



# EUBerry

FP7-KBBE-2010-4 265942

**The sustainable improvement of European berry production, quality and nutritional value in a changing environment: Strawberries, Currants, Blackberries, Blueberries and Raspberries**

**Work Package No.3**

## **FRUIT QUALITY CHARACTERIZATION AND DETERMINATION**

**P10: Geisenheim Research Center**



## **METHODOLOGIES**

**For fruit external quality, sensory evaluation and nutritional analysis**

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## *External Fruit Quality*

### Determination of External Fruit Quality - Strawberry

#### 1. Fruit Weight

##### Average fruit weight

- Average fruit weight at each picking date \_\_\_\_\_ g/fruit  
*(sample size: a minimum of 20 fruits (first quality))*

##### Balanced average weight

- Average over the entire fruiting season: \_\_\_\_\_ g/fruit

#### 2. Visual Aspects of the Fruit

##### Predominant fruit color

Intensity \_\_\_\_\_ by means of Ctifl-color-chart

Homogeneity (within a basket of fruits (500 g)):

3 = the colour is heterogeneous

5 = the colour is acceptably heterogeneous

7 = the colour is homogeneous

##### Brightness

3 = low

5 = medium

7 = high

(if necessary, notes 1 and 9 could be used additionally)

##### Predominant fruit shape



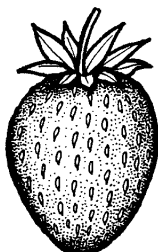
1 - oblate



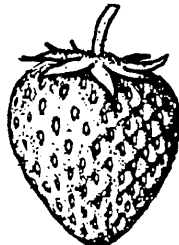
2 - globose



3 - globose conic



4 - ovoid



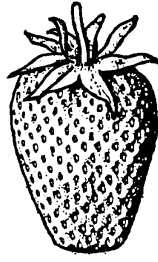
5 - cordiform



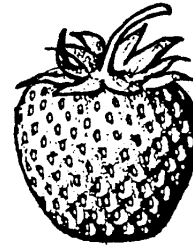
6 - long conic



7 - necked



8 - long wedge



9 - short wedge

#### Fruit homogeneity in the bin

3 = the fruits have different shapes  
 5 = the homogeneity is rather good  
 7 = the fruits in the basket are homogeneous  
 (if necessary, notes 1 and 9 could be used additionally)

#### Internal fruit colour

3 = pale red  
 5 = medium red  
 7 = dark red  
 (if necessary, notes 1 and 9 could be used additionally)

#### Uniformity of internal colour

3 = low  
 5 = medium  
 7 = high  
 (if necessary, notes 1 and 9 could be used additionally)

#### Size of internal cavity

3 = little  
 5 = medium  
 7 = big  
 (if necessary, notes 1 and 9 could be used additionally)

#### Achene position

3 = embedded  
 5 = at level  
 7 = prominent  
 (if necessary, notes 1 and 9 could be used additionally)

### **3. Flesh Firmness and Skin Resistance**

#### Flesh firmness

3 = soft  
 5 = medium  
 7 = firm  
 (if, necessary, notes 1 and 9 could be used additionally)

*flesh firmness is rated when eating the fruit (mouth feeling)*

#### Skin resistance

3 = low  
 5 = medium  
 7 = high  
 (if, necessary notes 1 and 9 could be used additionally)

*Skin resistance has to be evaluated by smoothly rubbing the fruit skin with the finger and rating the damage/wetness of the skin*

**The above mentioned fruit attributes have to be evaluated twice:**

- at the beginning of harvest
- in the middle of harvest

#### **4. Shelf-life Ability**

The conditions to evaluate the shelf-life ability of the fruits should be as follows:

- sample size: 500 g
- cold storage : 4-5 °C during 72 h
- additional storage at room temperature (20-25 °C) for 24 h

##### **To be evaluated after the mentioned storage conditions**

Brightness                      3 = low  
   5 = medium  
   7 = high  
   (if necessary, notes 1 and 9 additionally)

Resistance to bruising        3 = low  
   5 = medium  
   7 = high  
   (if necessary, notes 1 and 9 could be used additionally)

##### Predominant fruit colour

Intensity                              by means of Ctifl-color-chart

Calyx freshness                3 = low  
   5 = medium  
   7 = high  
   (if necessary, notes 1 and 9 could be used additionally)

Resistance to fruit rot         3 = low  
   5 = medium  
   7 = high  
   (if necessary, notes 1 and 9 could be used additionally)

**The above mentioned fruit parameters after storage have to be evaluated twice:**

- at the beginning of harvest
- in the middle of harvest

#### **5. Literature**

European network for strawberry cultivar evaluation, COST 836

## Determination of External Fruit Quality - Raspberry

### 1. Fruit Weight

#### Average fruit weight

- Average fruit weight at each picking date \_\_\_\_\_ g/fruit  
*(sample size: 50 fruits randomly selected)*

#### Balanced average weight

- Average over the entire fruiting season: \_\_\_\_\_ g/fruit

### 2. Visual Aspects of the Fruit

#### Fruit colour

Visual assessment	yellow	= 1,
	orange	= 2
	light red	= 3
	red	= 4
	dark red	= 5
	purpur	= 6
	black purpur	= 7
	black	= 9

#### For red cultivars only

Intensity of the colour

3 = the colour is light  
 5 = the colour medium  
 7 = the colour dark  
 (if necessary, notes 1 and 9 could be used additionally)

#### Homogeneity of the colour

(within a basket of fruits (500 g))

3 = the colour is heterogeneous  
 5 = the colour is acceptably heterogeneous  
 7 = the colour is homogeneous  
 (if necessary, notes 1 and 9 could be used additionally)

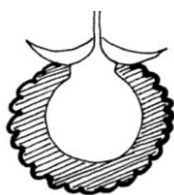
#### Brightness

3 = low  
 5 = medium  
 7 = high  
 (if necessary, notes 1 and 9 could be used additionally)

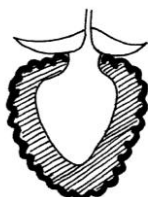
#### Bloom

1 = absent  
 3 = low  
 5 = medium  
 7 = high  
 (if necessary, note 9 could be used additionally)

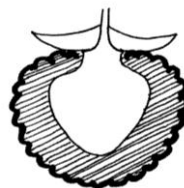
### Predominant fruit shape



1 - globose



3 - conic



2 - cordiform



4 - wedged

### Fruit shape homogeneity in the bin

3 = the fruits have different shapes  
5 = the homogeneity is rather good  
7 = the fruits in the basket are homogeneous  
(if necessary, notes 1 and 9 could be used additionally)

### Size of the druplet

3 = small  
5 = medium  
7 = big  
(if necessary, notes 1 and 9 could be used additionally)

### Crumbliness of the berries

3 = little  
5 = medium  
7 = severe  
(if necessary, note 9 could be used additionally)

## **3. Flesh Firmness and Skin Resistance**

### Firmness

3 = soft  
5 = medium  
7 = firm  
(if, necessary, notes 1 and 9 could be used additionally)

*Firmness is rated by hand*

## **4. Shelf-life Ability**

The conditions to evaluate the shelf-life ability of the fruits should be as follows:

- sample size: 500 g
- cold storage : 4-5 °C during 72 h
- additional storage at room temperature (20-25 °C) for 24 h

**To be evaluated after the mentioned storage conditions**

Brightness

3 = low  
5 = medium  
7 = high  
(if necessary, notes 1 and 9 additionally)

Resistance to bruising

3 = low  
5 = medium  
7 = high  
(if necessary, notes 1 and 9 could be used additionally)

Predominant fruit colour

Intensity

3 = the colour is light  
5 = the colour medium  
7 = the colour dark  
(if necessary, notes 1 and 9 could be used additionally)

Resistance to fruit rot

3 = low  
5 = medium  
7 = high  
(if necessary, notes 1 and 9 could be used additionally)

**The above mentioned fruit parameters after storage have to be evaluated twice:**

- at the beginning of harvest
- in the middle of harvest

## **5. Literature**

Richtlinie Obstbauliche Leistungsprüfungen

Herausgeber: Verband der Landwirtschaftskammern, Godesberger Allee 142-148, 53175 Bonn, 2003



## Determination of External Fruit Quality – Black currant

### 1. Raceme and Fruit Attributes

#### Weight of raceme

- Average raceme weight \_\_\_\_\_ g/raceme  
(sample size: 30-50 racemes randomly selected)

#### Length of raceme

- Average length of the racemes is given in [cm]
  - maybe the length of the peduncle can be given separately  
(sample size: 30-50 racemes randomly selected)

#### Average number of berry position

- Average number of potential berry position per raceme  
(sample size: 30-50 racemes randomly selected)

#### Average number of berries

- Average number of existing berries per raceme  
(sample size: 30-50 racemes randomly selected)

#### Drop off

- Calculating the degree of drop off; result is given in [%]  

$$\text{Drop off} = 100 - ([\text{number of existing berries} / \text{number of potential berry position}] * 100)$$

#### Fruit weight

- Average fruit weight \_\_\_\_\_ g/fruit  
(sample size: 50 berries randomly selected after removing them from 30 racemes )

#### Fruit size

- Measuring the diameter per fruit; result is given in [mm]  
(sample size: 50 berries randomly selected after removing them from 30 racemes )

#### Predominant Fruit Shape

1 = flat-globose  
2 = globose  
3 = drop-shaped

#### Fruit colour

##### Visual assessment

1 = blue-black  
2 = brown-black  
3 = black

#### Brightness

3 = low  
5 = medium  
7 = high  
(if necessary, notes 1 and 9 additionally)

Skin firmness:

3 = low

5 = medium

7 = high

(if, necessary notes 1 and 9 could be used additionally)

*Skin resistance has to be evaluated preferably sensorial by eating the fruits*

Skin resistance

3 = low

5 = medium

7 = high

(if, necessary notes 1 and 9 could be used additionally)

## **2. Literature**

Richtlinie Obstbauliche Leistungsprüfungen

Herausgeber: Verband der Landwirtschaftskammern, Godesberger Allee 142-148, 53175

Bonn, 2003

## ***Objective and Reproducible Measurements for external Fruit Quality Attributes***

### **Colour measurements - Tristimulus colorimeter**

#### **L\*a\*b\* space**

### **Intention**

Objective measurement of fruit surface colour from strawberry, raspberry, black currant and red currant

### **Material**

#### Equipment

- Spectralphotometer (Minolta 3500d)
  - Target mask 8 mm (strawberry, raspberry)
  - Target mask 3 mm (currants)
- Software Spectra-Magic NX

#### Sample

- Fruit samples (20-30 fruits) of strawberry, raspberry, black currant and red currant

### **Procedure**

#### Setting of the instrument

- Reflection/Transmission: Reflection
- Specular component: SCI
- Measurement Area: MAV (8 mm or 3 mm; depending on specie)
- UV-setting: 100%
- Target mask: (8 mm or 3 mm; depending on specie)

#### Calibration

Zero calibration

White calibration

#### Setting Observer and Illuminant

- Observer: 10 degree

- Primary: D 65
- Secondary: None

## Measuring

- Two sites of the fruits are measured; often the sunny and shady site of the fruit
- Average of both sites are calculated

## Results

Results are given as values of

- $L^*$  (lightness: 0 = black and 100 = white).
- $a^*$  (indicating in the maximum the red ( $+a^*$ ) and in the minimum the green colour ( $-a^*$ ))
- $b^*$  (indicating in the maximum the yellow ( $+b^*$ ) and in the minimum the blue colour ( $-b^*$ ))
- Hue angle (redness) is calculated as  $h = \arctan (b^*/a^*)$
- Chroma = colour saturation is calculated as  $C^* = (a^{*2} + b^{*2})^{0.5}$

## Fruit Firmness Measurements by FirmTech2

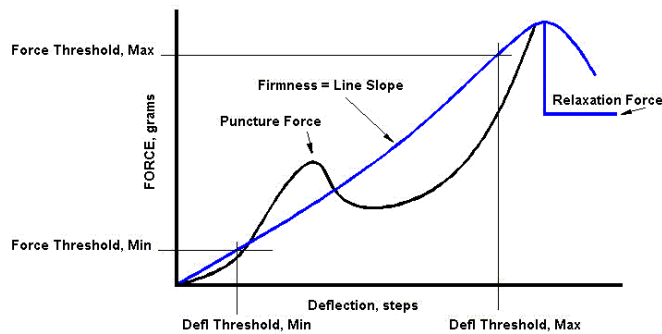
### Intention

Measuring non-destructive the firmness of strawberry, raspberry and currants

### Background

Measuring fruit firmness with FirmTech2 offers different possibilities:

- Compression by **Force Thresholds** firmness is measured by the compression of the fruit in a manner somewhat similar to squeezing it between the fingers. This type of measurement is the most common for soft fruits. A graphical representation of this is shown in the figure below which also shows the threshold measurement parameters. The slope of the line between the Minimum and Maximum **Force Thresholds** is defined as firmness. This line is calculated using linear regression of the data points. Firmness units are grams/mm. A firmness value of 250 grams/mm indicates that 250 grams of force would deflect or squeeze the fruit 1 mm. A higher instrument firmness value indicates greater fruit firmness.
- Compression by **Deflection Thresholds** firmness is measured similarly to that using Force Thresholds except the thresholds are based on Deflection. The slope of the line between the Minimum and Maximum Deflection Thresholds is defined as firmness. This procedure works well for fruit with display a great range of firmness such as blackberries.
- **Puncture** firmness measures the force required to rupture the skin or flesh of a fruit using a special rupture or puncture type probe. This procedure requires a puncture type probe that depends on the type of fruit you are testing. The probe is pressed into the fruit similar to the compression test up to the maximum force. Rupture force is extracted from the data and reported.
- **Relaxation** measurements combine Compression (Force Thresholds) measurement with an additional measured value that indicates the fruits resistance after a specified time (Time Delay). Two values are reported, the normal compression firmness and the relaxation force after the time delay.
- **Size measurement (optional in the Compression Force Threshold modus only)**  
Size measurement can be done at the same time as measuring firmness



Manual, Bioworks

## Material

### Equipment

- FirmTech2, Bioworks USA
- FirmTech 2 Software, Bioworks USA
- calibration weight (250g)
- Load cell (different Load cells are available)



- 
- Type 1      Type 2      Type 3 (puncture)
- Reference size
- turntable
  - for strawberries with 12 depths (indentures)
  - for raspberry, black currant and red currant with 25 depths (indentures);  
(the indentures help to hold the fruit during rotation and testing)

### Sample

- Fruit samples (30 fruits of uniform size) of strawberry, raspberry, black currant and red currant

## Procedure

### Setting of the instrument

- Select the right turntable

- Open program Firmtech Inst300 (general fruit testing: size and firmness)

#### Calibration

- Adjustment of the load cell height position at top (retract)
- For calibration follow the Manual

#### Select Load cell

- Type 2

#### Configuration (Inst300.exe)

- The last configuration saved will be automatically reloaded at startup
  - **Check the configuration!**
  - Force Treshold  
150 as maximum; 75 as minimum (for strawberry and raspberry)
  - 180 as maximum 50 as minimum for black and red currants
  - Sample Size: 30
  - Table positions: means the number of physical positions on the turntable; select 12 or 25, depending on turntable type/specie
- Load cell height  
The maximum extension of the linear stepper motor is 0.9 inches.
  - The stepper motor platform should be positioned so that the maximum extension of the load plate leaves a little over half of the average fruit diameter between the turntable and load plate. This will prevent the load plate from coming in contact with the turntable and generally limit the force if the load cell happens to malfunction or the calibration sequence was incorrect.

#### Measuring the Reference size:

### **Results**

Fruit firmness is given in [g/mm]

## **Fruit Firmness Measurements by Bareiss HPE FFF**

### **Intention**

Measuring the fruit firmness of strawberry

The Bareis HPE FFF instrument is said to be identical with the former Durofel DFT 100 (now the AGROSTA<sup>®</sup>100 ). It gives equal results like the Agrosta 100 when measuring time is fixed to 1 sec (). It is a portable penetrometer and destroys strawberries surface during measurements.

### **Material**

#### Equipment

- Bareiss HPE FFF instrument, 89610 Oberdischingen, Germany
- Plunger 0.50 cm<sup>2</sup> for strawberry

#### Fruit Sample

- 30 strawberry fruits of uniform size

#### Calibration

- According to the Manual

### **Procedure**

- Two sites of the fruits are measured by gently pressing the plunger on the fruit till the plunger disappears in the fruit ; often the sunny and shady site of the fruit are measured
- Average of both sites are calculated

### **Results**

Fruit firmness is given in Bareiss units (Durofel units)



## *Sensory Assessment*

### **Descriptive Sensory Assessment of Strawberry**

#### **Taste**

The attributes sugar, acidity, taste, flesh firmness and juicy are evaluated according to the following ratings:

- 1 = very weak
- 3 = weak
- 5 = medium
- 7 = high
- 9 = very high

The above mentioned fruit attributes have to be evaluated twice:

- at the beginning of harvest
- in the middle of harvest

---

#### **Ranking Test**

Additionally, **Ranking Tests** are performed sometimes.

- Simultaneous presentation of at least samples in random order, which then have to be arranged according to specified criteria (e.g. product properties, like/dislike)

## Descriptive Sensory Assessment of Raspberry

### Taste

The taste attributes sugar, acidity, unripe/green, fruity, juicy, untypical/strange taste, flesh firmness and general popularity are evaluated according to the following ratings:

1 = very weak

3 = weak

5 = medium

7 = high

9 = very high

The sugar : acid ratio was assessed according the following rating

1 = very acid

3 = acid

5 = well-balanced

7 = sweet

9 = very sweet

---

### Ranking Test

Additionally, **Ranking Tests** are performed sometimes.

- Simultaneous presentation of at least samples in random order, which then have to be arranged according to specified criteria (e.g. product properties, like/dislike)

## **Descriptive Sensory Assessment of Black Currant**

### **Odour**

The odour attributes intense odour, typical, untypical/strange, raspy, vegetative/green, flowery, fruity are evaluated according to the following ratings

- 1 = weak
- 2 = medium
- 3 = high

### **Taste**

The taste attributes typical, untypical/strange, astringent, aromatic, acid, bitter, fruity and mild are evaluated according to the following ratings:

- 1 = very weak
- 3 = weak
- 5 = medium
- 7 = high
- 9 = very high

In addition, the acceptance for fresh consumption was asked, giving yes or no as an answer.

## *Nutritional and other Internal Fruit Quality Analyses*

### **Extraction Method for Bioactive Compounds in Strawberry**

- 5 g of fruit is weighted and used for the extraction
- The extraction takes place by a solution of methanol and water (80% v/v) added to the pieces of strawberry in ratio of 1:5 (1 fruit:5 extraction phase, 5 g in 25 mL extraction phase):
  - **Double extraction** (twice with 10 mL and 7.5 mL of methanol)
  - Successively homogenization of the mixture has to be placed in continue agitation (or ultrasound assisted) along half an hour for two times. The extraction has to be in dark (cover the falcon tube with aluminum foil).
  - Separate the solid phase from the liquid phase by centrifugation at 4500g for 10 min.
  - Recover the supernatant and stock it in a falcon tube by a glass pasteur pipette for the TEAC and TPC analysis.
  - The vials have to be stored in a freezer at -20°C.
- **The determination of Anthocyanin content has to take place immediately after extraction.**

## Determination of Antioxidant Capacity (TEAC – Decolorization)

### Intention

The pre-formed blue/green radical of  $\text{ABTS}^{\bullet+}$  is generated by oxidation of ABTS with potassium persulfate. The radical cation has an absorption maximum at 734 nm. It is reduced in the presence of such hydrogen-donating antioxidants. The decolorization of the  $\text{ABTS}^{\bullet+}$  radical is determined as a function of concentration and calculated relative to the reactivity of Trolox, a water-soluble vitamin E analogue, as a standard under the same condition.

### Material

#### Equipment

- Photometer
- Cuvette 1 cm
- Stopwatch
- Ultrasonic bath/ Shaker

#### Chemicals

- ABTS (2,2'-azinobis
- Trolox (6-hydroxy-2,5,7,8 tetramethylchroman-2-carboxylic acid)
- Potassium persulfate (di-potassium peroxodisulfate)
- Dipotassium hydrogen phosphate
- Potassium dihydrogen phosphate
- Ethanol
- Phosphate buffered saline (**PBS**, 5mM, pH 7.2 – 7.4)  
7.14 g (41 mmol/L) of dipotassium hydrogen phosphate ( $\text{K}_2\text{HPO}_4$ ) and 1.23 g (9 mmol/L) Potassium dihydrogen phosphate ( $\text{KH}_2\text{PO}_4$ ) filled up with water to 1 L.
- ABTS stock solution  
77 mg ABTS are solvated in a 20 mL volumetric flask with a few ml PBS. 13 mg Potassium persulfate is weighed in a beaker and equally solvated with PBS (Ultrasound assisted!). Afterwards it is added to the ABTS. The flask is filled up with PBS to its mark. Before use it is necessary that the mixture has to be stand in dark

(aluminium foil) at room temperature for 12 to 16 hours (at night). The solution is stable in the dark for five days.

- **ABTS working solution**

The ABTS stock solution has to be diluted with PBS, then filtered with a paper filter, to an absorbance of 0.7 – 0.8. (1:50 to 1:70).

- **Trolox stock solution**

32 mg Trolox is weighed in a 50 ml volumetric flask and solvated with a few ml ethanol and filled up with PBS to its mark (2.5mM).

## **Procedure**

### Sample preparation

See extraction methodology. Supernatant is diluted 1:20 (100µL sample: 2000µL).

### Measuring

Only ten samples (repeat determination; all in all five samples) and one standard should be measured at once.

At first 1900 µL of ABTS working solution is pipetted in the cuvette. The reaction starts after addition of the sample solution respectively blank or standard (100 µL) and should be mixed immediately. The absorbance of the sample is measured after 6 minutes at 734 nm.

	Sample	Blank
ABTS Working Solution	1900 µL	1900 µL
PBS	-	100 µL
Sample solution	100 µL	-
total	2000 µL	2000 µL

### Calibration

The Trolox stock solution is diluted with PBS so that the final concentration of the dilution series ranges from 0.025 to 0.450 mmol/L. The Trolox solutions are measured like described above.

### Calculation

To obtain percentage of inhibition:

$$\%inhibition = \frac{Abs_{blank} - Abs_{sample/standard}}{Abs_{blank}} \times 100\%$$

The calibration is calculated by linear regression ( $\Delta A = ac + b$ ,  $c$  = concentration trolox mmol/l,  $\Delta A$  = %inhibition,  $a$  = %slope,  $b$  = %intercept).

$$TEAC - Value (mg Trolox eq / kg Fruit) = \frac{(\Delta A - b) \times F}{a \times E}$$

$\Delta A$  = %inhibition

$a$  = slope

$b$  = intercept

$F$  = Dilution factor (20)

$E$  = sample weight [kg/L extracting agent]

## Results

Declaration of TEAC-Value is given as [mmol Trolox equivalent/ kg] fruit with one decimal accuracy.

Attention: The TEAC-Value comprehends the antioxidative capacity of ascorbic acid.

## Literature

Miller NJ, Rice-Evans C, Davis MJ (1993): A novel method for measuring antioxidant capacity and its application to monitoring the antioxidant status in premature neonates, clin. Sci. 84, 407-412

Re R, Pellegrini N, Proteggente A, Pannala A, Yang M, Rice-Evans C (1999): Antioxidant activity applying an improved ABTS Radical Cation decolorization assay, Free Radical Biology & Medicine, vol. 26, No. 9/10, 1231-1237

## Determination of Total Phenolics by Folin-Ciocalteu Method

### Intention

The Total phenolics assay does not only determine phenolics but also reducing agents like ascorbic acid because the basic mechanism is an oxidation/reduction reaction. The exact chemical nature is not known but it is believed to contain heteropolphospho-tunstates molybdates. Molybdenum seems to be easier reduced in the complex. An electron-transfer reaction occurs between reductants and Mo(VI) under alkaline conditions which results as blue colour with an absorbance maxima about 720 nm.

### Material

#### Equipment

- Spectrophotometer
- Cuvette 1 cm
- Stopwatch

#### Chemicals

- Folin-Ciocalteu-Reagent
- Sodium carbonate
- Gallic Acid
- Sodium carbonate solution 20%  
200 g sodium carbonate is filled up with water to 1 L.
- Stock solution: 1000 Gallic Acid mg /L  
200mg Gallic Acid is solubilized in a few drops of Methanol and filled up with water to 200 mL.
- Standard: Gallic Acid  
The Gallic Acid stock solution is diluted with water so that the final concentration of the dilution series ranges from 10 to 50 mg Gallic Acid/L (0.1ml; 0.2ml; 0.3ml; 0.4ml; 0.5ml in 10ml volumetric flask)

### Procedure

#### Sample preparation

See extraction methodology. Supernatant is diluted 1:20 (100µL sample : 2000µL).



Measuring

A test tube (glas) is filled with 7.0 ml water. Afterwards 1 mL of the diluted sample (only water is used for the blank measurement) is added which is followed by 500 µL Folin-Ciocalteu-Reagent and vortexed. After 3 minutes 1.5 mL sodium carbonate is added and the tube is mixed one more time. The absorbance of the sample is measured after exactly 60 minutes at 760 nm.

Calibration

The Gallic Acid standards are measured like described above.

The calibration has to be repeated when a new Folin-Ciocalteu reagent is used.

Calculation

The calibration is calculated by linear regression ( $\Delta A = ac + b$ ,  $c$  = concentration Gallic Acid mg/l,  $\Delta A$  = absorbance,  $a$  = slope,  $b$  = intercept).

$$TP(\text{mg Gallic Acid eq / kg Fruit}) = \frac{(\Delta A - b) \times F}{a \times E}$$

$\Delta A$  =  $A_{\text{sample/standard}}$

$a$  = slope

$b$  = intercept

$F$  = Dilution factor (20)

$E$  = sample weight [kg/L extracting agent]

**Results**

Declaration of TP is given as [mg Gallic Acid equivalent/ kg] fruit without decimal place.

## **Determination of Total Anthocyanins in Strawberries** (by pH shift method)

### **Intention**

Anthocyanin pigments change hue and intensity with pH. At pH 1.0, anthocyanins exist in the colored oxonium or flavylum form and at pH 4.5 they are predominantly in the colorless carbinol form. An aliquot of an aqueous anthocyanin solution is adjusted to pH 1.0 and another aliquot to pH 4.5. The difference in absorbance is proportional to the anthocyanin content. Determination of anthocyanin content is based on Lambert-Beer's Law. Molar absorbance values for purified pigments taken from literature are used, making it unnecessary to determine them. Pelargonidin-3-glucoside is the major anthocyanin in Strawberry, so the total anthocyanin content is calculated as pelargonidin-3-glucoside.

### **Material**

#### Equipment

- Spectrophotometer
- Cuvette 1 cm
- Volumetric flasks

#### Chemicals

- Potassium chloride (KCl)
- Sodium acetate (NaAc)
- Hydrochloric acid (HCL)
- Acetic acid
- Buffer pH 1 (potassium chloride (M= 74.55 g/mol) solution)

A solution of 0,025 mol/L potassium chloride is produced. (1.86 KCl g/L) and adjusted to pH 1 with hydrochloric acid.

- Buffer pH 4.5 (sodium acetate (M= 82.03 g/mol) solution)

A solution of 0.4 mol/L sodium acetate is produced (32.81 NaAc g/L) and adjusted to pH 4.5 with acetic acid

### **Procedure**

#### Sample preparation

See Extraction methodologies as TEAC and TPC.

### Measurement

The supernatant is diluted 1:10 with each buffer solution. The absorbance maximum is determined (about 500 nm, depending on fruit variety). Each dilution is measured at the absorbance maximum and 700 nm. The spectrophotometer is zeroed with distilled water.

**Notice:** Dilute sample further if absorbance is greater than 1.0 AU.

### Calculation

Calculation of anthocyanins as Pg-3-glu/kg fresh weight (FW)

$$mg \text{ Pel - 3 - glu } / kg \text{ FW} = \frac{[(A_{\lambda_{\max}} - A_{700})_{pH1} - (A_{\lambda_{\max}} - A_{700})_{pH4,5}] \times MW \times F \times 1000}{\epsilon \times d \times E}$$

A = absorbance [-]

MW = molecular weight of pelargonidin-3-glucosid = 433.2 [g/mol]

F = dilution factor [-] = 10

d = cell pathlengths [cm]

$\epsilon$  = molar absorbance of Pel-3-glu = 15600 [ $\frac{L}{mol \times cm}$ ]

E = sample weight [kg/L extracting agent]

1000 = Factor for mg

### **Results**

Declaration of anthocyanins is given as Pel-3-gl [mg/kg FW] fruit.

### **Literature**

Giusti M. and Wrolstad R. E., Characterization and Measurement of anthocyanins by UV-visible Spectroscopy, Current Protocols in Food Analytical Chemistry, F1.2.1-F1.2.13 (2001)

## Determination of Ascorbic Acid in Strawberries

### Intention

Titrimetric determination of ascorbic acid in strawberries.

Ascorbic acid is stable in oxalic acid. The amount of ascorbic acid is determined in the oxalic acid extract of the fruits by the titration with iodide - iodate - solution.

### Material

#### Equipment

- 100 mL beaker
- 250 mL beaker
- 10 mL burette
- 50 mL Erlenmeyer flask without grinding (Beuta)
- 50 mL graduated cylinder
- 100 mL volumetric flask
- Hand blender
- 1 mL single volume pipette
- balance
- centrifuge
- centrifuge tips

#### Chemicals

- oxalic acid
- L (+) – ascorbic acid p.a.
- iodide – iodate – solution,  $c(I_2) = 1/128$  Molarity
- Oxalic acid solution, 2%ig  
40g of oxalic acid (56 g oxalic acid x will be weighed in a 250 mL beaker, transfer with water into a 2 L volumetric flask and filled up with water to its mark.
- Ascorbic acid standard  
About 100 mg ascorbic acid will be weighed accurately to 0,1 mg in a 100 mL volumetric flask and filled up with oxalic acid – solution to its mark.

### Sample extraction

- extraction takes place by a solution of oxalic acid 2% added to the pieces of strawberry in ratio of 1:5 (1 fruit:5 extraction phase, 10 g in 50 mL):
  - **Double extraction** (twice with 20 mL of oxalic acid)
  - Successively homogenization the mixture has to rest for 5 min in dark for both extraction.
  - Then it needs to separate the solid phase from the liquid phase by centrifugation at 4500g for 15 min.
  - At this point we have to recover the supernatant with help of a sieve and stock it in falcon tube.

**The ascorbic acid standard has to be prepared each time freshly.**

Iodide – iodate – solution,  $c(I_2) = 1/128$

Using Titrisol® solution, it has to be filled up with water to **2 L**.

If using Iodide – iodate – solution it has to be diluted with water in a 2 L volumetric flask at a ratio of 1:1.

**The iodide – iodate – solution has to be stored darkly.**

### **Procedure**

#### Determination of the Iodide – iodate – solution titre

1.0 mL of the ascorbic acid solution will be pipette into a 50 mL Erlenmeyer flask and mixed with 20 mL oxalic acid solution. After addition of some drops starch indicator, it has to be titrated with Iodide – iodate – solution to a permanent blue colour.

The titration has to be done quickly because of the susceptibility of oxidation of the ascorbic acid.

The determination of the titre has to be repeated daily with a freshly prepared ascorbic acid solution.

For blind testing 1.0 mL water will be used.

#### Sample preparation

About 250 g strawberries will be weighed exactly into a 2 L beaker and have to be completed with oxalic acid – solution to 1 kg. Then crush the fruits with a hand blender, transfer the mash in centrifuge tubes and centrifuge for 5 minutes at 10 000 U / min.

### Titration

20 mL supernatant is titrated with iodide-iodate-solution after adding the starch indicator.

### Calculation

$$mg \text{ ascorbic acid} / kg \text{ FW} = \frac{c \times MW \times V}{v \times E}$$

c = concentration of Iodide-iodate-solution [mol/L]

MW = molecular weight ascorbic acid 176.13 = [g/mol]

V = volume used Iodide – iodate [mL]

v = volume extract

E = sample weight [kg/L extract]

### **Results**

Declaration of ascorbic acid is given as [g / kg] fruit.

### **Literature**

R. Matissek, F. Schnepel, G. Steiner; Lebensmittelanalytik; Springer – Verlag; Berlin Heidelberg; 1989

## **Titrateable Total Acid in Strawberries**

### **Intention**

This method is used for the determination of titrateable total acid in strawberries.

The sample has to be titrated potentiometrically with 0.1 N NaOH (sodium hydroxide) to pH 8.1

### **Material**

#### Equipment

- 100 mL beaker (high size)
- 2 L beaker
- 1 L volumetric flask
- balance
- hand blender
- pH - measuring instrument
- single-rod measuring cell (storage in 3 mol/L potassium chloride – solution)
- magnetic stirrer
- 50 mL burette

#### Chemicals

- Water (aqua dest)
- 3 mol/L potassium chloride (KCl)
- buffer solutions for calibration the pH measuring instrument at pH 4,00 and 7,00
- 0.1 n sodium hydroxide (NaOH)

### **Procedure**

#### Calibration

Calibration of the pH measuring instrument is carried out with two buffer solutions with different but exact pH-values (two-point-calibration). The buffers have to be stirred during calibration.

### Sample Preparation

Approx. 10 g of mash strawberries are weighed exactly into a beaker and the weight is supplemented to 10 ml with water.

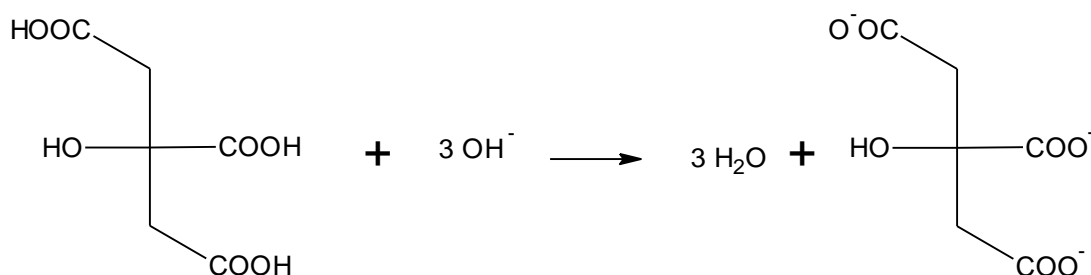
### Measuring

An aliquot of about 10 g strawberry mash, produced as described above is given into a 100 mL beaker (high size), weighed exactly and filled up to 10 mL with aqua dest. After immersion the single-rod measuring cell the sample has to be titrated with 0.1 N NaOH to pH 8.1 during constant stirring.

The addition of the volumetric standard solution has to be slow.

### Calculation

The content of total acidity will be calculated as citric acid at pH 8.1 as follows:



$$w(\text{total acid}) = \frac{V * c * M}{3 * E}$$

with:  $w(\text{total acid})$  = content of total acid calculated as citric acid [g / kg]

$V$  = volume of NaOH – solution [mL]

$c$  = concentration of NaOH-solution [mol/L]

$M$  = molecular weight of citric acid [g / mol] 192,12

$E$  = initial weight of the mash [kg]

## **Results**

Declaration of the total acidity at pH 8.1 is given as [g citric acid / kg].



## Determination of Brix in Strawberries

### Intention

Measuring the mass fraction sucrose (Brix) with a precision refractometer (p.ex. Abbé – refractometer).

### Material

- 1 L beaker
- balance
- hand blender
- Abbé – refractometer

### Procedure

#### Sample preparation

Approx. 250 g strawberries are weighed exactly into a 1 L beaker and crushed with a hand blender.

#### Measuring

For measuring take some drops of the fruit juice resulting from 3.1. For this purpose some drops are decanted over a glass rod into the measuring device. If necessary the mash has to be centrifugated and the supernatant can be used for the measurement.

Reference temperature is 20°C. The sample and the refractometer must have 20 °C. The refractometer has to be cleaned with water between two samples.

### Results

Declaration of °Brix with one decimal point.

°Brix is indicated as [g / 100g].