on the tongue, although smaller numbers of taste buds are found around the mouth and in the throat. Newly born children have about 10 000 taste buds but in adults, the number decreases to about 2000. The number of taste buds continues to decrease slowly with age, and at about 50–60 years of age, the loss may have a significant effect on the sensitivity of an individual to various taste stimuli.

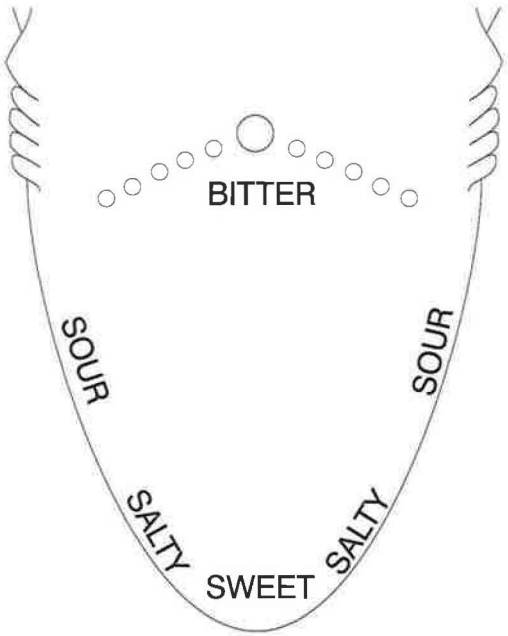


Figure 4.7 Basic tastes—sensory regions of the tongue

Principle

Each assessor is presented with aqueous solutions of reference substances (of known concentration) corresponding to the four primary tastes. After each tasting, the assessor records the results on a form.

Reagents

Water: Use neutral, tasteless, still, odour-free water of a purity close to that of distilled water and which will not affect the results.

Stock solutions of reference compounds: Stock solutions (ISO 3972) may be prepared from food grade reference substances (or analytical reagent grade).

Table 4.4 Stock solutions of taste reference compounds

Taste	Reference substance	Concentration (g/l)
Sweet	Sucrose	32
Salty	Sodium chloride	6
Acid	Citric acid	1
	Tartaric acid	2
Bitter	Caffeine	0.20
	Quinine hydrochloride	0.02

Apparatus

- Volumetric flasks—should be clean, dry and of a suitable capacity for stock solutions and dilutions.
- Vessels—should be clean, dry and with a capacity of about 50 ml for presentation of test solutions. Can be made of glass or disposable plastic.
- Chemical balance—accurate to two decimal places.

Procedure for determining ability to recognize basic tastes

Test solutions

For each primary taste, use the dilutions recommended below. These are made by diluting 250 ml of stock solution with 750 ml water.

Table 4.5 Test solutions for taste reference compounds

Taste	Reference substance	Concentration (g/l)
Sweet	Sucrose	8
Salty	Sodium chloride	1.5
Acid	Citric acid	0.25
	Tartaric acid	0.50
Bitter	Caffeine	0.050
	Quinine hydrochloride	0.005

For each assessor, prepare seven vessels each containing 30 ml of one of the basic tastes (including two repeats and a blank containing water only) and mark with a code.

Determination

Offer the seven vessels containing the reference solutions arranged at random. Provide a supply of water to enable the assessors to rinse their mouths before testing the samples. If the solutions are not to be swallowed, the assessors should take a sufficient amount to allow impregnation of all the internal surfaces of the mouth.

The water should be at room temperature (approximately 20 °C).

After tasting each sample, the results are entered on a score sheet, an example of which is shown below.

Form for basic tastes

BASIC TASTES

NAME _____

DATE _____

In front of you are seven samples of weak solutions representing the basic taste sensations, i.e., salt, sweet, sour (acid) and bitter. One of these may be a blank sample and others may be repeated.

Your task is to identify the dominant taste sensation of each sample.

Please rinse your mouth with water after tasting each sample.

SAMPLE CODE	BASIC TASTE DESCRIPTION
1	_____
2	_____
3	_____
4	_____
5	_____
6	_____
7	_____

Interpretation of results

Assessors should be able to identify the basic tastes correctly. If a basic taste is identified incorrectly, the following explanations are possible:

- taste testing technique—ensure that the assessor sips sufficient sample and moves it around the mouth to reach all parts of the tongue;
- sensory fatigue—although repeated tasting is useful, repeated retasting may lead to sensory fatigue;
- a few assessors may be 'blind' to the bitter taste and will not be suitable for tests in which bitterness is a component of the food product.

Assessors who are unable to recognize one or more basic tastes should be encouraged to retaste the solutions and, if necessary, the stock solutions.

Determination of the different types of threshold

Reference solutions of the primary tastes are presented in increasing orders of concentration. After each tasting, the assessor records his assessment on a score sheet.

Two series of dilutions, geometric and arithmetic, are generally used. The geometric series, with a ratio of 2, is for general use, and the arithmetic series with smaller intervals is used when greater accuracy is required.

Test solutions

Table 4.6 Geometric series of dilutions

Taste	Reference substance	Concentration of test solution (g/l) Volume of stock in 1000 ml					
		500	250	125	62	31	16
Sweet	Sucrose	16	8	4	2	1	0.5
Salt	Sodium chloride	3	1.5	0.75	0.37	0.18	0.09
Acid	Citric acid	0.5	0.25	0.125	0.062	0.030	0.015
	Tartaric acid	1.0	0.50	0.25	0.12	0.006	0.003
Bitter	Caffeine	0.10	0.05	0.025	0.012	0.006	0.003
	Quinine hydrochloride	0.01	0.005	0.002	0.0012	0.0006	0.0003

Table 4.7 Arithmetical series of dilutions

Taste	Reference substance	Concentration of test solution (g/l) Volume of stock in 1000 ml								
		250	225	200	175	125	100	75	50	25
Sweet	Sucrose	8	7.2	6.4	5.6	4.8	4.0	3.2	2.4	1.6
Salt	Sodium chloride	1.5	1.35	1.2	1.05	0.9	0.75	0.6	0.45	0.3
Acid	Citric acid	0.25	0.225	0.2	0.175	0.150	0.125	0.100	0.075	0.005
	Tartaric acid	0.5	0.45	0.4	0.35	0.3	0.25	0.2	0.15	0.1
Bitter	Caffeine	0.05	0.045	0.04	0.035	0.03	0.025	0.02	0.015	0.01
	Quinine hydrochloride	0.005	0.0045	0.004	0.0035	0.003	0.0025	0.002	0.0015	0.001

Determination of the threshold

For the primary tastes to be assessed, present the test solutions (about 15 ml) to the assessor in sequence starting with a sample of water and followed by the dilutions in increasing concentration. Several samples with the same concentration may be included. The results should be recorded on a form, an example of which is shown below.

Form for threshold test

THRESHOLD TEST

NAME _____

DATE _____

In front of you is a concentration series of *one* taste quality (sweet, salt, sour or bitter).

The samples are arranged in order of increasing concentration. First rinse your mouth out with control water to become familiar with its taste. Do not swallow the samples.

Start the test with the first sample and continue with the second, third, fourth, etc. RETASTING IS NOT ALLOWED. This refers not only to the sample you are in the process of tasting, but especially to previous samples.

Describe the taste of each sample, along with a possible mouthfeel (e.g. astringent). Determine also the taste intensity of each sample by using the following *intensity scale*:

0 = no taste or taste like control water
? = different from control water, the respective taste quality cannot, however, be recognized
1 = threshold, very weak
2 = weak
3 = pronounced (definite, distinct, clear)
4 = strong
5 = very strong

Sample No.	Taste quality (and mouthfeel)	Intensity

It is recommended that the assessor waits for 1 min between testings of successive samples. Before moving on to the next primary taste, the assessor should wait for a sufficient time to allow the primary taste tested to disappear completely.

Interpretation and analysis of results

For each assessor, the threshold for the primary tastes considered is determined. Although the assessor should have no previous knowledge of the result, when the four primary tastes are considered in succession, it is obvious that the fourth primary taste will be known in advance.

References

BS 5929 (1992) *Methods for sensory analysis of food. Part 7—Investigating sensitivity to taste.*

ISO 3972 (1991) *Sensory analysis. Methodology. Determination of sensitivity of taste.*

JELLINEK, G. (1985) *Sensory Evaluation of Food—Theory and Practice*. Chichester, UK: Ellis Horwood.

WATTS, B. M., YLIMAKI, G. L., JEFFERY, L. E. and ELIAS, L. G. (1989) *Basic Sensory Methods for Food Evaluation*. Ontario, Canada: International Development Research Centre.

ODOUR RECOGNITION

Odour is extremely important for overall flavour perception. Odour is perceived in the nasal cavity – if the nose is held or a cold is being suffered, food flavour is diminished (*see* Figure 4.8). Odour recognition of common compounds is useful for determining those assessors with good recognition and discrimination facilities.

The number of chemical substances that produce odour is very large; to date, some 17 000 have been identified. For certain natural flavours, particularly the fruity or sweet types, a single flavour character impact compound is present (e.g. vanillin in

vanilla, citral in lemon, menthol in peppermint). In other products, such as tea, coffee and cocoa, many compounds (between 400 and 800) are responsible for the flavour.

Moreover, the character and intensity of the perceived volatile aroma profile depends exclusively on the concentration of the individual flavour volatiles in the gaseous phase. Aldehyde C10 (*n*-decanal), for example, is a constituent of natural orange peel oil. At low concentrations, it imparts an orange-peely character to the flavour profile, but at much higher concentrations, the orange-peel note gives way to a fatty note. This is why this substance is used in orange flavourings as well as in chicken or other meaty-fatty flavourings.

Figure 4.8 Perception of aroma in the throat and nasal cavities

Principle

Each assessor is presented with odoriferous substances in various forms and concentrations. After smelling each sample, the assessor records the results on a form.

Reagents

Common household or factory substances may be used provided they are appropriate to the requirements of the test considered. Typical examples of compounds are given below.

Table 4.8 Common compounds to test odour recognition

Substance	Odour	Related odours
Acetic acid	Vinegar, sour	Pickles
Coffee	Coffee	Roasted
Onion	Onion	Sulphury
Cloves	Cloves, eugenol	Spicy
Anethole	Aniseed, anise	Liquorice
Cinnamon	Cinnamon, cinnanaldehyde	Spicy
Vanilla	Vanilla, vanillin	Sweet
Black pepper	Pepper	Pungent, spicy
Mustard	Mustard	Pickles
Acetone	Acetone	Nail polish remover
Almond extract	Almonds, benzaldehyde	Sweet
Garlic	Garlic	Sulphury
Lemon	Lemon, citral, acid	Citrus
Honey	Honey	Sweet

Apparatus

Dark coloured (or clear, wrapped in aluminium foil) individual glass vials (10 to 125 ml) with a glass stopper or screw cap to hold the products to be tested. Alternatively, beakers fitted with a watch glass can be used. If plastic containers are used, they must be odour free.

Procedure for determining the ability to recognize odours

The odoriferous substances should be placed inside the vials and covered with a clean cotton wool ball. Liquids may be poured onto the cotton wool ball. Vessels should be filled one-quarter to one-half full in order to leave a headspace above the sample for the volatiles to concentrate.

Determination

Present each assessor with the series of vials. The assessor should be instructed to open the vials one by one, with the mouth closed, and then to sniff the vapour in order to identify the product. Once the assessor has come to a decision, the vial should be closed and the answer entered on the score sheet, an example of which is shown below.

Form for odour recognition

ODOUR RECOGNITION

NAME _____

DATE _____

In front of you are seven coded odour bottles. Please remove the cap and try to assess the odour from the bottle.

Describe the odour below.

Please replace the cap after use.

BOTTLE CODE	DESCRIPTION
1	_____
2	_____
3	_____
4	_____
5	_____
6	_____
7	_____

Interpretation of results

The results may be interpreted in different ways according to the objectives of the test. For example, the correct reply for each substance may be the chemical name (where this is known), the common name, an association or even a descriptive expression.

References

BS 5929 (1992) *Methods for sensory analysis of food. Part 7—Initiation and training of assessors in the detection and recognition of odours.*

ISO 5496 (1992) *Sensory analysis. Methodology. Initiation and training of assessors in the detections and recognition of odours.*

JELLINEK, G. (1985) *Sensory Evaluation of Food—Theory and Practice*. Chichester, UK: Ellis Horwood.

WATTS, B. M., YLIMAKI, G. L., JEFFERY, L. E. and ELIAS, L. G. (1989) *Basic Sensory Methods for Food Evaluation*. Ontario, Canada: International Development Research Centre.

SAMPLING AND PRESENTATION OF MATERIAL TO BE TESTED

General principles for product sampling should be applied to test samples. Valid conclusions for a product can only be drawn if the samples tested are representative of the whole.

Methods of preparation and presentation of samples should be appropriate to the product and to the objectives of the test. Care is needed, however, to ensure maximum uniformity between sub-samples for each assessor.

Carriers may sometimes be used for tests on the evaluation of products where direct tasting is not feasible (see ISO 5497), for example, hot sauces, etc.

Containers should be chosen so as not to affect the test, and lighting should be controlled when appearance is assessed.

References

BS 5929 (1986) *Methods for sensory analysis of food. Part 1—General guide to methodology.*

ISO 6658 (1985) *Sensory analysis. Methodology. General guidance.*

TEST ROOM AND FACILITIES

The minimum requirements for a sensory laboratory are:

- a test area in which work may be carried out individually in testing booths and in groups;
- a sample preparation area.

In addition, an office, cloakroom, rest room and lavatories are recommended.

Testing area

Sensory analysis is best conducted in a special test room.

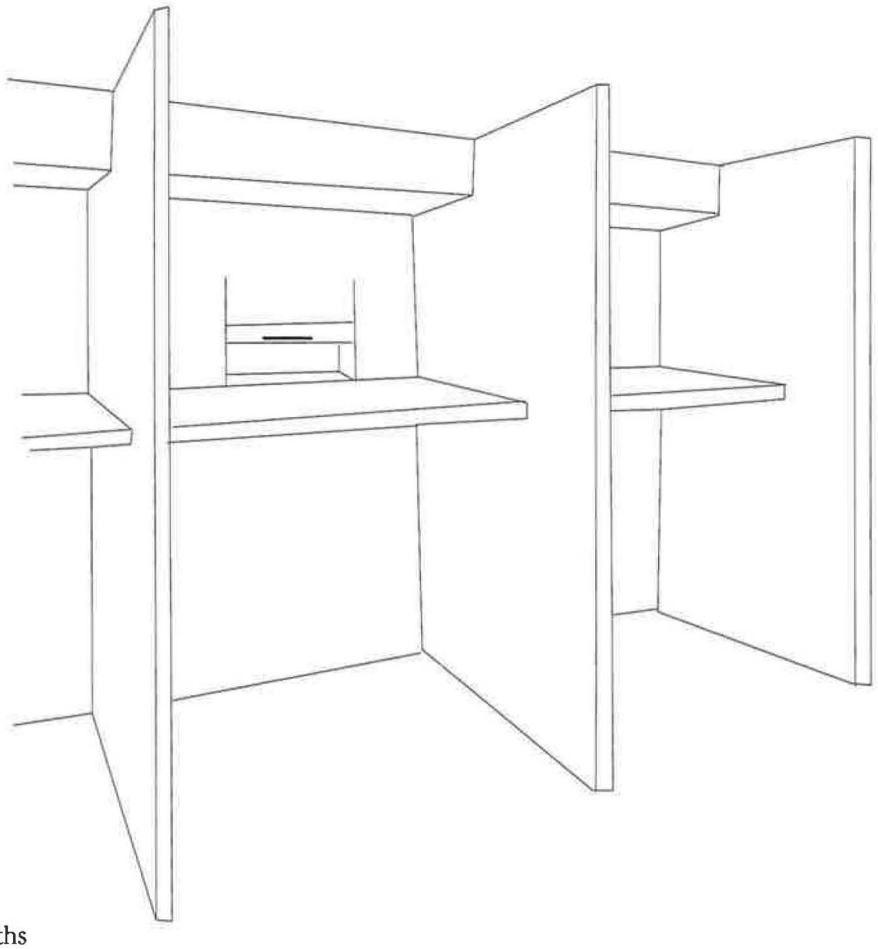
The objective of using a test room is to create a separate environment for each assessor with the minimum distraction, so that each can quickly adjust to the task. The room should be at a comfortable temperature, of neutral decor (matt off-white or light neutral grey is recommended), and ventilated with odour-free air. Persistent odours, such as tobacco or cosmetics, should be avoided.

The test room should be quiet. A low background noise is less disturbing than a fluctuating level of noise. Conversation and interruptions cause the greatest distractions.

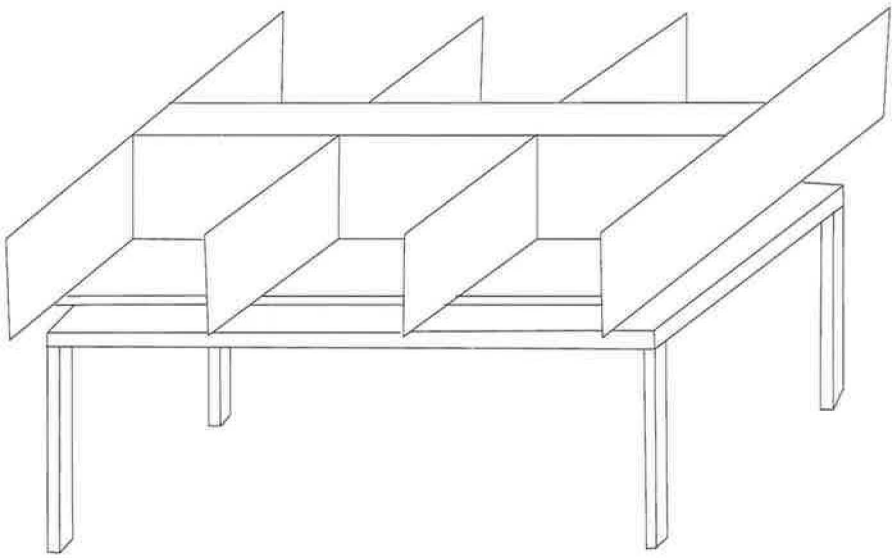
It is often useful to have control over both the intensity and colour of the lighting. Coloured lights help to mask differences in appearance.

The surfaces with which the food comes into contact should be clean and non-absorbent. The dimensions of the tasting booths are important. Very low ceilings and very narrow booths can feel oppressive. Comfortable seating is recommended.

The test room can be permanent or temporary. Permanent booths can be constructed with dividers and a hatch for passing the samples through on trays. Temporary booths can be constructed from light wood or heavy cardboard and can sit on table tops between assessors. Figure 4.9 illustrates examples of permanent and temporary booths.



a) Permanent booths



b) Temporary booths

Figure 4.9 Design of panel booths (permanent and temporary)

Preparation area

A laboratory (or kitchen) for the preparation of samples should be located in the immediate vicinity of the testing area. Its location must be such that the assessors do not have to pass through the preparation area to gain access to the testing room. The preparation area should be well ventilated so that odours do not affect the testing area. The materials selected for the construction of the room, and furnishings, should be easy to maintain, clean and odour free.

Apparatus and equipment

The preparation area must contain equipment suitable for the range of products to be processed. The main elements are:

- a work surface;
- a sink with running hot and cold water;
- equipment required for the preparation and presentation of the samples (containers, dishes, balance, etc.);
- electrical equipment for cooking (stove, oven), storage (refrigerator, freezer) and cleaning (waste disposal equipment, dishwasher);
- storage facilities.

References

ISO 8589 (1988) *Sensory analysis. General guidance for the design of test rooms.*

CONDUCTING TESTS

How the test is conducted depends on the test method and the type of sample.

The samples should be coded with random 3-digit numbers (see Table 4.9) to minimize bias. The order of assessment should also vary. With small numbers of samples and assessors, the order can be balanced so that every possible order occurs an equal number of times. In larger experiments, the order is best randomized.

Hunger and satiety may influence an assessor's performance. If panels are held too frequently, performance may deteriorate. Expectoration of samples may be recommended with trained assessors. If possible, assessors should be asked to refrain from smoking and to consume only water for 1 h before the test. It is recommended that assessors do not wear cosmetics, scents, after-shaves, or any other foreign odours which may influence the results of the panel.

The time of day at which the test is carried out is important, the best times being at mid-morning and mid-afternoon. It is preferable to avoid meal times.

References

BS 5929 (1986) *Methods for sensory analysis of food. Part 1—General guide to methodology.*

ISO 6658 (1985) *Sensory analysis. Methodology. General guidance.*

Table 4.9 Table of random numbers

813	332	025	203	562	949	123	958
560	210	096	585	648	937	786	697
419	576	438	337	866	121	010	549
277	423	558	210	018	805	847	922
353	137	469	554	456	472	581	088
780	071	586	579	680	348	239	175
226	737	787	854	647	483	459	661
802	570	306	656	786	385	103	447
889	304	510	327	323	055	245	312
565	080	591	186	407	307	979	758
775	073	775	412	345	126	654	737
999	685	894	132	585	205	188	862
274	723	440	475	258	400	514	568
883	659	725	347	243	395	801	274
635	458	776	816	808	876	414	358
448	333	948	273	400	580	226	807
058	460	658	064	669	582	843	676
208	165	495	724	807	670	344	164
742	004	237	435	012	593	534	127
122	135	695	460	998	770	942	054
886	706	010	953	766	158	497	970
302	013	674	124	186	830	960	946
062	900	632	985	227	757	030	919
684	068	331	159	712	645	159	490
478	479	886	286	039	700	114	334
878	830	273	210	279	918	706	291
906	033	481	549	365	466	687	397
237	288	564	686	200	454	391	526
393	861	017	805	464	123	859	421
343	123	328	675	333	293	220	466
224	578	005	581	036	626	853	332
114	913	181	835	062	430	076	518
046	335	194	693	077	613	568	795
313	271	948	396	465	161	994	404
280	349	701	697	918	721	409	408
483	858	633	371	416	617	202	192
763	408	043	397	660	754	788	311
908	339	344	607	892	928	593	221
383	994	322	419	290	081	772	335
562	713	071	044	405	832	654	369
431	282	727	420	464	984	495	575
666	711	581	058	760	795	200	363
092	037	049	053	741	163	341	492
591	966	863	633	823	429	081	340
412	893	607	384	891	610	701	495
706	373	990	077	421	984	543	481

TEST METHODS IN SENSORY EVALUATION

Types of test

The most common sensory evaluation tests are categorized into three.

- Difference tests to determine whether a sensory difference exists between two products.
- Tests using scales and categories. These estimate the order or size of differences or the categories or classes to which samples should be allocated.
- Descriptive or profiling tests. These identify the specific sensory attributes present in a sample and may be quantitative.

Types of panel and assessor

The type of panel and expertise of the assessors will vary according to the objectives of the sensory test.

- *Expert panel*—usually a small panel of highly trained and experienced expert assessors. They will be capable of discriminating between and describing subtle differences between samples.
- *Trained panel*—usually larger than an expert panel and the assessors will not be as highly trained or experienced as those on an expert panel. They will be able to discriminate and describe subtle differences between samples.
- *Untrained panel*—a panel larger than those consisting of expert or trained assessors. Untrained assessors are less sensitive to small differences between samples.
- *Consumer panel*—these make up the largest panels since the sensory abilities of assessors in a consumer panel will vary widely. Consumer panels do not readily describe in detail what they perceive.

DIFFERENCE TESTS

The following difference tests are used:

- paired comparison test;
- triangular test;
- duo-trio test;
- two-out-of-five test;
- 'A'-'not A' test.

Paired comparison test

Definition

A paired comparison test is one in which samples are presented in pairs for comparison and detection of differences on the basis of some defined criteria.

Application

The test is recommended for:

- determination of the difference, if there is a difference, and if there is, the direction of the difference between the two samples;
- determination of whether there is a preference;
- selection and training of assessors.

Assessors

The recommended number of assessors is as follows:

- 7 or more experts;
- 20 or more trained assessors;
- 30 or more untrained assessors;
- 100 or more consumers in consumer tests.

Procedure

The assessor is presented with one or more pairs of coded samples (*see* Table 4.9 for random numbers) in a controlled or random order. The two samples in each pair may be the same or different. The assessor is asked specific relevant question(s) referring to the difference, the direction of difference or preference. The person supervising the test can opt either for the forced choice or allow for the no-preference replies. If the forced-choice technique is chosen, the assessors must indicate which sample they find more intense, or prefer, even if they cannot find a difference. Additionally, the assessor can be asked how confident he is in his choice. An example score sheet is given below.

Form for paired comparison test

PAIRED COMPARISON TEST

NAME _____

DATE _____

SAMPLE CODES | 883 | | 408 |

In front of you are two samples. These may be the same or different.
Please taste the samples in the order given. Rinse your mouth with water before tasting each sample.

Can you detect any difference? _____

Please describe any differences:

_____

_____

_____

How confident are you in your choice of sample? Please tick one of the below.

- Absolutely sure ☐
- Fairly sure ☐
- Not very sure ☐

Analysis and interpretation of results

Forced-choice technique

For a one-sided test (this is concerned with the direction and the determination of the direction of a specified difference), note the total number of responses (taking the larger of the two figures) and refer to Table 4.10. If the number of responses in the test is larger than the figure given in the table for the total number of assessors, the result of the test is significant (three different levels of significance are given).

For a two-sided test (this is only to find out whether there is a difference in intensity or preference), the total number of responses (taking the larger of the two figures) is read from Table 4.11. If the number of responses in the test is larger than the figure given in the table for the total number of assessors, the result of the test is significant (three different levels of significance are given).

Table 4.10 Critical values for the paired comparison test (P=0.5): one-sided test

No. of replies	Minimum correct/incorrect judgements to establish significant difference		
	$P = 0.05$	$P = 0.01$	$P = 0.001$
7	7	7	–
8	7	8	–
9	8	9	–
10	9	10	10
11	9	10	11
12	10	11	12
13	10	12	13
14	11	12	13
15	12	13	14
16	12	14	15
17	13	14	16
18	13	15	16
19	14	15	17
20	15	16	18
21	15	17	18
22	16	17	19
23	16	18	20
24	17	19	20
25	18	19	21
26	18	20	22
27	19	20	22
28	19	21	23
29	20	22	24
30	20	22	24
31	21	23	25
32	22	24	26
33	22	24	26
34	23	25	27
35	23	25	27
36	24	26	28
37	24	27	29
38	25	27	29
39	26	28	30
40	26	28	31
41	27	29	31
42	27	29	32
43	28	30	32
44	28	31	33
45	29	31	34
46	30	32	34
47	30	32	35
48	31	33	36
49	31	34	36
50	32	34	37
60	37	40	43
70	43	46	49
80	48	51	55
90	54	57	61
100	59	63	66

No-difference or no-preference option

If no-difference or no-preference replies have been permitted, the results can be treated according to one of the following procedures.

- Ignore them, that is, subtract them from the total number of replies from the panel.
- Allocate half of the no-difference or no-preference replies to each of the two categories of replies.

The proportion of no-difference or no-preference replies provides important additional information which may be useful in subsequent tests. It can indicate: that the difference between the two samples is just below the detection threshold; an imperfect experimental technique; variation between the assessors; or lack of panel motivation.

Confidence in choice option

This information is useful for determining how confident individual assessors and the group as a whole are in assessing a difference, especially if the statistical decision is borderline. It may also be important for the panel leader in deciding whether further training is necessary.

Table 4.11 Critical values for the paired comparison test (P=0.5): two-sided test

No. of replies	Minimum correct/incorrect judgements to establish significant difference		
	$P = 0.05$	$P = 0.01$	$P = 0.001$
7	7	–	–
8	8	8	–
9	8	9	–
10	9	10	–
11	10	11	11
12	10	11	12
13	11	12	13
14	12	13	14
15	12	13	14
16	13	14	15
17	13	15	16
18	14	15	17
19	15	16	17
20	15	17	18
21	16	17	19
22	17	18	19
23	17	19	20
24	18	19	21
25	18	20	21
26	19	20	22
27	20	21	23
28	20	22	23
29	21	22	24
30	21	23	25
31	22	24	25
32	23	24	26
33	23	25	27
34	24	25	27
35	24	26	28
36	25	27	29
37	25	27	29
38	26	28	30
39	27	28	31
40	27	29	31
41	28	30	32
42	28	30	32
43	29	31	33
44	29	31	34
45	30	32	34
46	31	33	35
47	31	33	36
48	32	34	36
49	32	34	37
50	33	35	37
60	39	41	44
70	44	47	50
80	50	52	56
90	55	58	61
100	61	64	67

References

BS 5929 (1986) *Methods for sensory analysis of food. Part 1—General guide to methodology.*

BS 5929 (1982) *Methods for sensory analysis of food. Part 2—Paired comparison test.*

ISO 5495 (1983) *Sensory analysis. Methodology. Paired comparison test.*

ISO 6658 (1985) *Sensory analysis. Methodology. General guidance.*

JELLINEK, G. (1985) *Sensory Evaluation of Food—Theory and Practice*. Chichester, UK: Ellis Horwood.

Triangular test

Definition

In the triangular test, three coded samples, two of which are identical, are presented to an assessor simultaneously. The assessor is asked to select the odd sample.

Application

The triangular test is recommended for the following:

- detection of slight differences between samples;
- situations when only a limited number of assessors are available;
- selection and training of assessors.

Disadvantages of the method are that:

- it is not economical for the assessment of a large number of samples;
- it may be more influenced by sensory fatigue than the paired comparison with intensely flavoured samples;
- it may be difficult to ensure that two samples are identical.

Assessors

The recommended numbers of assessors are:

- 6 or more experts;
- 15 or more trained assessors;
- 25 or more untrained assessors.

Procedure

Each assessor is presented with one set of three coded samples (*see* Table 4.9 for random numbers), two of which are identical. The assessor is asked to select the odd sample. Samples should be presented an equal number of times in each of the two sets of three distinct permutations of order which are BAA, AAB, ABA, ABB, BBA and BAB. The assessor is asked specific relevant question(s) referring to the difference, the direction of difference or preference. The person supervising the test can opt either for forced choice or no-preference replies. If the forced-choice technique is chosen, the assessors must indicate which sample they find more intense, or prefer, even if they cannot find a difference. Additionally, the assessors may be asked how confident they are in their choice. An example score sheet is given below.

Form for triangular test

TRIANGULAR TEST

NAME _____

DATE _____

SAMPLE CODES | 883 | | 408 | | 958 |

Of the three samples in front of you, two are the same and one may be different.
Please taste the samples in the order given and select the odd one. Rinse your mouth with water before tasting each sample.

Can you detect any difference? _____

If so, which is the odd sample? _____

Which do you prefer? _____

Please describe any differences:

_____

_____

_____

How confident are you in your choice of sample? Please tick one of the below.

- Absolutely sure ☐
- Fairly sure ☐
- Not very sure ☐

Analysis and interpretation of results

Forced-choice technique

The number of correct answers is counted and compared with the minimum number of replies for the given panel size in Table 4.12. If, at the specified level of significance, the number of replies is higher than that given in the table, the result is significant.

Table 4.12 Critical values for the triangle test (P=1/3)

No. of replies	Minimum correct/incorrect judgements to establish significant difference		
	$P = 0.05$	$P = 0.01$	$P = 0.001$
5	4	5	–
6	5	6	–
7	5	6	7
8	6	7	8
9	6	7	8
10	7	8	9
11	7	8	9
12	8	9	10
13	8	9	11
14	9	10	11
15	9	10	12
16	9	11	12
17	10	11	13
18	10	12	13
19	11	12	14
20	11	13	14
21	12	13	15
22	12	14	15
23	12	14	16
24	13	15	16
25	13	15	17
26	14	15	17
27	14	16	18
28	15	16	18
29	15	17	19
30	15	17	19
31	16	18	20
32	16	18	20
33	17	18	21
34	17	19	21
35	17	19	22
36	18	20	22
37	18	20	22
38	19	21	23
39	19	21	23
40	19	21	24
41	20	22	24
42	20	22	25
43	20	23	25
44	21	23	26
45	21	24	26
46	22	24	27
47	22	24	27
48	22	25	27
49	23	25	28
50	23	26	28
60	27	30	33
70	31	34	37
80	35	38	41
90	38	42	45
100	42	46	49

No-difference or no-preference option

If no-difference or no-preference replies have been allowed, the results may be treated according to one of the following procedures.

- Ignore them, that is, subtract them from the total number of replies from the panel.
- Allocate one-third of the no-difference or no-preference replies to the categories of correct replies.
- Allocate two-thirds to the incorrect replies.

- Consider them separately.

The proportion of no-difference or no-preference replies provides important additional information which may be useful in subsequent tests. It can indicate: that the difference between the two samples is just below the detection threshold; an imperfect experimental technique; variation between the assessors; or lack of panel motivation.

Confidence in choice option

The confidence in choice information is useful for determining how confident individual assessors and the group as a whole are in assessing a difference, especially if the statistical decision is borderline. This information may be important for the panel leader in deciding whether further training is necessary.

References

BS 5929 (1986) *Methods for sensory analysis of food. Part 1—General guide to methodology.*

BS 5929 (1984) *Methods for sensory analysis of food. Part 3—Triangular test.*

ISO 4120 (1983) *Sensory analysis. Methodology. Triangular test.*

ISO 6658 (1985) *Sensory analysis. Methodology. General guidance.*

JELLINEK, G. (1985) *Sensory Evaluation of Food—Theory and Practice*. Chichester, UK: Ellis Horwood.

Duo-trio test

Definition

In the duo-trio test, the reference sample is given first. It is followed by two samples, one of which is identical to the reference sample and which the assessor is asked to identify.

Application

The duo-trio test is used to determine whether there is a sensory difference between a given sample and the reference sample. It is particularly suitable when the reference sample is well known to the assessors.

Assessors

At least 20 assessors are recommended.

Procedure

The assessor is first given the identified reference sample. This is followed by the two coded samples (*see* Table 4.9 for random numbers), one of which is identical to the reference sample. The assessor is asked to identify this sample. A typical score sheet is shown below.

Form for duo-trio test

DUO-TRIO TEST

NAME _____

DATE _____

You will receive a control sample and several pairs of samples.

In each pair, circle the number of the sample which tastes different to the control. Retasting is allowed.

C	408	958
	883	187

Please describe any differences:

_____

_____

_____

Thank-you for testing these samples.

Analysis and interpretation of results

Interpretation is the same as the one-sided test for the paired comparison method.

References

BS 5929 (1992) *Methods for sensory analysis of food. Part 8—Duo-trio method.*

ISO 10399 (1991) *Sensory analysis. Methodology. Duo-trio test.*

Two-out-of-five test

Definition

The two-out-of-five test involves five coded samples (*see* Table 4.9 for random numbers), two of which are of one type and three of another. The assessor is asked to group the two sets of samples.

Application

This test is recommended when:

- there are only a small number of selected assessors (i.e., fewer than 10) available;
- there is a need to establish a difference more economically than by other tests.

The disadvantages of this test are similar to those in the triangular method, but this method is more strongly affected by sensory and memory fatigue. Its principal use is in visual, auditory and tactile tests.

Assessors

The recommended number is 10 or more assessors.

Procedure

The assessor is presented with one set of five coded samples (*see* Table 4.9 for random numbers), two of which are of one type and three of the other. The assessor is asked to group the two sets of samples. When the number of assessors is less than 20, the order of presentation should be chosen at random from the following 20 distinct permutations: AABBB, ABABB, ABBAB, ABBBA, BAABB, BABAB, BABBA, BBAAB, BBABA, BBBAA, BBAAA, BABAA, BAABA, BAAAB, ABBAA, ABABA, ABAAB, AABBA, AABAB, AAABB. A typical score sheet is given below.

Form for two-out-of-five test

TWO-OUT-OF-FIVE TEST

NAME _____

DATE _____

SAMPLE CODES	883	408	958	187	723

Of the five samples in front of you, two are the same and the other three are different.

Please taste the samples in the order given and determine the two groups, one group of two samples the same and the other of three samples the same. Rinse your mouth with water before tasting each sample.

Which two are similar? _____

Please describe any differences:

_____

_____

_____

How confident are you in your choice of sample? Please tick one of the below.

Absolutely sure ☐

Fairly sure ☐

Not very sure ☐

Analysis and interpretation of results

The number of correct answers is compared with the corresponding number in Table 4.13. If the number of correct answers is greater than the equivalent value in the table, the result is significant.

Table 4.13 Critical values for the two-out-of-five test (P=1/10)

No. of replies	Minimum correct/incorrect judgements to establish significant difference
	$P = 0.05$
5	3
6	3
7	3
8	3
9	4
10	4
11	4
12	4
13	4
14	4
15	5
16	5
17	5
18	5
19	5
20	5
21	6
22	6
23	6
24	6
25	6
26	6
27	6
28	7
29	7
30	7

References

BS 5929 (1986) *Methods for sensory analysis of food. Part 1—General guide to methodology.*

ISO 6658 (1985)*Sensory analysis. Methodology. General guidance.*

'A' – 'not A' test

Definition

In this difference test, the assessor is presented with a series of samples, after he has learnt to recognize sample 'A', and is then asked to indicate which sample is 'A'.

Application

The 'A' – 'not A' test may be used for the assessment of samples which have variations of appearance or which leave a persistent after-taste. It is useful when strictly similar repeated samples are not available.

Assessors

The recommended number of assessors is:

- 20 selected assessors;
- 30 assessors.

Procedure

The assessor is presented with the samples one at a time. Firstly, he is presented with the reference sample 'A' until he can recognize it. This is followed by several samples presented at random, which may be 'A' or 'not A'; the assessor is then asked to say which they are. Only a few samples should be assessed during each session. A typical score sheet is shown below.

Form for 'A' – 'not A' test

'A' – 'not A' TEST

NAME _____

DATE _____

Sample 'A'

Please taste the sample marked 'A'. You can retaste several times until you are confident you can recognize its taste. When you are confident please inform the panel leader who will remove sample 'A' and provide you with five coded samples.

Coded samples

Please taste the samples in the order given and tick as 'A' or 'not A'. Rinse your mouth with water before tasting each sample.

	'A'	'not A'
831	—	—
692	—	—
276	—	—
756	—	—
138	—	—

Thank-you for testing these samples.

Analysis and interpretation of results

The results of this test may be analysed in the same way as those for the paired comparison test.

TESTS USING SCALES AND CATEGORIES

Quantification of sensory data on the basis of the perceived intensity of attributes requires the use of some form of scaling procedure. Most detailed analyses and interpretations of sensory characteristics require the intensities to be given a numerical value.

Types of tests used to estimate the order or size of differences, or the categories or classes to which samples should be allocated, are as follows:

- ranking;
- classification;
- rating;
- scoring;
- grading.

Ranking test

Definition

A ranking test is one in which a series of three or more samples are presented to an assessor at the same time and which is to be arranged in order of intensity, degree or preference.

Application

This method has a wide range of applications.

- Screening tests to aid the planning of more precise methods.
- Selection of products.
- Consumer tests for acceptance and preference.

- Training assessors.

The ranking method is rapid and suitable for the assessment of small numbers of samples (approximately six) in quality and flavour evaluations, and a larger number of samples (approximately 20) for appearance evaluations.

Assessors

The recommended number of assessors is:

- 2 or more experts;
- 5 or more trained assessors;
- 10 or more assessors;
- 100 or more consumers.

Procedure

Before starting the test, it is important to ensure that the assessors understand and agree on the attribute or criterion to be evaluated. Each assessor examines the coded samples (*see* Table 4.9 for random numbers) in a prescribed order, and, as a result, assigns a preliminary ranking. This ranking can be checked and adjusted by re-examination of the samples. The assessor records the finding on a score sheet, an example of which is shown below.

Form for ranking test

RANKING

NAME _____

DATE _____

Please taste each of the samples in the order below, and place them in increasing order of *sweetness*.

472	918	251	463

Please rinse your mouth with water before tasting each sample.

Please write the codes in increasing order of sweetness in the boxes below.

	Least			Most
Code				

Please write any comments below.

_____

Thank-you for completing this form

Analysis and interpretation of results

Calculation of the rank sums

Decode the samples and tabulate the rank orders to each sample given by each assessor. Where there are tied rankings, record the mean rank. Calculate the rank sum for each sample by summing the ranks for each assessor.

By comparing the rank sums for the samples, it is possible to obtain an evaluation of the differences between the samples.

Statistical interpretation

Friedman test This test is used where the order of the rankings is not predetermined, for example, in preference tests (BS 5929, ISO 8587).

The Friedman value (F) can be calculated as follows:

$$F = \frac{12}{JP(P+1)} (R^2_1+R^2_2+...R^2p)-3J(P+1)$$

where: J =number of assessors
P =number of samples (or products)
$R_1,R_2...Rp$ =rank sums attributed to the P samples for the J assessors.

The value F is then compared with the critical values in Table 4.14. If F is equal to or greater than the critical value corresponding to the number of assessors, the number of samples and the selected level of significance (P=0.05, 0.01), it can be concluded that there is a significant overall difference between the samples.

Table 4.14 Approximate critical values from the Friedman test

Number of assessors J	Number of samples (or products) P					
	3	4	5	3	4	5
	Significance=0.05			Significance=0.01		
2	–	6.00	7.60	–	–	8.00
3	6.00	7.00	8.85	–	8.20	10.13
4	6.50	7.50	8.80	8.00	9.30	11.00
5	6.40	7.80	8.96	8.40	9.96	11.52
6	6.33	7.60	9.49	9.00	10.20	13.38
7	6.99	7.62	9.49	8.85	10.37	13.38
8	6.25	7.65	9.49	9.00	10.35	13.28
9	6.22	7.81	9.49	8.66	11.34	13.28
10	6.20	7.81	9.49	8.60	11.34	12.28
11	6.54	7.81	9.49	8.90	11.34	12.28
12	6.16	7.81	9.49	8.66	11.34	12.28
13	6.00	7.81	9.49	8.76	11.34	12.28
14	6.14	7.81	9.49	9.00	11.34	12.28
15	6.40	7.81	9.49	8.93	11.34	12.28

Number of samples or products	Number of degrees of freedom (v=P–1)	Level of significance	
		0.05	0.01
3	2	5.99	9.21
4	3	7.81	11.34
5	4	9.49	13.28
6	5	11.07	15.09
7	6	12.59	16.81
8	7	14.07	18.47
9	8	15.51	20.09
10	9	16.92	21.67
11	10	18.31	23.21
12	11	19.67	24.72
13	12	21.03	26.22
14	13	22.36	27.69
15	14	23.68	29.14

However, as the number J of assessors becomes greater, F approximately follows the chi-squared distribution with (P—1) degrees of freedom (Table 4.15).

Table 4.15 Critical values of the chi-squared distribution

Number of assessors	Number of samples					
	P=0.05					
	3	4	5	6	7	8
2	28	58	103	166	252	362
3	41	84	150	244	370	532
4	54	111	197	321	487	701
5	66	137	244	397	603	869
6	79	163	291	474	719	1037
7	91	189	338	550	835	1204
8	104	214	384	625	950	1371
9	116	240	431	701	1065	1537
10	128	266	477	777	1180	1703
11	141	292	523	852	1295	1868
12	153	317	570	928	1410	2035
13	165	343	615	1003	1525	2201
14	178	368	661	1078	1639	2367
15	190	394	707	1153	1754	2532
16	202	420	754	1228	1868	2697
17	215	445	800	1303	1982	2862
18	227	471	846	1378	2097	3028
19	239	496	891	1453	2217	3193
20	251	522	937	1528	2325	3358

If one or more rankings are tied, F can be replaced by

$$F' = \frac{F}{1-\{E / [JP(P^2-1)]\}}$$

where: $E=(n^3_1-n_1) + (n^3_2-n_2) +...+ (N^3_k-n_k)$

Where an overall difference between the samples has been statistically demonstrated, the rank sums of each sample can be used to identify significant differences for the sample pairs.

Let i and j be two samples and R_i and R_j their rank sums. Two samples are different if:

$$|R_i-R_j| > 1.960 \frac{JP(P+1)}{6} \text{ (0.05 significance level)}$$

$$|R_i-R_j| > 2.576 \frac{JP(P+1)}{6} \text{ (0.01 significance level)}$$

Note: This test provides an indication of the order in which the samples are hierarchically ranked. However, the risk of an incorrect conclusion increases rapidly when these tests are carried out simultaneously.

Page test: This test is used when the order of the ranking is predetermined, for example, during storage trials (BS 5929, ISO 8587).

When the samples are structured by a natural order, for example, by different storage times, temperatures etc., the Page test is recommended rather than the Friedman test:

$$L=R_1 + 2R_2 + 3R_3 +...PR_p$$

where: R_1 to R_r are the rank sums for the samples.

If L is equal to or greater than the critical value (Table 4.16) corresponding to the number of assessors, the number of samples and the chosen level of significance (*P*=0.05 or *P*=0.01), it can be concluded that the ranking drawn up by the assessors corresponds to the predetermined order of the samples.

Table 4.16 Critical values for the Page test from P=0.01 degrees of freedom

Number of assessors	Number of samples					
	P=0.01					
	3	4	5	6	7	8
2	—	60	106	173	261	376
3	42	87	155	252	382	549
4	55	114	204	331	501	722
5	68	141	251	409	620	893
6	81	167	299	486	737	1063
7	93	193	346	563	855	1232
8	105	220	393	640	972	1401
9	119	246	441	717	1088	1569
10	131	272	487	793	1205	1736
11	144	298	534	869	1321	1905
12	156	324	584	946	1437	2072
13	169	350	628	1022	1553	2240
14	181	376	674	1098	1668	2407
15	194	402	721	1174	1784	2574
16	206	427	767	1249	1899	2740
17	218	453	814	1325	2014	2907
18	231	479	860	1401	2130	3073
19	243	505	906	1476	2245	3240
20	256	531	953	1552	2360	3406

References

BS 5929 (1989) *Methods for sensory analysis of food. Part 6—Ranking.*

ISO 8587 (1988) *Sensory analysis. Methodology. Ranking.*

Classification test

Definition

A classification test is one in which samples are assigned to predetermined categories or classes.

Application

Classification is recommended for use where, for example, defects in a product are to be assessed.

Assessors

The recommended number of assessors is three or more experts or trained assessors.

Procedure

The classification to be applied should be clearly defined and understood. Each assessor examines the samples and assigns them to one of the predetermined categories.

Analysis and interpretation of results

The results may be summarized as frequencies for each category. The chi-squared test can then be used to compare the distributions of two or more types of a product in the different categories.

References

BS 5929 (1986) *Methods for sensory analysis of food. Part 1—General guide to methodology.*

ISO 6658 (1985) *Sensory analysis. Methodology. General guidance.*

Rating test

Definition

A rating test is a method of classification involving categories. Each category is composed of an ordered scale. No assumption is made about the size of the difference between the numbers.

Application

Rating can be used to evaluate the intensity of one or more attributes of a sample, or to determine the degree of preference.

Assessors

The recommended numbers of assessors are:

- 1 or more experts;
- 5 or more trained assessors;
- 20 or more assessors;
- 50 or more consumers (two samples);
- 100 or more consumers (three or more samples).

Procedure

The classification method should be understood by all the assessors. The scale may be graphic or descriptive, unipolar or bipolar. If numbers are assigned, it should not be assumed that they are scores.

Examples of scales in sensory analysis

The choice of an appropriate scale in sensory evaluation depends on the type and quantity of information required. The more detailed the information, the more complex the scales.

Inexperienced panellists are more comfortable with a small number of categories, for example, five. Nine categories are common for experienced panellists; above this number the divisions are too close for the panellist to discriminate between them. If more than nine categories are required, unstructured line scales are recommended. These give wider flexibility in terms of statistical analysis, and after a short explanation, most panellists can use these scales immediately.

A few examples are given below.

Scale A—Category scale

☐	extremely strong
☐	very strong
☐	moderately strong
☐	slightly strong
☐	neither weak nor strong
☐	slightly weak
☐	moderately weak
☐	very weak
☐	extremely weak

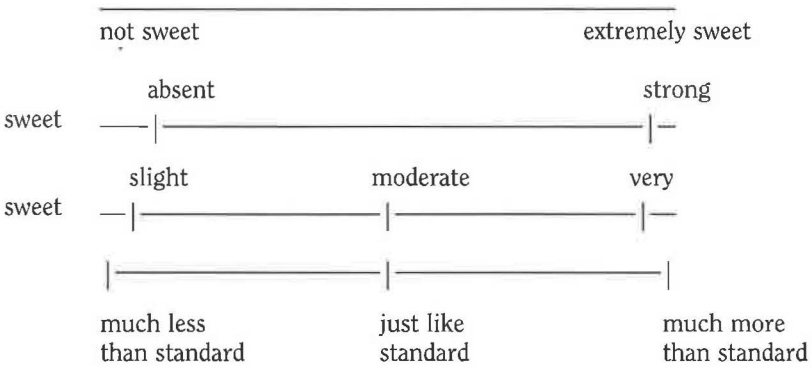
Scale B

0 = not present
1 = just recognizable, or threshold
2 = weak
3 = moderate
4 = strong
5 = very strong

Scale C

Weak ☐☐☐☐☐☐☐☐☐☐ Strong

Scale D—Graphic scales



It is recommended that the lines are 100 mm long. The assessors place a mark on the line to indicate intensity. Numerical values are then attributed by measuring the distance in millimetres between the mark made by the assessor and the left hand end of the line.

Sources of bias in using scales

All scales should be used critically; the sensory analyst should be aware of difficulties that the assessors may experience in using the scales. Common sources of bias are:

- *the central tendency* effect where there is a reluctance on the part of the panellist to use scale extremes;
- *contrast and convergence* effects resulting from the presence of a sample that is very different from the others.

Analysis and interpretation of results

For rating on a discrete scale with a small number of points, the results for one sample may be treated as for classification. When the data set is large, frequency distributions are recommended.

References

BS 5929 (1986) *Methods for sensory analysis of food. Part 1—General guide to methodology.*

ISO 4121 (1987) *Sensory analysis. Methodology. Evaluation of food products by methods using scales.*

ISO 6658 (1985) *Sensory analysis. Methodology. General guidance.*

Scoring test

Definition

A scoring test is a form of rating using a numerical scale. The numbers used in scoring form an interval or ratio scale (that is, the different scores have a defined and mathematical relationship to each other).

Application

Scoring is used for evaluating the intensity of one or more attributes.

Assessors

The recommended number of assessors is:

- 1 or more experts;
- 5 or more trained assessors;
- 20 or more assessors.

Procedure

The assessor assigns to each sample a value according to a predetermined scale, such as descriptive, line, etc. (*see Examples of scales in sensory analysis,* above).

Analysis and interpretation of results

The results obtained for one sample may be summarized as a median or a mean with some method of variation such as the standard deviation.

If only two samples are involved and the distribution of the results is normal, the t-test may be used to compare means and the F-test to compare variances.

If more than two samples are involved, the analysis of variance (ANOVA) procedure is recommended.

References

BS 5929 (1986) *Methods for sensory analysis of food. Part 1—General guide to methodology.*

ISO 6658 (1985) *Sensory analysis. Methodology. General guidance.*

Grading test

Definition

A grading test is the classification of a product according to quality, on the basis of one or more attributes. The assessors are usually experts or trained assessors.

Application

This method requires previous selection of attributes, a definition of the scale for these attributes, a weighting for each attribute and a definition of the grades.

Assessors

The number of assessors will vary according to the grading method applied.

Procedure

The procedure depends upon the particular grading method. A typical grading scale is shown below in Table 4.17.

Table 4.17 Example of a grading scale

Grade	Description
1	Bland
2	Trace of flavour, not identifiable
3	Nutty, beany, corny
4	Oxidized
5	Rancid

Analysis and interpretation of results

The results can be summarized as for the classification method and may be used for making a decision about a food product, such as its price, whether to accept or reject it, and so on.

References

BS 5929 (1986) *Methods for sensory analysis of food. Part 1—General guide to methodology.*

ISO 6658 (1985) *Sensory analysis. Methodology. General guidance.*

DESCRIPTIVE OR PROFILING TESTS—QUANTITATIVE DESCRIPTIVE ANALYSIS

Definition

Descriptive tests indicate the sensory properties of a product in a reproducible manner. The separate attributes contributing to the formation of the overall impression of the product are identified, and their intensities assessed, in order to establish a description of the flavour, appearance and/or texture.

Application

The tests are recommended for:

- product development;
- establishing the nature of differences between products;
- quality control;
- provision of sensory data for correlation with instrumental techniques.

Assessors

A panel of five or more experts or trained assessors is required.

Procedure

A preliminary set of trials is carried out with a range of qualities of the product to be tested in order to establish its important sensory properties by consensus.

The ability of the assessors to work together as a group and to express their opinions clearly is crucial to the success of this method.

The panel leader directs the assessors' discussions until agreement is reached on each component, thus enabling a description of the sensory attributes of the product.

If a consensus is not obtained, it is possible to use reference substances to aid the group in reaching an agreement. The panel leader produces a score sheet, and reports on and interprets the results.

Methods of assessing the intensity are given in *Examples of scales in sensory analysis,* above. Examples of typical score sheets are given below.

Examples of descriptive profiling score sheets (cassava flour biscuits)

DESCRIPTIVE PROFILING OF CASSAVA FLOUR BISCUITS

NAME: _____ DATE: _____

Sample code _____

Please evaluate the biscuit samples from left to right. Identify the attributes below and judge their intensity. Place a clear vertical mark on each line at the appropriate position.

APPEARANCE

INTENSITY

	light	dark
Colour	—|———————————|—	
	soft	hard
Hardness	—|———————————|—	
	fine	coarse
Texture	—|———————————|—	

TEXTURE

	chewy	brittle
Chewiness	—|———————————|—	
	dry	wet
Moist	—|———————————|—	
	fine	coarse
Fibrousness	—|———————————|—	

FLAVOUR

	absent	strong
Fruitiness	—|———————————|—	
	absent	strong
Nuttiness	—|———————————|—	
	absent	strong
Sweetness	—|———————————|—	

DESCRIPTIVE PROFILING OF FUFU

NAME: _____ DATE: _____

Sample code _____

Please evaluate the fufu samples from left to right. Identify the attributes below and judge their intensity. Place a clear vertical mark on each line in the appropriate position.

APPEARANCE

INTENSITY

	light	dark
Yellowness	—|———————————|—	
Greyness	—|———————————|—	

TEXTURE

	absent	strong
Chewiness	—|———————————|—	

FLAVOUR

	absent	strong
Cassava	—|———————————|—	
Cocoyam	—|———————————|—	
Plantain	—|———————————|—	

In the test sessions, the assessors check the samples (usually three or four) against the glossary of terms, scoring each attribute on an intensity scale. It is common to note the order in which the factors are perceived, including the presence of an after-taste, and to score the overall impression of the aroma, flavour, appearance or texture.

Analysis and interpretation of results

- After the assessors have completed their assessments, the panel leader tabulates the results and initiates a discussion to resolve differences. In the light of the discussion and, if necessary, after re-examination of the samples, the panel arrives at a group decision on the profile.
- A profile is obtained from the average of the scores assigned by each assessor displayed in the form of a spider chart (*see* Figure 4.10) or a line diagram.
- There is no simple way of treating the results statistically. Analysis of variance has been used to determine consensus among the assessors for each single attribute. Multivariate techniques of analysis (Principal Components Analysis,

Canonical Variates Analysis, Generalized Procrustes Analysis) have been used to explore the meaning and significance of dfferences between products and assessors. Multiple stepwise linear regression has been used to model sensory and instrumental methods.

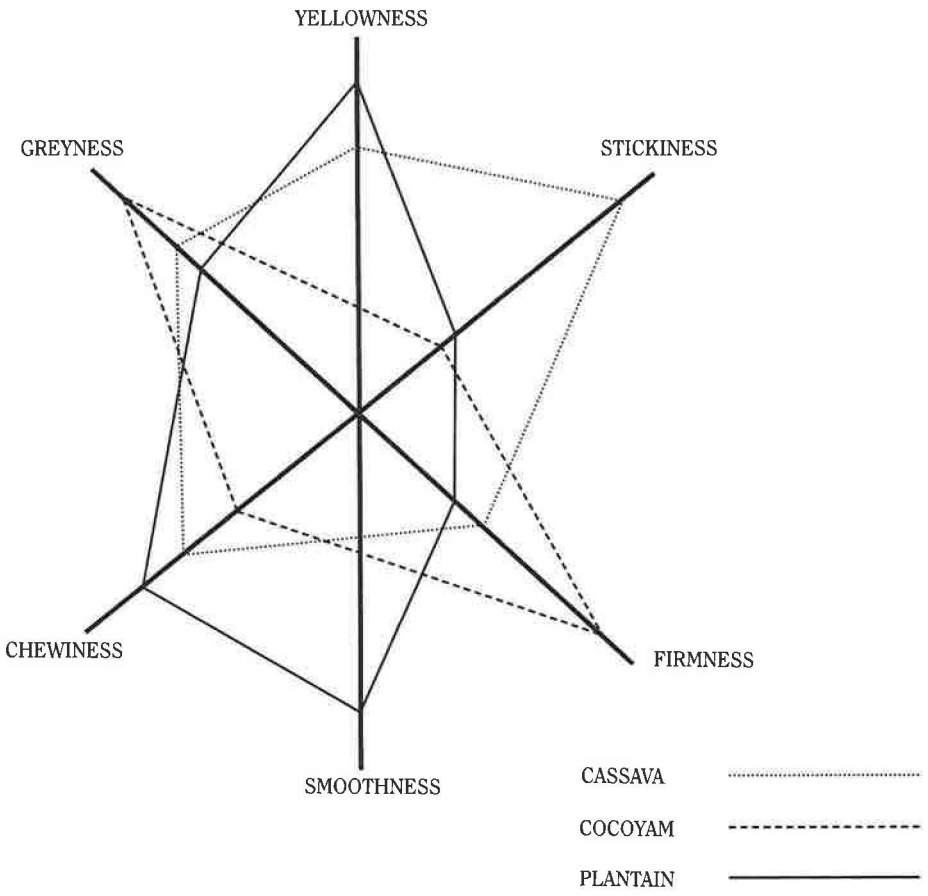


Figure 4.10 Descriptive profiling—spider chart

References

BS 5929 (1986) *Methods for sensory analysis of food. Part 4—Flavour profile methods.*

ISO 6564 (1985) *Sensory analysis. Methodology. Flavour profile methods.*

STANDARDS

British standards

BS 5098 (1975) *Glossary of terms relating to sensory analysis of food.*

BS 5929 (1986) *Methods for sensory analysis of food. Part 1—General guide to methodology.*

BS 5929 (1982) *Methods for sensory analysis of food. Part 2—Paired comparison test.*

BS 5929 (1984) *Methods for sensory analysis of food. Part 3—Triangular test.*

BS 5929 (1986) *Methods for sensory analysis of food. Part 4—Flavour profile methods.*

BS 5929 (1986) *Methods for sensory analysis of food. Part 5—'A'-'not A' test.*

BS 5929 (1989) *Methods for sensory analysis of food. Part 6—Ranking.*

BS 5929 (1992) *Methods for sensory analysis of food. Part 7—Investigating sensitivity to taste.*

BS 5929 (1992) *Methods for sensory analysis of food. Part 8—Duo-trio method.*

BS 5929 (1992) *Methods for sensory analysis of food. Part 9—Initiation and training of assessors in the detection and recognition of odours.*

International standards

ISO 3972 (1991) *Sensory analysis. Methodology. Determination of sensitivity of taste.*

ISO 4120 (1983) *Sensory analysis. Methodology. Triangular test.*

ISO 4121 (1987) *Sensory analysis. Methodology. Evaluation of food products by methods using scales.*

ISO 5492 (1992) *Sensory analysis. Methodology. Vocabulary.*

ISO 5495 (1983) *Sensory analysis. Methodology. Paired comparison test.*

ISO 5496 (1992) *Sensory analysis. Methodology. Initiation and training of assessors in the detection and recognition of odours.*

ISO 5497 (1982) *Sensory analysis. Methodology. Guidelines for the preparation of samples for which direct sensory analysis is not feasible.*

ISO 6564 (1985) *Sensory analysis. Methodology. Flavour profile methods.*

ISO 6658 (1985) *Sensory analysis. Methodology. General guidance.*

ISO 8587 (1988) *Sensory analysis. Methodology. Ranking.*

ISO 8588 (1987) *Sensory analysis. Methodology. 'A'-'not A' test.*

ISO 8589 (1988) *Sensory analysis. General guidance for the design of test rooms.*

ISO 10399 (1991) *Sensory analysis. Methodology. Duo-trio test.*

American standards

ASTM (1988) *Standards on sensory evaluation of materials and products.* Philadelphia, US: ASTM.

SUGGESTED FURTHER READING

Books

BIRCH, G. G., BRENNAN, J. G. and PARKER, K. J. (1977) *Sensory Properties of Foods.* London: Applied Science Publishers.

JELLINEK G. (1985) *Sensory Evaluation of Food—Theory and Practice.* Chichester, UK: Ellis Horwood.

LAWLESS H. T. and KLEIN B. P. (1991) *Sensory Science Theory and Applications in Foods.* New York, US: Marcel Dekker Inc.

LYON, D. H., FRANCOMBE, M. A., HASDELL, T. A. and LAWSON, K. (1992) *Guidelines for Sensory Analysis in Food Product Development and Quality Control.* London: Chapman and Hall.

MEILGAARD, M., CIVILLE, G. V. and CARR, B. T. (1987) *Sensory Evaluation Techniques.* Florida, US: CRC Press, Inc.

MOSKOWITZ, H. R. (1983) *Product Testing and Sensory Evaluation of Foods.* Connecticut, US: Food and Nutrition Press Inc.

MOSKOWITZ, H. R. (1985) *New Directions for Product Testing and Sensory Analysis of Foods.* Connecticut, US: Food and Nutrition Press Inc.

MOSKOWITZ, H. R. (1988) *Applied Sensory Analysis of Foods. Volume I.* New York, US: CRC Press Inc.

MOSKOWITZ, H. R. (1988) *Applied Sensory Analysis of Foods. Volume II.* New York, US: CRC Press Inc.

O'MAHONY, M. (1986) *Sensory Evaluation of Food. Statistical Methods and Procedures.* New York, US: Marcel Dekker, Inc.

PIGGOTT, J. R. (1988) *Sensory Analysis of Foods: 2nd edn.* London: Elsevier Applied Science.

STONE, H. and SIDEL, J. L. (1985) *Sensory Evaluation Techniques.* London: Academic Press Inc.

THOMSON, D. M. H. (1987) *Food Acceptability.* London: Elsevier Applied Science.

WATTS, B. M., YLIMAKI, G. L., JEFFERY, L. E. and ELIAS, L. G. (1989) *Basic Sensory Methods for Food Evaluation.* Ontario, Canada: International Development Research Centre.

WILLIAMS, A. A. and ATKIN, R. K. (1983) *Sensory Quality in Foods and Beverages: Definition, Measurement and Control.* Chichester, UK: Ellis Horwood Ltd.

Suggested journals

Primarily for sensory evaluation:

Food Quality and Preference
Journal of Sensory Studies
Journal of Texture Studies

Other sensory evaluation publications often appear in the following:

Journal of Food Science
Journal of Food Quality
Journal of Food Technology
Journal of Science of Food and Agriculture

Section 4.6 Starch characterization

INTRODUCTION

The methods for extraction and analysis of starch given here are taken from published scientific literature. They have been selected for their applicability for the analysis of non-grain starch staples.

The purpose of these methods is primarily for research and development work in, for example, determining changes in the properties of starch during storage and processing. Some of the methods have not been validated by NRI experts. Individual researchers should validate procedures and make appropriate modifications for their own needs.

PRESERVATION OF CASSAVA ROOTS, YAM, SWEET POTATO TUBERS AND BANANAS BEFORE STARCH EXTRACTION

Preservation of cassava roots, yam and sweet potato tubers

Introduction

Biochemical changes occurring post-harvest in roots and tubers can have a considerable effect on the properties of starch recovered from their tissues. Ideally, starch should be extracted immediately after harvest; this, however, is not always practicable. The methods described below are designed to reduce post-harvest changes in those root and tuber crops that need to be stored for several days or weeks before processing.

Accurate records should be kept of the time elapsed between harvesting and processing, and of the method used for preservation of the sample post-harvest.

Rationale

The techniques described are designed to limit enzymatic activity within the plant tissue and prevent the growth of spoilage micro-organisms during storage.

Suitability

Four methods are given for preservation of root and tuber crops, in order of their suitability for use in the field: preservation by curing; preservation by sun drying; preservation by refrigeration; and preservation by freeze drying.

Limitations

There are limitations to these techniques:

- curing: access to suitable fungicides, and response of tissue to curing process;
- sun drying: changes occurring during drying of tissue, availability of sunlight;
- refrigeration: not suitable for use in the field;
- freeze drying: not suitable for use in the field.

Principles

Curing. This process creates conditions suitable for repair of superficial wounds, thus preventing entry of micro-organisms into the plant tissue. A further barrier to microbial attack is provided by a layer of fungicide applied to the outside of the root or tuber.

Sun drying. This process uses sunlight to reduce the moisture content of the sample to a low level, which prevents enzymatic activity in the plant tissue and growth of spoilage organisms.

Refrigeration/freezing. Storage at temperatures of 0–5 °C will reduce microbial and enzymatic activity in the plant tissue to a low level. Activity can be reduced almost to zero by storing samples frozen at –20 °C.

Freeze drying. This process removes all water from samples by a process of sublimation under controlled conditions. Freeze-dried samples exhibit no enzymatic activity and are not susceptible to spoilage as long as they remain dehydrated.

Requirements

Equipment

Curing

- None

Sun drying

- None

Refrigeration/freezing

- Refrigerator (4 °C)
- Freezer (–20 °C)

Freeze drying

- Freeze drying unit
- Freezer (–70 °C)

Consumables

Curing

- Thiabendazole fungicide ('Mertect' Ciba-Geigy, Colombia)
- Household bleach
- Sisal sacks
- Plastic bags
- Plastic buckets
- Water

Sun drying; refrigeration/freezing; freeze drying

- Plastic bags

Procedure

Preservation by curing

Harvest roots or tubers incurring minimal damage. Wash in water to remove surface dirt and grade according to the following criteria:

- roots/tubers acceptable for curing should not be bruised and should only have superficial wounds located away from the stem (cut) end of the root/tuber. Cut away ragged areas of wounded tissue carefully with a knife;
- reject roots/tubers with bruises or deep wounds and any with wounds near the cut end.

Place clean roots or tubers in sisal sacks and immerse for up to 5 min in 0.4% thiabendazole solution. If thiabendazole is not available, immerse in 10–15% bleach solution. After treatment, empty onto a shaded area to dry for 15–30 min.

For curing, pack treated roots/tubers into plastic bags to create conditions of high humidity (>95% relative humidity). Roots/tubers should be maintained at 25 °C for 4–6 days to allow curing to take place.

After curing, remove roots/tubers from the curing environment and repack in plastic bags for storage until processed.

Reference

BALAGOPALAN, C., PADMAJA, G., NANDA, S. K. and MOORTHY, S. N. (1988) pp. 46–47. In: *Cassava in Food, Feed and Industry*. Florida, US: CRC Press Inc.

Preservation by sun drying

Spread washed roots/tubers on a drying area and leave until dry. The drying process must be accelerated by cutting the roots/tubers into several pieces. Rapid drying is essential to prevent adverse changes in the starch. Pack dried roots/tubers into plastic bags for storage until required.

Preservation by refrigeration/freezing

Pack washed roots/tubers in plastic bags and store either at 4 °C or –20 °C. Samples will keep for several weeks at 4 °C, and almost indefinitely when deep frozen.

Preservation by freeze drying

Cut washed roots/tubers into small pieces, freeze to –70 °C and then freeze dry using a freeze drier. The heat exchanger should be free of ice before the drier is turned on, to ensure efficient drying. Freeze-dried material can be stored in plastic bags under dehydrated conditions indefinitely.

Preservation of bananas

Introduction

Bananas continue to undergo biochemical changes after harvest because of the process of ripening. During ripening, starch is gradually converted into sugars, thus leading to an increase in sweetness.

Rationale

The techniques described below are designed to slow or prevent the process of ripening of the bananas, during the time between harvest and starch extraction.

Suitability

Refrigeration of bananas at temperatures of 4–14 °C with the relative humidity controlled at 80% will slow down the ripening process. Freeze drying is necessary if post-harvest changes are to be prevented.

Limitations

There are limitations to these techniques:

- refrigeration: storage at low temperatures will slow down the ripening process, although biochemical changes will continue to occur;
- freeze drying: freeze drying will prevent post-harvest changes, but has the disadvantage of high cost.

Principles

Refrigeration. Storage at low temperature reduces the rate of activity of enzymes involved in the ripening process.

Freeze drying. Freeze drying removes all water from samples by a process of sublimation under controlled conditions. Freeze-dried samples exhibit no enzymatic activity and are not susceptible to spoilage as long as they remain dehydrated.

Requirements

Equipment

Refrigeration

- Refrigerator

Freeze drying

- Freezer (–70 °C)
- Freeze drier unit

Consumables

Refrigeration; freeze drying

- Plastic bags

Procedure

Preservation by refrigeration

Store bananas in plastic bags or cardboard boxes, cover in polythene and store at 4–14 °C. The relative humidity should be maintained at approximately 80%.

Preservation by freeze drying

Cut the bananas into small pieces, freeze to –70 °C and then freeze dry in a freeze drier. The heat exchanger should be free of ice before the drier is turned on, to ensure efficient drying. Freeze-dried material can be stored in plastic bags under dehydrated conditions indefinitely.

Reference

STOVER, R. H. and SIMMONDS, N. W. (1987) pp. 373–376. In: *Bananas*. Harlow, UK: Longman Scientific and Technical.

EXTRACTION OF STARCH

Extraction of starch from cassava roots, yam and sweet potato tubers, and bananas

Introduction

It is necessary for starch granules to be released from the plant tissue, and separated from any impurities which may interfere with subsequent analysis.

Rationale

The techniques described below are intended to maximize the efficiency of extraction and purification of starch from cassava roots, yam, sweet potato tubers and bananas.

Suitability

Extraction of cassava and sweet potato starch presents few difficulties, although sweet potato starch takes longer to settle and gives poorer yields when compared to cassava. Yams (and aroids) contain mucilaginous materials which interfere with the extraction process, although this can be overcome by using 0.03 M ammonia solution. A special technique is required for the recovery of banana starch.

Limitations

The techniques for extraction of cassava, sweet potato and yam starch do not have any significant limitations. The extraction of banana starch presents difficulties: the efficiency of extraction is approximately 3%; and phenolic browning can occur during the extraction process.

Principles

All the extraction techniques described in this section rely on mechanical action to disrupt the plant tissue and release starch granules, followed by filtration to separate starch from impurities.

Requirements

Equipment

Extraction of starch from cassava roots, yam and sweet potato tubers

- Waring blender

Extraction of starch from bananas

- Dewar for liquid nitrogen
- Waring blender
- Refrigerator (0–5 °C)
- Refrigerated centrifuge
- Oven (optional)

Consumables

All reagents are analytical grade unless otherwise stated.

Extraction of starch from cassava roots and sweet potato tubers

- Muslin
- Nylon or metal filters (200 and 150 μm)
- Plastic or metal trays
- Distilled water
- Heavy duty plastic bags
- Rolling pin or bottle
- Bristle hair brush
- Plastic bottles or bags

Extraction of starch from yam tubers

- Muslin
- Nylon or metal filters (200 and 150 μm)
- Plastic or metal trays
- Distilled water
- Heavy duty plastic bags
- Rolling pin or bottle
- Bristle hair brush
- Plastic bottles or bags
- Ammonia

Extraction of starch from bananas

- Tris-HCl (Trizma HCl)
- EDTA
- Magnesium chloride
- Polyvinylpyrrolidone (Molecular weight 10 000)
- Distilled water
- Muslin
- Nylon or metal filters (250, 180 and 90 μm)
- Plastic or metal trays
- Heavy duty plastic bags
- Rolling pin or bottle
- Bristle hair brush
- Plastic bottles or bags

Procedure

Extraction of starch from cassava roots and sweet potato tubers

Peel clean roots or tubers and blend (with excess water) in a Waring blender. Use the blender in short bursts to avoid heating the starch. Filter starch milk through muslin followed by 200 and 150 μm filters to remove impurities. Sediment rapidly in shallow trays and wash several times with water to remove impurities from surface. Dry starch either by sun drying, or oven drying at 30 °C overnight.

Pack dry starch into heavy duty plastic bags, roll with a rolling pin or glass bottle and then seive starch through a clean dry 250 μm sieve. Use a soft bristle hair brush to brush starch through mesh.

Pack starch into plastic bottles or bags and store under dry conditions.

Extraction of starch from yam tubers

Prepare as described in *Extraction of starch from cassava roots and sweet potato tubers* above, except that extraction should take place in 0.03 M ammonia solution to separate starch from mucilaginous material.

Extraction of starch from bananas

Peel enough bananas to provide a minimum 100 g of pulp. Slice pulp into cubes (2 × 2 cm) and freeze in liquid nitrogen.

Blend frozen pulp in a Waring blender with at least 100 ml of extraction medium (*see* below) at 1 °C for a few seconds to form a homogenous slurry.

Force the slurry through a sheet of muslin, and filter through 250, 180 and 90 μm filters, allowing 15 min for each filtration step. Add extraction medium to the slurry during each step to improve filtration, and collect the filtrate in a flask containing 100 ml of extraction medium at 1 °C.

Centrifuge at 3000 *g* for 5 min at 1 °C. Discard the upper pigmented layer of material from the starch pellet, wash starch with 50 mmol Tris-HCl and resuspend in distilled water. Repeat the centrifuge and washing steps four times to purify the starch.

Resuspend the purified starch in distilled water, spread on a shallow tray and then either sun dry or dry in an oven overnight at 30 °C.

Pack dry starch into heavy duty plastic bags, roll with a rolling pin or glass bottle and pass through a clean dry 250 μm sieve. Use a soft bristle hair brush to brush starch through mesh.

Pack starch into plastic bottles or bags and store under dry conditions.

Extraction medium for banana starch: 50 mmol Tris-HCl (pH 7.5), 10 mmol EDTA, 10 mmol magnesium chloride, 0.75% (w/v) polyvinylpyrrolidone.

References

ASAOKA, M., BLANSHARD, J. M. V. and RICKARD, J. E. (1992) Effect of cultivar and growth season on the gelatinisation properties of cassava (*Manihot esculenta*) starch. *Journal of the Science of Food and Agriculture*, **59**: 53–58.

MOORTHY, S. N. (1991) Extraction of starches from tuber crops using ammonia. *Carbohydrate Polymers*, **16**: 391–398.

STEELE, A. (1996) *Characterisation of Banana Starches*. PhD thesis. UK: University of Bath.

AMYLOSE AND AMYLOPECTIN

Introduction

Starch is composed of two polymers, amylose and amlyopectin. The relative percentage of each polymer is responsible for the characteristic properties of a particular type of starch.

Rationale

The techniques described below are for the determination of the percentage of each polymer fraction in a starch sample. The chromatographic procedure can be used for determining the molecular weight of the polymers in the sample, and for separating the various polymer fractions for more detailed study.

Suitability

The techniques described below are applicable to any pure sample of starch. Flours need extensive purification to remove contaminating materials such as protein and lipids.

Limitations

The iodo-colorimetric assay is susceptible to interference by lipids, and samples may require defatting if amylose values are very low. The iodo-colorimetric assay only gives limited information about the polymeric composition of the starch.

The chromatographic procedure is capable of providing detailed information on the molecular composition of starch polymers. However, this technique is susceptible to interference from contaminants in both sample and reagents, and is also limited by high capital and consumable costs. Gel permeation chromatography (GPC) is limited by its inability to separate the components of the high molecular weight amylose fraction.

Principles

The iodo-colorimetric assay depends on the relative affinities of amylose and amylopectin for iodine (tri-iodide ion). Amylose adsorbs iodine strongly, forming a deep blue complex which adsorbs strongly at 620 nm. Amylopectin has a weak affinity for iodine, forming a purple complex, which adsorbs at 620 nm.

GPC relies on the principle of size exclusion to separate the polymer fractions on a bed of swollen particles of dextran or agarose gels. Large molecules are unable to enter the spaces between the swollen particles of gel and thus elute quickly. Smaller molecules enter the spaces between the gel particles and interact with the gel, causing them to elute more slowly.

Requirements

Equipment

Iodo-colorimetric assay

- Analytical balance (accurate to four decimal places)
- Spectrophotometer

GPC

- Analytical balance (accurate to four decimal places)
- Water-bath (thermostatically controlled)
- Rotary evaporator
- Refrigerator (optional)
- Centrifuge
- Vacuum pump
- Peristaltic (micro-tube) pump
- 2 × GPC columns (100 × 2.2 cm)
- Fittings for GPC columns
- Fraction collector
- Spectrophotometer
- Refractive index detector (optional)
- Chart recorder (optional)

Consumables

All reagents are analytical grade unless stated otherwise.

Iodo-colorimetric assay

- Ethanol (95%)
- Sodium hydroxide (1 M)
- Distilled water
- Acetic acid (1 M)
- Iodine solution (0.2 g iodine + 2 g potassium iodide in 100 ml distilled water)
- Potato amylose
- Potato amylopectin

GPC

- Distilled water
- Sodium hydroxide (2 M)
- Hydrochloric acid (0.5 M)
- pH indicator papers
- Isoamylase (Hayashibara Biochemical Labs, Japan)
- Acetate buffer (60 mmol)
- Ethanol (100%)
- Fractogel TSK HW 55 (Merck)
- Fractogel TSK HW 50 (Merck)
- Sodium chloride
- Sodium azide
- Blue dextran 2000 (Sigma)
- Pullulan standards P800, P400, P200, P100, P50, P20, P10 and P5 (Polymer Labs, UK)
- Acetic acid
- Sodium acetate
- Glucose
- Phenol
- Sulphuric acid

Procedure

Iodo-colorimetric assay

Total amylose

Weigh starch (100 mg) into a screw-cap universal bottle (28 ml capacity). Add 95% ethanol (1 ml) and 1 M sodium hydroxide (9 ml). Replace the cap and maintain at room temperature for 18–24 h to permit dispersion and gelatinization of starch granules; do not mix.

Transfer gel to a 100 ml volumetric flask and make up to volume with distilled water (take care to ensure that all gel is transferred). Shake vigorously to dissolve the gel.

Transfer 5 ml of dissolved starch gel to a 100 ml volumetric flask containing distilled water (50 ml). Add 1 M acetic acid (1 ml), iodine solution (2 ml), and make up to volume with distilled water. Keep at room temperature for 20 min.

Read absorbance of the solution against a control solution in a spectrophotometer at 620 nm. Determine concentration of amylose using equation derived from a standard curve (see below).

Preparation of control for iodo-colorimetric assay

In a 100 ml volumetric flask mix distilled water (50 ml), 1 M sodium hydroxide (9 ml) and 95% ethanol (1 ml). Make up to volume with distilled water.

Transfer 5 ml of above solution into a 100 ml volumetric flask, mix with distilled water (50 ml), acetic acid (1 ml) and iodine solution (2 ml). Make up to volume with distilled water.

Preparation of standard curve for iodo-colorimetric assay and calculation of percentage of amylose and amylopectin in samples of starch

Weigh potato amylose (100 mg) and potato amylopectin (100 mg) into separate screw-cap universal bottles. Add 95% ethanol (1 ml) and 1 M sodium hydroxide (9 ml). Do not mix contents. Keep at room temperature for 18–24 h.

Transfer solutions of amylose and amylopectin into separate 100 ml volumetric flasks and make up to volume with distilled water. Use these solutions to prepare the following standard mixtures of amylose/amylopectin:

% amylose	amylose (ml)	amylopectin (ml)
0	0	20
10	2	18
20	4	16
25	5	15
30	6	14

Add 5 ml of each standard to 50 ml distilled water in separate 100 ml volumetric flasks. Add 1 M acetic acid (1 ml) and iodine solution (2 ml), make up to 100 ml with distilled water and keep for 20 min at room temperature.

Read absorbance of each standard in a spectrophotometer at 620 nm. Plot absorbance against percentage of amylose. Carry out linear regression analysis to derive the equation for determination of percentage of amylose in samples of starch.

Gel permeation chromatography (GPC)

Preparation of eluent for GPC

Dissolve sodium hydroxide (0.2%), sodium chloride (0.2%) and sodium azide (0.02%) in distilled water. The pH of the eluent should be adjusted to pH 11. The eluent must be degassed overnight under vacuum before use.

Interpretation of results

The ratio of amylose to amylopectin has a significant effect on the cooking properties of starches. Starches containing a higher percentage of amylopectin have a higher peak viscosity and paste stability; this means that the starch will produce a thicker paste which will be less likely to break down during cooking.

References

SOWBHAGYA, C. M. and BHATTACHARYA, K. R. (1971) A simplified colorimetric method for determination of amylose content in rice. *Starch*, **23**: 53–56.

SHANTY, A. P., SOWBHAGYA, C. M. and BHATTACHARYA, K. R. (1980) Simplified determination of water-insoluble amylose content of rice. *Starch*, **32**: 409–411.

GPC system and calibration

Vertically mount two GPC (100 × 2.2 cm) ascending glass columns (Amicon, UK) on a stable stand, in an environment where temperature fluctuations are minimal (ideally, in an air-conditioned room). Soak the packing materials (Fractogel TSK-55(S) and TSK-50(S), Merck, UK) separately in eluent. Determine the volume of gel required to fill a column experimentally. Soak the gel in a volume of eluent three times the volume of gel required. Use a glass rod to disperse the gel gently. Magnetic stirrers must not be used since they disrupt the gel network. Degas the dispersed gel suspension overnight under vacuum. After degassing, the supernatant is poured off and replaced with fresh eluent to 75% of the volume used for soaking the gel.

Load the columns by pouring gel suspension down the inside walls of the columns with the help of a glass rod, to avoid entrapping air bubbles. Remove excess eluent evident as supernatant liquid in the column slowly by draining as the gel settles under atmospheric pressure. Check the homogeneity of the packing and the presence of any air bubbles by injecting blue dextran 2000 (2 mg/ml) onto the column. If the packing is not homogenous, remove and repack.

After packing, equilibrate with eluent. Then connect the columns in series so that samples injected into the system pass down the bed of the column packed with TSK-55 gel before entering the column packed with TSK-50 gel. Eliminate any eluent head-space in the second column to prevent sample dilution during fractionation. Connect the eluent reservoir, from which eluent is pumped into the system at the required flow rate with a peristaltic pump. Particular care must be taken to avoid introducing air bubbles. Insert a three-way valve into the eluent line downstream from the pump to allow injection of starch samples. Connect a fraction collector downstream from the second column (see Figure 4.9). When all connections have been made, equilibrate with eluent at the operating flow rate (27–30 ml/h) for 3 days before use. Determine the void volume of the system by injecting blue dextran (2 mg/ml) into the column and measuring the volume of eluent eluted before the dextran. Monitor the blue dextran elution with a spectrophotometer. A refractive index detector and chart recorder could also be connected to the column if these items of equipment are available.

After equilibriation, calibrate to enable the molecular weight of sample fractions to be determined. This is carried out by injecting a range of pullulan standards under operational conditions. The normal range of molecular weight standards is 853 000–5800. Calculate the degree of polymerization (DP) of sample fractions from a linear calibration curve of molecular weight plotted against elution volume.

Sample analysis by GPC

Weigh starch (40 mg) into a screw-cap universal bottle. Add distilled water (0.25 ml) and 2 M sodium hydroxide (0.25 ml), mix carefully, replace cap and incubate at 40 °C for 3 h to allow time for dispersion and dissolving.

Dilute and neutralize (pH 6.2–6.4) with distilled water (3.5 ml) and 0.5 M hydrochloric acid (approximately 0.9 ml). Check pH with indicator papers.

Add 60 mmol acetate buffer (5 ml, pH 3.5), isoamylase (30 µl) and incubate at 40 °C for 22–24 h to debranch sample.

Concentrate debranched sample with 100% ethanol (2 × 10 ml) at 40 °C in a rotary evaporator until dry. Store sample at 5 °C until required.

Add distilled water (0.33 ml) and 2 M sodium hydroxide (0.53 ml) to redissolve sample.

Add distilled water (1.24 ml) and centrifuge at 3300 *g* for 20 min to remove contaminants.

To inject the sample (1 ml) onto the column, turn off the peristaltic pump and allow time for the gel in the first column to expand in order to prevent a void volume at the head of the column. This would dilute the sample. Inject the sample and allow the system to run for approximately 6.5 h to allow time for the first fraction to pass through the system. After 6.5 h, set the fraction collector to collect 80 fractions (2.5 ml fraction) over a period of 13 h.

Interpretation of results

Data collected from the GPC analysis is used to construct a graph in which carbohydrate concentration is plotted against the elution volume. Each amylopectin fraction will have a characteristic elution volume determined by molecular weight. Amylose and high molecular weight amylopectin fractions have lower elution volumes than lower molecular weight amylopectins. If the GPC system has been calibrated with amylopectin standards, the molecular weight of each sample fraction can be determined. The carbohydrate content of each fraction gives a direct measure of the percentage of that fraction in the original sample. GPC data give a detailed picture of the molecular structure of starch granules, and may help to explain why cooking properties vary between samples of apprently similar starch.

References

DUBOIS, M., GILLES, K. A., HAMILTON, J. K., REBERS, P. A. and SMITH, F. (1956) Colorimetric method for determination of sugars and related substances. *Analytical Chemistry*, **28**: 350–356.

IKAWA, Y., GLOVER, D. V., SUGIMOTO, Y. and FUWA, H. (1981) Some structural characteristics of starches of maize having a specific genetic background. *Starch*, **33**: 9–13.

INOUCHI, N., GLOVER, D. V., TAKAYA, T. and FUWA, H. (1983) Development changes in fine structure of starches of several endosperm mutants of maize. *Starch*, **35**: 371–376.

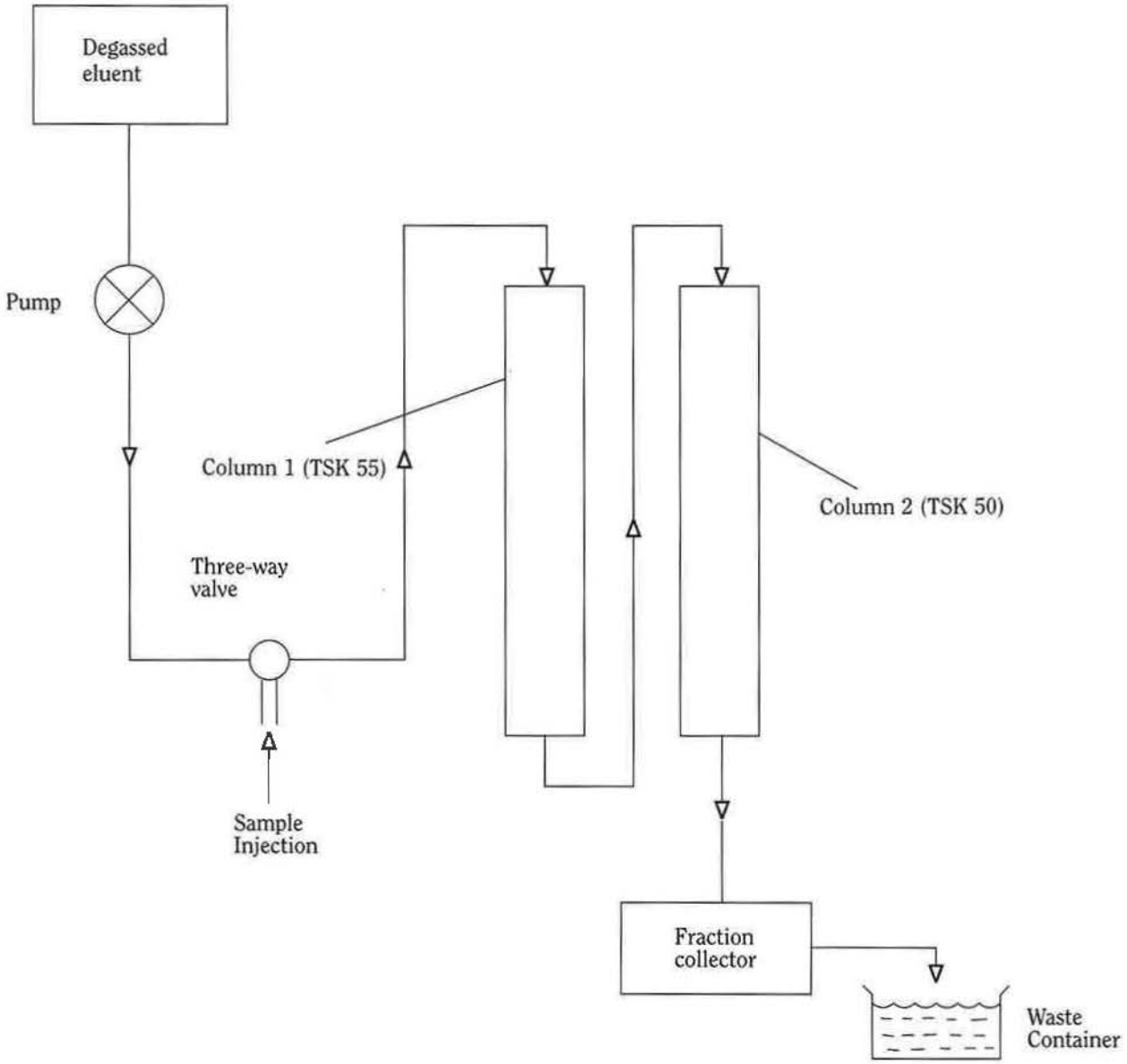


Figure 4.11 GPC system

ALKALI REDUCING VALUE

Introduction

Theoretically, amylose and amylopectin in fresh starch contain only one reducing end group/molecule. If the starch molecules degrade, the number of reducing end groups will increase. Reducing value can therefore be useful in indicating the degradation of starch granules at a molecular level.

Rationale

The most effective method for estimating reducing value is the ferricyanide method (Schoch, 1964) in which the number of reducing end groups in a sample is estimated by measuring the amount of ferricyanide that will bind to starch molecules under alkaline conditions.

Suitability

This technique is applicable to any pure starch sample. Flours and impure starches may give spurious results.

Limitations

The ferricyanide method cannot give an absolute value for reducing end groups because of unwanted side reactions which occur under alkaline conditions. This technique results in the reducing value being over-estimated, although it provides useful information which can be used for comparative purposes.

Principles

This procedure is dependent upon the ability of ferricyanide to react with reducing end groups on starch molecules under alkaline conditions.

Requirements

Equipment

- Analytical balance accurate to four decimal places
- Water-bath
- Waring blender

Consumables

- Distilled water
- Potassium ferricyanide
- Sodium carbonate
- Acetic acid (glacial)
- Zinc sulphate (heptahydrate)
- Potassium chloride
- Potassium iodide
- Potato starch
- Sodium thiosulphate

Procedure

Reducing value

Reagents

Prepare the reagents listed below before beginning the determination.

Alkaline ferricyanide: dissolve potassium ferricyanide (16.5 g) and anhydrous sodium carbonate (22 g) in distilled water (1 l). Store at room temperature for 2 days, then filter to remove sediment. Filtered reagent should be stored in a dark glass bottle at room temperature. The reagent should remain stable for approximately 2 months.

Zinc sulphate-acetic acid: dissolve potassium chloride (70 g), zinc sulphate (20 g) and acetic acid (200 ml) in distilled water (1 l).

Potassium iodide solution (20%): dissolve potassium iodide (100 g) in distilled water (500 ml). Store in a dark glass bottle away from light and discard when signs of a yellow iodine colour are observed.

Starch indicator: prepare a potato starch paste by heating potato starch (2 g) in distilled water (400 ml) for 15 min; stir occasionally to keep the starch granules in suspension until pasting has occurred. Cool the paste to room temperature and homogenize in a Waring blender for 5 min.

Sodium thiosulphate solution: prepare 1 l of 0.05 M sodium thiosulphate solution in distilled water.

Determination of reducing value

Weigh sample starch (200–250 mg) into a Pyrex bottle containing distilled water (50 ml). Heat in a boiling water-bath for 3 min, with continuous agitation to disperse the starch. Add alkaline ferricyanide reagent (25 ml), close the bottle with a

ventilated stopper and maintain in the boiling water-bath for exactly 15 min, with occasional agitation. Remove the bottle and cool rapidly under running water. Add zinc sulphate solution (60 ml) and mix well. Add potassium iodide solution (20 ml) and titrate with sodium thiosulphate.

Run blanks (without heating) using the same quantities of reagents as for actual samples. Titrate the solution as previously described but with the addition of starch indicator just before the end point (that is, when the yellow ferricyanide colour starts to fade). Determine moisture by the normal method on a separate sample of starch.

Calculate the reducing value as ml of 0.1 M thiosulphate/g of starch on a dry weight (d.w.) basis using:

$$\text{ferricyanide number} = \frac{(\text{blank titre} - \text{sample titre}) \times \text{molarity of thiosulphate} \times 10}{\text{sample weight (g d.w.)}}$$

Interpretation of results

In fresh starch, the amylose and amylopectin theoretically only contain one reducing end group per molecule. For this reason, the reducing value/ferricyanide number for fresh starch should always be less than one. Reducing values greater than one indicate that the starch molecules have been partially degraded, leading to an increase in the number of reducing end groups. Starch with a high reducing value/ferricyanide number will have poor cooking properties, typically forming a weak and unstable paste. The most common reason for increased reducing value is microbial spoilage. However, chemical adulteration can also lead to an increase in reducing value.

References

SCHOCH, T. J. (1964) Determination of reducing value. pp. 64–66. In: *Methods in Carbohydrate Chemistry (Starch)* WHISTLER, R. L. and PASCHALL, E. F. (eds.) New York: Academic Press.

FUNCTIONAL PROPERTIES OF NON-GRAIN STARCHES

Swelling volume, swelling power and solubility

Introduction

As the temperature of an aqueous suspension of starch is raised above the gelatinization range, hydrogen bonds continue to be disrupted, water molecules become attached to the liberated hydroxyl groups and the granules continue to swell. As a direct result of granule swelling, there is a parallel increase in starch solubility.

Rationale

The swelling power of starch can be determined by heating a weighed dry starch sample in excess water. Swelling power is defined as the swollen sediment weight/g dry starch. Swelling volume is simply the volume of the swollen sediment. Starch solubility is expressed as the percentage (by weight) of the starch sample that is dissolved molecularly after heating. Swelling power and solubility values provide evidence of the level of non-covalent bonding between molecules within the starch granules.

Suitability

The procedure for swelling power/volume and solubility is a simple technique which can be applied to any sample of pure starch or flour.

Limitations

No limitations have been reported for this technique.

Principles

This procedure measures the changes during irreversible swelling which occur if starch granules in aqueous suspension are heated above their gelatinization temperature.

Requirements

Equipment

- Analytical balance (accurate to four decimal places)

- Water-bath
- Centrifuge
- Drying oven

Consumables

- Distilled water

Procedure

Procedure for solubility, swelling power and swelling volume

Prepare an aqueous 1% (w/v) starch suspension by weighing starch (0.15 g equivalent dry weight) into a 40 ml screw-cap universal bottle containing enough distilled water to make 15 g total weight. It is important that the moisture content of the sample is known so that the weight of starch and volume of water can be adjusted to ensure that there is exactly 1% (w/v) dry starch. Heat the suspension to 95 °C in a water-bath, shaking gently to ensure that the starch granules remain in suspension until gelatinization occurs (5 min). The gelatinized sample should be held at 95 °C in the water-bath for 1 h.

Cool the sample to room temperature under running water, transfer to a graduated conical centrifuge tube and centrifuge for 20 min at 1000 *g*.

Swelling volume is obtained directly by reading the volume of the swollen sediment in the tube. Swelling power is determined by weighing the sediment and expressing swelling power as the weight (g) of swollen sediment/g dry starch. Solubility is determined by evaporating the supernatant and weighing the residue. Solubility is expressed as the percentage (by weight) of the starch sample that is dissolved molecularly after heating.

Interpretation of results

Good quality starch with a high starch content and paste viscosity will have a low solubility and high swelling volume and swelling power. High solubility, low swelling volume and swelling power are indicative of poor quality starches that produce thin, low stability pastes when cooked.

References

LEACH, H. W. and SCHOCH, T. J. (1961) Structure of the starch granule II. Action of various amylases on granular starches. *Cereal Chemistry*, **38**: 34–46.

MAT-HASHIM, D. B., MOORTHY, S. N., MITCHELL, J. R., HILL, S. E., LINFOOT, K. J. and BLANSHARD, J. M. V. (1992) The effect of low levels of antioxidants on the swelling and solubility of cassava starch. *Starch*, **44**: 471–475.

Pasting characteristics

Introduction

The transition from a suspension of starch granules to a paste, when heat is applied, is accompanied by a large increase in viscosity. Changes in viscosity also accompany the formation of gels upon cooling of starch pastes.

Rationale

Various types of viscometer are available for studying the pasting characteristics of aqueous suspensions of starch granules, but the Brabender Visco-Amylograph offers the most information at a lower capital cost. The Brabender Visco-Amylograph measures the amount of torque on a sensing element inserted into a rotating vessel containing an aqueous suspension of starch granules. During heating, viscosity increases, thus increasing the level of torque on the sensing element. Changes in viscosity are recorded on a chart recorder which forms part of the Visco-Amylograph.

Suitability

The Brabender Visco-Amylograph is suitable for use with any sample of pure starch. Flours and impure samples of starch often fail to paste properly, or they give anomalous results and should not be used with the Visco-Amylograph.

Limitations

The Brabender Visco-Amylograph requires a relatively large amount of starch per sample (25 g) which may be limiting for use with starches difficult to obtain in large quantities (such as banana starch). Use of a rotating sample bowl introduces shear

forces which will affect the viscosity data obtained. Oscillatory viscometers have been developed to overcome this problem, but the capital and running costs of these instruments are very high.

Principles

The Brabender Visco-Amylograph measures changes in viscosity occurring during a controlled cooking and cooling cycle.

Requirements

Equipment

- Brabender Visco-Amylograph
- Drying oven
- Analytical balance (accurate to four decimal places)

Consumables

- Distilled water

Procedure

Brabender Visco-Amylograph

NB: The moisture content (% m.c.) of the sample must be known in order to calculate the amount of sample required.

Switch on the power supply and control unit at least 20 min before beginning the analysis. Switch on the mains water supply and ensure that the waste pipe is inserted into a sink or drain to avoid flooding.

Set the start temperature, hold temperature and hold time. Check that the correct sensitivity cartridge ('head') is used. For sweet potato, cassava, yam and cooking banana analyses, the following settings should be used:

Start temperature:	50 °C	Hold temperature:	95 °C
Hold time:	20 min	Sensitivity head:	700 cmg
Rate of temperature increase:	1.5 °C/min	Digit switch:	set to 2

Prepare a 5% suspension of the sample as follows:

W = weight of sample required = [22.5/(100–% m.c.)] × 100, in 450 ml suspension (g);
weight of distilled water required (g) = 450–W.

Weigh the sample into a 250 ml beaker. Pour about 150 ml of the distilled water onto the sample and mix well. Pour into the viscograph bowl. Use the remaining water to rinse out the beaker into the viscograph bowl. Attach the measuring sensor and lower into the bowl. Lower the cooling probe down into the suspension.

Remove the protective cap from the recording pen. Switch on the viscograph and check that the r.p.m. setting is 75. The suspension will now be heated until it reaches 50 °C. When the start temperature is attained, flick the marker pen to mark the start of the run on the recording chart, and press the start switch on the Brabender control unit. The suspension will now heat up to the hold temperature at a constant rate of 1.5 °C/min and remain at the hold temperature for 20 min.

Once the suspension has cooled down to 50 °C, leave the instrument running for a further 20 min to measure the potential for set back (retrogradation) of the paste on cooling. Turn off the viscograph, lift the cooling probe, detach the measuring sensor and remove the bowl. Replace the protective cap on the marker pen, and turn off the mains water supply.

Clean the bowl and measuring sensor using hot water and a soft brush. DO NOT USE DETERGENT OR ANY FORM OF ABRASIVE ON THESE ITEMS.

Analysis of results

To determine the pasting characteristics of a sample, viscosity values (in Brabender units) are read from six significant points on the visco-amylogram produced by the Brabender Visco-Amylograph. These points are:

1. pasting temperature; initiation of paste formation;
2. peak viscosity and temperature at which peak viscosity is attained;

3. viscosity at 95 °C;
4. viscosity after 20 min at 95 °C; indicates paste stability;
5. viscosity at 50 °C; measures set back on cooling;
6. viscosity after 20 min at 50 °C; indicates stability of paste.

Interpretation of results

The pasting temperature is the temperature at which irreversible swelling of the starch granules occurs leading to the formation of a viscous paste in aqueous solution. Starches with lower pasting temperatures are generally considered to be easier to cook. However, lower pasting temperatures are also associated with low paste stability, which is usually considered to be an undesirable property. Low pasting temperature and low paste stabilty indicate that fewer associative forces and cross-links are present within the starch granule.

The peak viscosity value and viscosity at 95 °C are measures of the ability of the starch to form a paste during cooking. The higher the value the thicker the paste will be. Paste stability is determined by subtracting the viscosity value after 20 min at 95 °C from the value for paste viscosity at 95 °C. Paste stabilty gives a measure of the tendency of the paste to break down during cooking. High paste stabilty is frequently a requirement for industrial users of starch.

The viscosity value at 50 °C gives a measure of the set-back or retrogradation of the starch paste on cooling. Retrogradation is caused as crosslinks re-form between starch molecules within starch granules during cooling. A high setback value is useful if the starch is to be used for preparation of products such as foofoo that need to have a high viscosity and paste stability at lower temperatures. The paste stability at 50 °C is determined by subtracting the viscosity value after 20 min at 50 °C from the initial value for paste viscosity at 50 °C.

References

MAZURS, E. G., SCHOCH, T. J. and KITE, F. E. (1957) Graphical analysis of the Brabender viscosity curves of various starches. *Cereal Chemistry*, **34**: 141–152.

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