Carotenoid

ANALYSIS OF BIOFORTIFIED FOODS

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CAROTENOIDS DETERMINATION

Introduction

Carotenoids are natural liposoluble pigments synthesized by plants and some microorganisms. Some of them are precursors of vitamin A. The carotenoids present in animals come from their diet, especially fruits and vegetables. Carotenoids have different functional properties. These are primarily colorants widely used by the food industry, but also functional molecules active in the prevention of certain diseases.

Applicability

This method is used for the determination of carotenoids present in biofortified foods.

Principle of the analysis

Extract and quantify plant carotenoids. The method is based on the extraction of carotenoids with an organic solvent, and the subsequent determination of their concentration by spectrophotometry.

Experimental procedure

1. SAMPLING

The plants to be analyzed must be collected according to certain rules. The samples to be analyzed for carotenoid content can be collected directly from the retail sector or even from the production. The number of samples collected will depend on the cultivated area.

2. SAMPLE PREPARATION

Each sample taken should be thoroughly washed under running water using brushes or sponges to ensure that the cleaning procedure is complete and effective.

Samples should be cleaned, peeled, cut, crushed, homogenized in processors/blenders before being extracted. They must be weighed in duplicate, by removing aliquots of each sample in function of the likely amount of carotenoids present in each matrix according to the table below.

TABLE 1. Sample quantity

Sample	Mass (g)	Volumetric flask (mL)
Carrot	2	100
Sweet potato	2 a 5	50
Cassava	5 a 15	50
Cassava flour	5 a 20	25
Maize	5 a 10	50
Maize flour	2 a 3	50
Tomato	2 a 5	100

In HarvestPlus, we work on the determination of carotenoids in several matrices. The specific procedure for preparing each type of sample studied is described below.

Matrix: Cassava

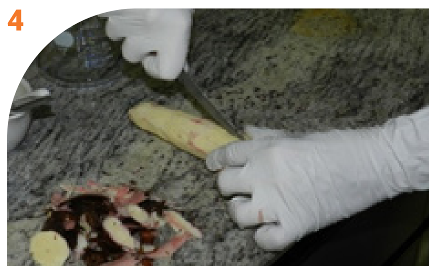
1

2

3

Each sample should be peeled and then cut lengthwise into four equal parts.

4



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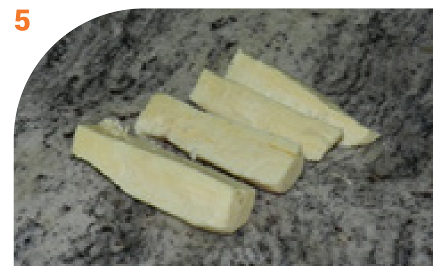

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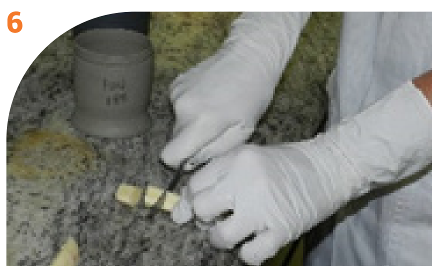

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Choose two parts that are in the opposite position and grind them into a mixer.

Ground samples can be stored in the freezer in sealed pots in the dark.

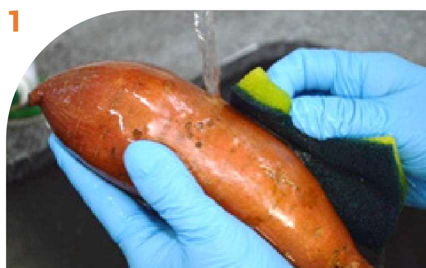
8

9

Matrix: Sweet potato

Biofortified sweet potatoes are thoroughly washed under running water with brushes or sponges to ensure that the cleaning procedure is adequate.

1

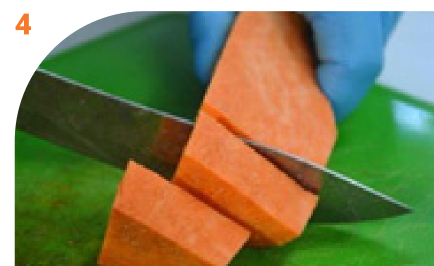


2

3

Each sample must be peeled and cut lengthwise into four equal parts.

4



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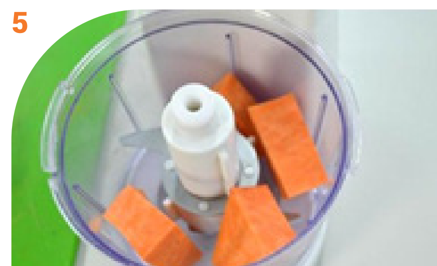

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After cleaning, the samples are peeled, cut and then, using a processor, the sweet potato samples are crushed to obtain a homogeneous mixture.

3. EXTRACTION OF SAMPLES

The samples are weighed using an analytical balance (0.1 mg accuracy) by taking aliquots for analysis having a weight as described in Table 1.

1

2

3

Samples are transferred to a mortar to begin extraction. 3 g of celite and 25 ml of acetone are added and the mixture is homogenized/crushed with a pestle until a paste is obtained.

4

This suspension, which contains the extracted carotenoids, is transferred to a filter plate funnel coupled to a 250 ml vacuum flask for filtration.

5

It is washed at least three times with acetone until the extraction of the sample is complete, i.e. the sample remains colorless.

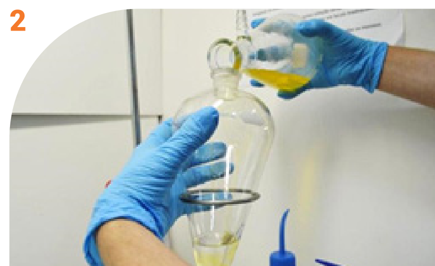
4. OBTAINING THE EXTRACT IN PETROLEUM ETHER

The extract containing the carotenoids (acetone) is transferred to a 500 ml extraction funnel containing about 40 ml of petroleum ether.

1

This step must be carried out so that carotenoids solubilized in acetone can be transferred to another solvent — petroleum ether.

2



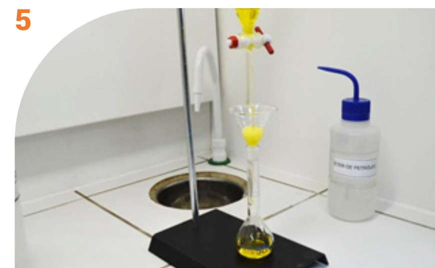
To remove acetone, ultrapure water is slowly added to prevent emulsion formation. The formation of two phases is observed: a) petroleum ether + carotenoids (1 and 2) and b) water + acetone. Phase b is then eliminated. This procedure is repeated up to three times or more.

3

The carotenoid extract in petroleum ether should be transferred to a volumetric flask for quantification. It is preferable to use amber glass to prevent degradation of the molecules.

4

5



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A funnel containing a filter paper with 3 g of anhydrous sodium sulfate is placed on the volumetric flask. All carotenoid extract from the extraction funnel should be transferred to the 50 ml flask by passing it through the sulphate layer.

7

After washing the sulphate and the funnel with the solvent at least four times, the volume of the flask is completed with petroleum ether. Make sure the total volume of ether used does not exceed the capacity of the flask, in this case 50 ml.

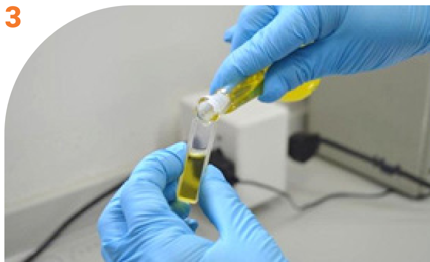
5. SPECTROPHOTOMETRIC QUANTIFICATION

1

The carotenoid content of the extracts must be quantified by spectrophotometry.

2

3



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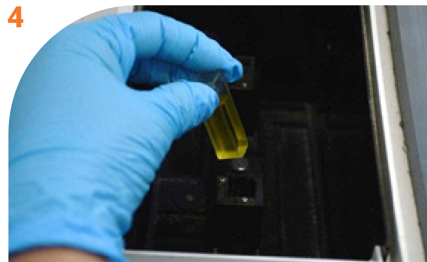


The reading of the sample is performed using a spectrophotometer at a wavelength of 450 nm using petroleum ether as "blank".

To calculate the total carotenoid content, use the following formula:

$$\text{Carotenoids } (\mu g|g) = \frac{A \times V\,(mL) \times 10^4}{A^{1\%}_{1cm} \times P\,(g)}$$

A = absorbance
V = total volume of the extract (ml)
P = weight of the sample (g)
$A^{1\%}_{1cm}$ = 2592 – molar absorptivity coefficient of β-carotene (in the petroleum ether)

RESULTS SHEET

Analyst: Date:

Sample:

Origin:

Observations:

Note: take note of the facts as they occurred.

Quantification by spectrophotometry

Absorbance	Total volume of the extract (ml)	Weight of the sample (g)	Molar absorptivity coefficient

To calculate the total carotenoid content, use the following formula:

$$\text{Carotenoids } (\mu g|g) = \frac{A \times V\,(mL) \times 10^4}{A_{1cm}^{1\%} \times P\,(g)}$$

A = absorbance
V = total volume of the extract (ml)
P = weight of the sample (g)
$A_{1cm}^{1\%}$ = 2592 – molar absorptivity coefficient of β-carotene (in the petroleum ether)

Sample	Result

ANNEX

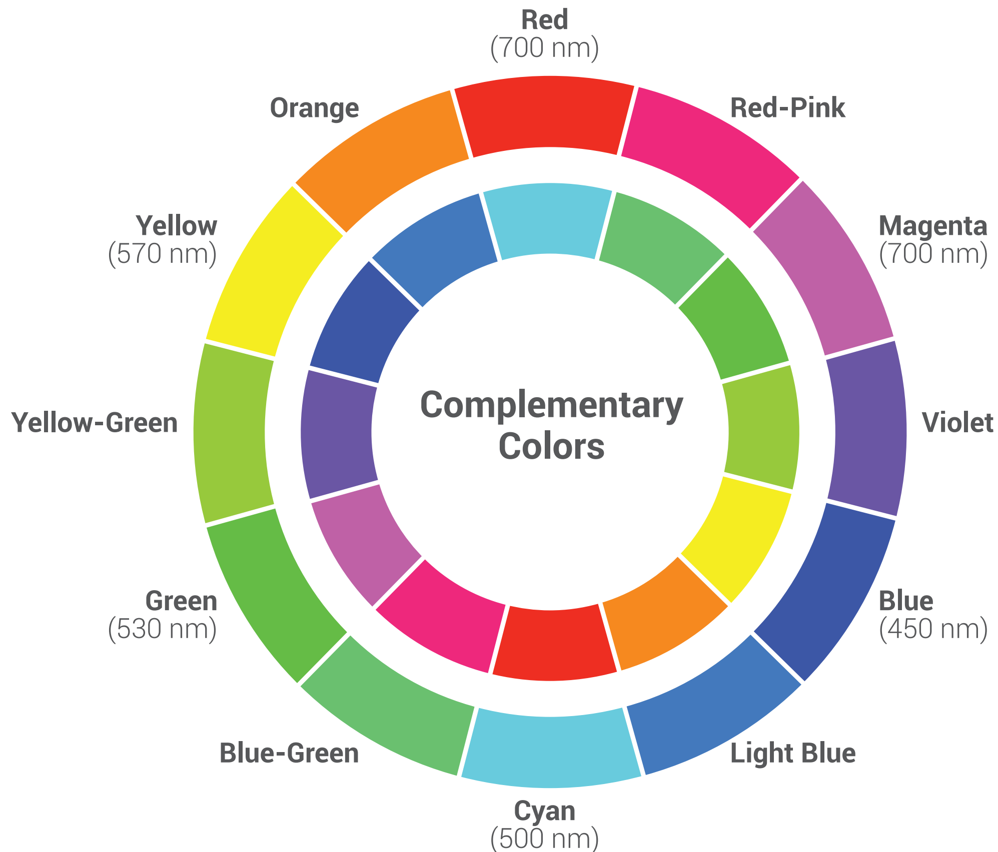
Spectrophotometry

1. LIGHT-MATTER INTERACTION

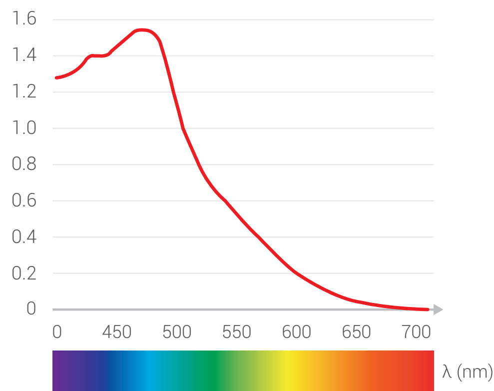
Spectrophotometry is the study of the interaction between matter and radiation. When light passes through a substance, it is partly transmitted and partly absorbed. If a substance absorbs in the visible range (400 nm < λ < 800 nm), then it is colored. If it is illuminated by white light, it will take the color of the radiations that manage to cross, the complementary colors of the absorbed colors.

We can use the chromatic circle showed below which allows to quickly visualize the complementary colors.

The colors are placed in ascending order of wavelength along the circle. Two complementary colors are facing each other.



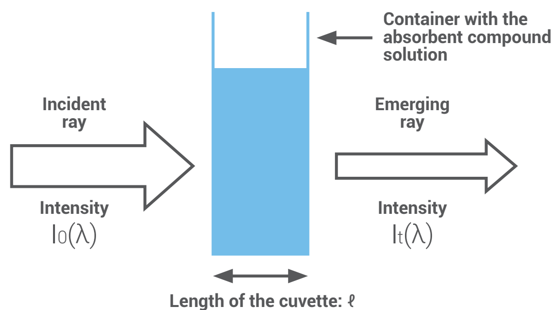
Example: a solution of diiodine I_2 absorbs mainly wavelength radiations between 450 and 500 nm (blue-violet): the solution is then yellow-orange (complementary colors of the absorbed radiations, located opposite in the chromatic circle).



2. BEER-LAMBERT LAW

The spectrophotometry is a quantitative and qualitative analytical method to measure the absorbance or optical density of a chemical substance, usually a solution. The more concentrated the sample, the more it absorbs light, within the proportionality limits set out by the Beer-Lambert law.

Consider an incident monochromatic radiation (of wavelength λ), intensity $I_0(\lambda)$. This radiation passes through a thickness of solution of compound X of concentration **c** which absorbs the light partially. The transmitted intensity is $I_t(\lambda) < I_0(\lambda)$.



Where:

- The solution transmittance:
  $T = \frac{I_t(\lambda)}{I_0(\lambda)}$; $0 < T < 100\%$
- The solution absorbance (or optical density):
  $A = -\log T$ (A, is a dimensionless positive quantity).

For a solution with low concentration of absorbing substance, the following relation, called Beer-Lambert law, is verified:

$$A = \varepsilon(\lambda) \cdot \ell \cdot c$$

A: absorbance
ℓ: length of the cell
c: concentration of the absorbing substance
ε(λ): molar absorptivity coefficient (in function of the nature of the substance, wavelength of the light λ, nature of the solvent and temperature T).

Units

The dimensional analysis shows that the molar absorptivity coefficient has the dimension of a length squared divided by a quantity of material. Therefore, its unit in the SI is $m^2 \cdot mol^{-1}$. But this is not the most common unit:

- ℓ is measured in cm (the cuvette is usually 1 cm)
- c is a concentration in $mol \cdot L^{-1}$
- A is without a unit
- ε(λ) is therefore in $L \cdot mol^{-1} \cdot cm^{-1}$

If the solution contains several absorbing substances, there is an additivity of the optical densities:

$A = \Sigma_i A_i = \Sigma\, \varepsilon_i(\lambda) \cdot \ell \cdot c_i$ therefore $A = \ell \cdot \Sigma_i\, \varepsilon_i(\lambda) \cdot c_i$

ℓ is the length of the cuvette, c_i the concentration of the substance X_i and $\varepsilon_i(\lambda)$ is the molar absorptivity coefficient of the substance X_i.

Limits

The value of A is between 0 y +∞, but experimentally we cannot use all the absorbance values.

For low A values:
The smaller the A values, the more light the detector receives and the smaller the difference between the incident intensity and the transmitted intensity. The values would be

close to the value of the inherent uncertainty of the device and could not be used.

For high A values:
There are two limits when the values of A increase.

- The first is due to the spectrophotometer itself. The higher the A values are, the less light the detector receives. When approaching the detection limit, the detector can no longer distinguish two values close to A. You will see a clipping of the curve, we call it saturation of the device even if the term is not well chosen (it could make believe that the detector receives too much light, but it is the opposite). This limit depends on the device used.
- The second limit is at the level of the Beer-Lambert law. This is no longer valid when the concentration of absorbing species is too high (the molecules no longer behave independently of each other). The limit depends on the absorbing molecules.

Experimentally, with the spectrophotometers of the lab, for reasonable measures we will stay in the following limits:
$0,1 < A < 1$.

3. EXPERIMENTAL PRACTICE

If we set ℓ and c, we obtain the curve A (λ), which is called the absorption spectrum of X.

If we set λ, A must be proportional to c with ℓ constant; we then trace the curve A = f(c) to see if the Beer-Lambert law is confirmed (calibration curve).

For a solution containing only one absorbing species, the measurement of optical density is therefore a non-destructive method of measuring the concentration of the substance of interest. Spectrophotometry can therefore be used in kinetic studies or as an analytical method for determining concentrations.

Choice of working wavelength

To increase the precision and to limit the uncertainty on the measurements, choose the wavelength for which the molar absorptivity coefficient of the substance is maximum.

Note 1: When the absorption maximum leads to an excessively high absorbance (saturation, leaving the range of validity of the Beer-Lambert law), choose another wavelength at a relative maximum of lower absorbance, or at a shoulder of the spectrum.

Note 2: If several colored substances are likely to absorb the incident light, try to set a wavelength for which only one of the species absorbs the light. We can then follow the variation of its concentration, without taking into account other species.

Need of a blank

If only the absorbance of the test compound is to be measured, all the other sources of absorption must be omitted: the walls of the cuvette and the solvent.

Here is the procedure for a fixed λ measurement:

1. Place a measuring vessel (referred to as a reference cuvette) containing only the solvent in the device.
2. Read the absorbance, and calibrate the device to indicate that this absorbance should be zero (this is done automatically with the digital devices you have).
3. Remove the reference cuvette and place the cuvette containing the absorbent substance. Read the measurement. For wavelength scanning, place the reference cuvette for the device to measure its absorbance at each λ. It will subtract the curve obtained from that of the sample.

Use of the cuvette

- Hold the cuvettes by the frosted faces.
- Care must be taken to eliminate the fine air bubbles that may be formed during the filling of the cuvette.
- The cuvette faces that are crossed by the light beam must be carefully wiped with soft paper in order to eliminate all fingerprints.
- If the cuvette is plastic, it should be discarded when it has scratches.

Spectrophotometry requires a lot of precision in the preparation of solutions. It is a very sensitive method.

Quality assurance and quality control

Good practices includes the implementation of quality assurance and quality control (QA/QC) procedures when compiling records.

Quality control (QC) is a system of systematic technical activities designed to measure and control the quality of the documentation during its development. The purpose of a QC system is: (i) to provide systematic and consistent checks to ensure the integrity, accuracy and completeness of the data; (ii) identify and rectify errors and omissions; (iii) Document and archive material documentation and record all QC activities. Quality Control (QC) activities include general methods, such as data acquisition accuracy checks and calculations, and the use of standard procedures. Quality assurance (QA) activities include a planned system of review procedures implemented by individuals who were not directly involved in the compilation or development of the inventory.

Practical considerations for the development of QA/QC systems

In practice, the QA/QC system is only one component of the documentation process, and the agencies responsible for documentation do not have unlimited resources. There is a demand to reconcile the need for quality control, improved accuracy and reduced uncertainty, with the needs for timeliness and cost-effectiveness. A good practice system aims to balance these needs and enable continuous improvement of documentation estimates.

The quality assurance standard expects the laboratory to undertake work in accordance with good professional practices and regulations, which should lead to a secure framework for operators.

Organization

The laboratory must have planned and formalized the precautions taken to ensure the constancy of the equipment used on the site or in its mobile laboratories (transport, storage, use, etc.).

Validation of methods

Explanations of the validation requirements of methods or recommendations on the same subject in particular technical fields may be given in the technical accreditation guides or the documents of specific requirements associated with the involved technical fields.

Accreditation of laboratories

Accreditation of laboratories according to international standards is intended to attest to the competence of laboratories to perform calibrations or tests with specified means. Interlaboratory comparisons are one of the reliable and powerful tools for achieving this goal.

The results of interlaboratory comparisons also allow accreditation bodies such as Cofrac to ensure consistency of test results and benchmarking between laboratories at both national and international levels.

Interlaboratory comparisons

Accredited laboratories must participate in interlaboratory comparisons to demonstrate their competence and ensure the quality of their results. The accredited laboratory must establish a plan for participation in interlaboratory comparisons by determining the representative circuits of all the fields of activity related to its scope of accreditation. Frequency of participation arises from an anlysis of needs that considers, among other things, other quality assurance measures. This plan is reviewed regularly in light of significant changes, such as changes in methods, equipment and personnel.

Interlaboratory comparisons are necessary because they are a tool of progress for laboratories and can be used as one of the elements to demonstrate their competence.

Situations where the laboratory performs the tests but not the sampling

When the laboratory carries out the sampling operation without accreditation, and under accreditation the tests on the sample(s), and reports on a single report the result of the sampling and processing operations, test results may be reported as covered by accreditation only if they contain a restrictive statement that relate only to the samples tested.

As a corollary, in this situation, any notice or declaration of conformity extrapolating the results to the object from which the samples are derived cannot be given under accreditation.

Control of records

The retention period of the records must meet the needs of the laboratory, customers, general management and the public authorities and cannot be less than 18 months.

Quality assurance

Inventory control

- Glassware cleaning
- Inventory control of organic solvents
- Control of the inventory of glassware
- Quality of reagents used:
  1. Characteristics of the product, the solvent used in the extraction process
  2. Certificate or purity % (example: Spectro UVvis)
  3. Purity % of celite
  4. Distilled or ultra-pure water

Documents of records and archive preservation

Samples for the analysis of carotenoids:

- You have to register them and associate a number, a code, a classification.
- Number of samples - a description / origin.
- Write down the description of each sample, i.e., origin, responsible person, date of harvest, weight, etc.
- A technician must be responsible for each activity.

Materials and reagents			
Number	**Item**	**Detail**	**Quantity**
1	Washing material, brushes, foams		
2	Knife		
3	Washer bottle	500 ml	
4	Volumetric pipettes	1, 5, 10 ml	
5	Volumetric matrax	25, 50, 100 ml,	
6	Kitassato (Filtering Erlenmeyer)	250, 2000 ml	
7	Test tube	50 ml	
8	Separation funnel	500 ml	
9	Funnels		
10	Mortar and pistil		
11	Sintered glass funnel		
	Reagents and chemicals		
1	Celite HyFlo Super Cel		
	Equipment		
1	Spectrophotometer		
2	Analytical scale		
3	Ultrapure or distilled water		
4	Vacuum pump		
	Local shopping		
	Reagents and chemicals		
1	Acetone		
2	Petroleum ether		
3	Sodium sulfate anhydrous		

HarvestPlus is a leader in a worldwide initiative to improve nutrition and public health by developing and promoting biofortified food crops that are rich in vitamins and minerals, and providing global leadership on biofortification evidence and technology. HarvestPlus is part of the CGIAR Research Program on Agriculture for Nutrition and Health (A4NH).

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MKTPLACE

CIAT
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Since 1967 Science to cultivate change