PROCEDURES FOR CHEMICAL ANALYSIS of potato and sweetpotato samples at CIP's Quality and Nutrition Laboratory

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PROCEDURES FOR **CHEMICAL ANALYSIS** of **potato** and **sweetpotato** samples at CIP's Quality and Nutrition Laboratory

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International Potato Center (CIP)
Global Program Genetics and Crop Improvement

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Quality and Nutrition Laboratory

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PROCEDURE 1

Analysis of ascorbic acid in fresh potato tubers

1. Sampling and sample preparation

- For each genotype collect 4 to 5 potato tubers per replication. The tubers must be representative of each genotype and replications can be from the field or from laboratory, depending on the interest of the scientist.
- Wash the tubers with plenty of tap water, removing any soil residue; rinse with distilled water and dry with paper towel.
- Cut each tuber in four sections longitudinally; then take two opposite sides of each tuber, and with the help of a stainless steel slicer cut 3–5 slices from each section.
- Cut these slices into small cubes, mix, weigh a portion and analyze immediately for the ascorbic acid concentration, as indicated below.

2. Extraction

- Weigh 7.5 g of the cut and mixed sample directly in the extraction tube.
- Add 38 ml of extracting solution (0.4% oxalic acid and 20% acetone) and homogenize in the Ultra Turrax for 1 minute.
- Rinse the mixer with the extracting solution and also the walls of the glass tube (approximately 3 ml).
- Filter the extract through Whatman # 2 filter paper using vacuum pump, and rinse the walls of the glass tube and filter.
- Transfer the filtered extract into a volumetric flask (protected from light), bring to 50 ml with extracting solution and mix.

3. Determination

- Mix 1 ml of the extracting solution with 9 ml of 2.6 DCIP diluted solution of and after 1 minute read the absorbance (Reagent Blank: RB) at 520 nm.

- Mix 1 ml of the extract (filtered solution of the sample) with 9 ml of 2.6 DCIP diluted solution, and read the absorbance (Sample Absorbance: SAbs) at 520 nm (read twice for each volumetric flask) after 1 minute.
- Mix 1 ml of the extract with 9 ml of distilled water, and read the absorbance (Sample Blank: SB) at 520 nm.
- Subtract the sample blank from the sample absorbance and then subtract this value from the reagent blank. This final value (real absorbance) is used to estimate the concentration of ascorbic acid.

4. Standard curve preparation of ascorbic acid

- Prepare a stock solution of ascorbic acid (1000 µg / ml). For this purpose, weigh 100 mg ascorbic acid in a beaker and dissolve in 50 ml of extracting solution. Transfer the solution to a volumetric flask protected from light and bring to 100 ml with extracting solution.
- Prepare ascorbic acid standard solutions (5, 10, 20, 30, 40 and 50 µg / ml) by taking aliquots of 0.25, 0.5, 1.0, 1.5, 2.0 and 2.5 ml from the stock solution of ascorbic acid and bringing to 50 ml with the extracting solution.
- Mix 1 ml of extracting solution with 9 ml of 2.6 DCIP diluted solution, and read the absorbance (Reagent Blank) after 1 minute at 520 nm.
- Mix 1 ml of each standard solution with 9 ml of the diluted solution of 2.6 DCIP, stir and after 1 minute read the absorbance (standard absorbance) at 520 nm (read twice).
- Then, mix 1 ml of the standard solution with 9 ml of distilled water and after 1 minute read the absorbance (standard blank) to 520 nm.
- Subtract the standard blank from the standard absorbance and then subtract this value from the reagent blank. This final value (real absorbance) is used to build the standard curve: Real absorbance vs. concentration (µg /ml).

5. Preparation of reagents

5.1 Extracting solution: oxalic acid 0.4% and acetone 20%

Weigh 4 g of oxalic acid and dissolve in 100 ml distilled water (stir with the help of a magnet until completely dissolved). Add 500 ml of distilled water and add

200 ml of acetone. Stir slowly and bring to pH 1.1 with concentrated sulfuric acid (to prevent evaporation of acetone, cover the beaker with aluminum foil). Finally, transfer the extracting solution to a volumetric flask and bring to 1 l with distilled water.

5.2 Stock Solution of 2.6 4.2 dichloroindophenol (DCIP)

Weigh 100 mg of 2.6 DCIP and dissolve in 100 ml of warm water (40-50 °C). Add 84 mg of $NaHCO_3$ and stir until all particles are dissolved. Transfer the solution into a volumetric flask and bring to 500 ml with distilled water. Filter with suction and store the solution in an amber glass flask. This preparation must be done avoiding the light.

The stock solution should be diluted with distilled water before using. When 1 ml of the extracting solution and 9 ml of diluted 2,6 DCIP solution are reacted, the absorbance obtained at 520 nm should be between 0.300 and 0.350. This absorbance value is usually obtained when 1 ml of the diluted stock solution of 2.6 DCIP is dissolved in 13 ml of distilled water.

6. References

- Egoaville, M.; Sullivan, J.; Kozempel, M.; Jones, W. 1988. Ascorbic acid determination in processed potatoes. *American Potato Journal*. 65, 91-97.

- Burgos, G.; Auqui, S.; Amoros, W.; Salas, E.; Bonierbale, M. 2009. Ascorbic acid concentration of native Andean potato varieties as affected by environment, cooking and storage. *Journal of Food Composition and Analysis*. 22, 533–538.

PROCEDURE 2
Analysis of total and individual carotenoids in fresh potato tubers

1. Sampling and sample preparation

- For each genotype, collect 4 to 5 potato tubers per replication. The tubers must be representative of each genotype and replications can be from field or laboratory, depending on the scientist's interest.
- Wash the tubers thoroughly with tap water, removing any soil residue; then rinse with distilled water and dry with paper towel.
- Cut each tuber longitudinally into four sections, take two opposite sides of each potato, cut into squares, homogenize in a food processor and analyze immediately.

2. Extraction

- Weigh a portion of homogenized sample in a glass tube. The weight of the sample depends on its coloration (6-8 g for the pale yellow flesh samples; 4-5 g for intermediate yellow flesh samples; and 2-3 g for deep yellow flesh samples).
- Add 40 ml of cold acetone, homogenize in the Ultra-Turrax for 1 minute at 21000 rpm, filter with suction through a sintered glass funnel, and receive the extract in a flask protected from light. Wash the Ultra-Turrax and the residue with a small amount of acetone.
- Repeat the extraction until the residue is devoid of color. Usually only 3 extractions are required.

3. Partitioning to petroleum ether

- Place 30 ml of petroleum ether and a small amount of distilled water in a 500 ml separatory funnel.

- Transfer the acetone extract to the petroleum ether in 4 fractions. After each transference, add 200 ml of distilled water allowing it to fall through the separatory funnel walls. Let the two phases separate, and discard the lower aqueous phase every time.
- Wash 4 times with 200 ml of a dilute solution of sodium chloride (about 1%) to prevent formation of emulsions. Let the two phases separate for 3 minutes and discard the aqueous phase.

4. Saponification

- Collect the transferred ether extract in a 125 ml volumetric flask and add a few crystals of the antioxidant BHT (2,6-di-tert-butyl-4-methylphenol).
- Add 40 ml of 20% potassium hydroxide methanol solution. Cover the flask with aluminum foil and let the mixture stir in the dark at room temperature for 3 hours.
- After the 3 hours transfer the saponified extract to a 500 ml separatory funnel. Collect the methanolic phase in the same flask and add an equivalent volume of acetone.
- Add 25ml of diethyl ether to the ethereal phase and transfer the methanolic phase into 4 fractions.
- After each transfer, shake the separatory funnel and add 200 ml of distilled water, allowing it to fall through the walls. Let it stand 3 minutes and discard the aqueous phase every time.
- Wash 4 times with 200 ml of distilled water. Let the two phases separate for 3 minutes, and discard the aqueous phase.
- Concentrate with nitrogen gas until the ethereal extract has a volume of approximately 25 ml.
- Filter the ethereal extract through a glass funnel containing anhydrous sodium sulfate, and collect in a 25 ml volumetric flask with petroleum ether.

5. Spectrophotometric analysis of total carotenoids

- Read the ethereal extract at 450 nm and calculate the total carotenoid concentration using the recommended absorption coefficient for mixtures of carotenoids (2500).

6. Chromatographic analysis (HPLC) of individual carotenoids

- Place 15 ml of the extract in a tube, dry the extract with nitrogen gas (N2) at 35 °C and store at -70 °C. Defrost a few minutes prior to the injection, add 1 ml of acetone HPLC grade, sonicate, filter through a PTFE filter of 0.22 mm directly into the sample vial and inject 10 µl into the chromatograph.

  HPLC Chromatographic conditions:

  Polymeric C30 Column: YMC C30, 3µm, 4.6 x 250 mm
  Mobile phase: Eluent A: methanol (HPLC grade) and eluent B: methyl tert-butyl ether (HPLC grade)
  Gradient Elution: 100:0 to 97:3 for 15 minutes, then 90:10 for 20 minutes, then 60:40 for 10 minutes, and finally at 40:60 for 15 minutes.
  Flow rate: 0.8 ml / min
  Re-equilibration time: 15 minutes

- Determine the area of each carotenoid at its maximum wavelength absorbance and calculate the carotenoid concentration using the standard curve of each one.

7. Preparation of the standard curve of carotenoids

- Dissolve the standard in petroleum ether (stock). In some cases it is necessary to add a little acetone to make the carotenoid more soluble.
- Read the stock in the spectrophotometer at the appropriate wavelength for each carotenoid and make the calculation of the concentration using the extinction coefficient corresponding to the wavelength and the solvent used.

- Inject 1 ml of the stock to HPLC under the same conditions mentioned for the sample. Integrate each carotenoid at the corresponding wavelength and determine its purity. With the specific area, make the corresponding calculation of the points of the standard curve that cover the range of area found in the samples, and perform the appropriate dilutions for each point on the curve.
- Build the standard curve for each carotenoid by plotting the concentration (µg/ml) vs. the area.

8. Preparation of Reagents

8.1. Methanolic solution of KOH 20 %

Weigh 100 g of potassium hydroxide in 1 liter of methanol (stir with the aid of a magnetic bar until completely dissolved).

9. References

- Rodriguez-Amaya, D.; Kimura, M. 2004. HarvestPlus Handbook for carotenoid analysis. HarvestPlus Technical Monograph 2. Washington, DC and Cali: International Food Policy Research Institute (IFPRI) and International Center for Tropical Agriculture (CIAT).
- Burgos, G.; Salas, E.; Amoros, W.; Auqui, M.; Muñoa, L.; Kimura, M.; Bonierbale, M. 2009. Total and individual carotenoid profiles in the Phureja group of cultivated potatoes: I. Concentrations and relationships as determined by Spectrophotometry and High Performance Liquid Chromatography (HPLC). *Journal of Food Composition and Analysis*. 22, 503–508.
- Burgos, G.; Salas, E.; Amoros, W.; Muñoa, L.; Sosa, P.; Díaz, C.; Bonierbale, M. 2012. Carotenoid concentrations of native Andean potatoes as affected by cooking. *Food Chemistry*. 133, 1131-1137.

PROCEDURE 3
Analysis of total phenolics compounds in freeze-dried and milled potatoes

1. Sampling and sample preparation

As described in Annex 1.

2. Extraction

- Weigh a portion of freeze-dried and milled sample in a glass tube. The weight depends on the color of the sample (0.8 to 1.0 g for white or cream color samples, 0.5 to 0.7 g for yellow or red samples, and 0.1 to 0.4 g for purple samples).
- Add 10 ml of 70% methanol, homogenized for 10 seconds and sonicate for 10 minutes (first extraction).
- Filter the methanol extract through Whatman # 4 filter paper into a volumetric flask.
- Add 10 ml of 70% methanol over the residue, homogenized on a vortex and dispensing in a water bath at 80 °C for 5 minutes (second extraction).
- Filter the extract into volumetric flask and finally add extracting solution up to a final volume of 25 ml.

3. Determination

- Dispense 400 µl of sample in a test tube. In the case of a blank sample, dispense 400 µl of distilled water. After this, follow the same steps for the samples and the blank sample.
- Add 8 ml of distilled water and 0.5 ml of 2N Folin Ciocalteau, mix and let stand for 6 minutes
- Add 1500 µl of 20% sodium carbonate and mix thoroughly. Place the tubes in a water bath at 40 °C for 30 minutes.

- Read absorbance at 765 nm and calculate the concentration of total phenolic compounds based on the standard curve and express the results in mg chlorogenic acid / 100 g sample.

4. Preparation of calibration curve

- Prepare a stock solution of chlorogenic acid at a concentration of 5000 mg/l. For this purpose, weigh 250 mg of chlorogenic acid, dissolve with 5 ml of methanol, transfer the solution into a volumetric flask protected from light and add distilled water up to a final volume of 50 ml.
- Prepare standard solutions with concentrations of 50, 100, 200, 300, 400, 500, 700, 1000 and 1500 mg/l by taking volumes of 0.25, 0.5, 1, 1.5, 2, 2.5, 3.5, 5, 7.5 ml of the stock and bringing to a volumetric flask of 25 ml with distilled water. Analyze each standard duplicate.
- Dispense 100 µl of each standard solution into test tubes (in the case of blank reagent use 0.1 ml of distilled water) and mix with 8 ml of distilled water and 500 µl of Folin-Ciocalteu reagent. Mix and let stand for 6 minutes.
- Add to each tube 1.5 ml of 20% sodium carbonate, and mix.
- Place the tubes in a water bath at 40 °C for 30 minutes.
- Read at 765 nm and build the standard curve by plotting the concentration (mg/l) vs. absorbance.

5. Preparation of reagents

5.1. Extractant solution: 70 % Methanol

Place 300 ml of distilled water into a volumetric flask and add methanol up to a final volume of 1L.

5.2. Saturated solution of sodium carbonate (20%)

Weigh 50 g of anhydrous sodium carbonate, add 200 ml of distilled water and place in the agitator heater until completely dissolved, and boil. Allow the solution to cool at room temperature and add one teaspoon (approximately 1.5 g) of sodium carbonate to saturate the solution, stir until completely dissolved**.** After 24 hours, filter the solution using two Whatman # 2 filter papers, transfer the filtered

solution to a volumetric flask and dilute with distilled water up to a final volume of 250 ml. Transfer the solution to an amber bottle.

6. References

- Waterhouse, A. 2002. Determination of total phenolics. Current Protocols in Food Analytical Chemistry. University of California, U.S.A. I1.1.1-I.1.1.8.
- Burgos, G.; Amoros, W.; Muñoa, L.; Sosa, P.; Cayhualla, E.; Sanchez, C.; Díaz, C.; Bonierbale, M. 2013. Total phenolic, total anthocyanin and phenolic acid concentrations and antioxidant activity of purple-fleshed potatoes as affected by boiling. *Journal of Food Composition and Analysis*. 30, 6–12.

PROCEDURE 4
Analysis of total anthocyanins in freeze-dried and milled potatoes

1. Sampling and sample preparation

As described in Annex 1.

2. Extraction

- Weigh a portion of freeze-dried and milled sample in a glass tube. The weight depends on the color of the sample (1.0 g for white or cream color samples, 0.2g for yellow or red samples, and 0.05 to 0.1 g for purple samples).
- Add 5 ml of the extracting solution (80:20 95% ethanol: 1.5M hydrochloric acid), shake for 10 seconds and sonicate for 10 minutes.
- Add 5 ml of extracting solution and repeat the extraction.
- Protect the sample from light and refrigerate (4 ^{0}C) overnight.
- Centrifuge at 7000 rpm for 10 minutes at 4 ^{0}C, filter the supernatant through Whatman #2 paper in a 25 ml volumetric flask.
- Add 5 ml of extracting solution to the pellet, sonicate for 5 minutes, place in a water bath at 80 °C for 5 minutes, and filter.
- Add 5 ml of extracting solution to the pellet and repeat the extraction. Filter the extract in volumetric flask and bring to 25 ml using the extractant solution.

3. Quantification

- Read the absorbance at 700, 545 and 515 nm and calculate the total anthocyanins concentration using as reference the extinction coefficient (3.02 x 104) and the molecular weight (718.5 g L-1) of malvidin-3-pcoumarilglucoside for purple samples and using the extinction coefficient (2.73 x 104) and molecular weight (486.5g L-1) of pelargonidin-3-glucoside for red samples.
- Perform autozero with extracting solution and, if necessary, dilute with the extractant solution.

4. Preparation of reagents

4.1. Extractant Solution 80:20 (95% Ethanol : HCl 1.5 M)

Add about 500 ml of 95% ethanol into a volumetric flask. Add 200 ml of 1.5M hydrochloric acid and 95% ethanol up to a final volume of 1L.

4.2. Ethanol (95%)

Add 50 ml of distilled water in a volumetric flask. Add ethanol up to 1L and mix thoroughly. Cover, mix and pour into a pre-labeled amber bottle.

4.3. HCl 1.5M Solution

Add about 500 ml of distilled water and then 124.35 ml of 37% hydrochloric acid. Add distilled water up to a final volume of 1L.

5. References

- Jansen, G.; Flamme, W. 2006. Coloured potatoes (Solanum tuberosum L.) anthocyanin content and tuber quality. Genetic Resources and Crop Evolution. 53, 1321-1331.
- Burgos, G.; Amoros, W.; Muñoa, L.; Sosa, P.; Cayhualla, E.; Sanchez, C.; Díaz, C.; Bonierbale, M. 2013. Total phenolic, total anthocyanin and phenolic acid concentrations and antioxidant activity of purple-fleshed po tatoes as affected by boiling. *Journal of Food Composition and Analysis*. 30, 6–12.

PROCEDURE 5

Evaluation of hydrophilic antioxidant activity in freeze-dried and milled potato tubers and sweetpotato roots

1. Sampling and sample preparation

As described in Annex 1.

2. Extraction

- Weigh 0.08 g of sample (previously well mixed)
- Add 5 ml of 80% methanol
- Shake and sonicate for 30 minutes.
- Centrifuge at 5000 rpm for 10 minutes.
- Remove the supernatant in a plastic tube (first extraction)
- Add 5 ml of extracting solution to the pellet.
- Let stand overnight protected from light and at room temperature.
- Centrifuge at 5000 rpm for 10 minutes.
- Finally, add the supernatant to the plastic tube and bring to 10 ml.

3. Determination

- Dispense 150 µl of sample into a test tube and 150 µl of 80% methanol into another tube (Sample Blank). Follow the next steps for samples and sample blank.
- Add 2850 µl of ABTS diluted solution, shake the tube and wait for 10 minutes. Repeat this step and read the absorbance at 734 nm. If the absorbance is below 0.2, make dilutions and repeat the reading to obtain values within the range.
- Calculate the final concentration of hydrophilic antioxidant activity based on the Trolox standard curve, and express the results in µg Trolox equiv/g sample.

4. Preparation of calibration curve

- Prepare a stock solution of Trolox (2000 µM / L). For this purpose weigh 0.025 g of Trolox, dissolve with 30 ml of methanol and bring to a final volume of 50 ml using the same methanol. Perform this step protected from light.
- Prepare Trolox standard solutions (200, 300, 400, 500, 600, 700 and 800 µM Trolox/l) by taking aliquots of 2.5, 3.75, 5, 6.25, 7.5, 8.75 and 10 ml from the stock solution and bringing to 25 ml with methanol.
- Dispense 150 µl of each standard solution into test tubes and continue with the evaluation of the antioxidant activity, as described for the samples. In this case the control sample is called standard blank.
- Build the standard curve for Trolox by plotting the concentration (µM / L) vs. absorbance.

5. Preparation of reagents

5.1. Extractant solution of 80% methanol

Put 50 ml of distilled water into a volumetric flask and add methanol up to 250 ml. Mix thoroughly.

5.2. Stock coloring (Reagent "A" & "B")

Reagent A: 7 mM 2,2'-Azino-bis(3-ethylbezothiazoline-6-sulfonic acid (AZBT): Add a tablet (10 mg) of AZBT in a screw cap tube protected from light. Dissolve with 2.8 ml distilled water using vortex.

Reagent B: 2.45 mM sodium persulfate ($K_2S_2O_8$): Weigh 6.62 mg of $K_2S_2O_8$ in a beaker, protecting from the light; add 10 ml of distilled water and stir until completely dissolved to finally transfer to a screw cap tube.

5.3. Stock Solution (Chromogenic radical ABTS2+)

The stock solution is prepared by mixing the two solutions, reagent A with B in equal quantities (1:1). Shake and allowed them to react for 16 h at room temperature in the dark. (This mixture can only be used up to 8 hours). The volume of the working solution depends on the number of samples to be analyzed.

5.4. ABTS2+Diluted Solution (96% ethanol)

Dispense 3.9 ml of ABTS stock solution in a flask protected from light and diluted with 110 ml of 96% ethanol to obtain an absorbance of 1.1 ± 0.02 units at 734 nm.

6. References

- 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 and Medicine*. 26, 1231-1237.
- Burgos, G.; Amoros, W.; Muñoa, L.; Sosa, P.; Cayhualla, E.; Sanchez, C.; Díaz, C.; Bonierbale, M. 2013. Total phenolic, total anthocyanin and phenolic acid concentrations and antioxidant activity of purple-fleshed potatoes as affected by boiling. *Journal of Food Composition and Analysis*. 30, 6–12.

PROCEDURE 6
Analysis of individual phenolic acids in freeze-dried and milled potato tubers

1. Sampling and sample preparation

As described in Annex 1.

2. Extraction

- Weigh 0.1 g of the freeze-dried and milled sample in a glass tube.
- Add 4.5 ml of the extractant solution (80% methanol, Mili Q water 19.5%, acetic acid 0.5%), homogenize in a vortex for 10 seconds, sonicate for 20 minutes, and centrifuge at 7000 rpm for 10 min.
- Transfer the supernatant directly to a test tube with screw cap and reextract the pellet using 3 ml of the extractant solution and following the same procedure as described above.
- Add 3 ml of the extractant solution to the pellet, mix in a vortex, heat at 80 ° C for 5 min and transfer the supernatant into the same test tube.
- Place the glass tube with the collected supernatant at 36 °C and reduce the volume of the extract using nitrogen gas.
- Bring the extract to a final volume of 5 ml using Milli Q water.

3. Chromatographic analysis (HPLC) of individual phenolic acids

- Filter the extract through a 0.45-mm PVDF filter directly into the sample vial and inject 25 µl into the chromatograph.

HPLC Chromatographic conditions:

Symmetry C18 column: 5 um, 4.6 mm x 250 mm

Mobile phase: Eluent A: 1% Acetic acid in water and eluent B: Acetonitrile with 0.1% Acetic acid

Gradient elution: 97:3 to 95:5 for 7 minutes, then 60:40 for 38 minutes, then 0:100 for 6 minutes, and finally at 97:3 for 6 minutes.

Flow rate: 0.7 ml/min

Re-equilibration time: 13 minutes

- Determine the area of each phenolic acid at its maximum wavelength of absorbance, and calculate the phenolic acid concentration using the standard curve of each phenolic acid.

4. Preparation of the standard curve of phenolic acids

- Weigh 5 mg of the chlorogenic acid and/or cafeic acid standards, dissolve with methanol HPLC grade, and adjust to 50 ml (Stock 1).
- Take 25 ml of Stock 1 and bring to 50 ml with MilliQ water (Stock 2)
- Inject 25 µl of the stock 2 into the HPLC under the same conditions mentioned for the sample.
- Integrate each phenolic acid at its corresponding wavelength, and based on the area calculate the dilutions in order to cover the range of areas found in the samples.
- Build the standard curve for each phenolic acid by plotting the concentration (mg /ml) vs. area.

5. Preparation of reagents

5.1. Extracting solution (80% methanol, Water Mill Q, 19.5%, 0.5% glacial acetic acid): Add 195 ml of Milli Q water and 5 ml of glacial acetic acid to volumetric glass and bring to 1L with Methanol HPLC grade.

6. References

- Xu, X.; Li, W.; Lu, Z.; Beta, T.; Hydamaka, A. 2009. Phenolic content, composition, antioxidant activity, and their changes during domestic cooking of potatoes. *Journal of Agricultural and Food Chemistry*. 57 (21), 10231–10238.
- Andre, C.M; Ghislain, M.; Bertin, P.; Oufir, M.; Herrera, R.; Hoffmann, L.; Hausman, J.F.; Larondelle, Y.; Evers, D. 2007. Andean potato cultivars (*Solanum tuberosum* L.) as a source of antioxidant and mineral micronutrients. *Journal of Agricultural and Food Chemistry*. 55, 366–378.
- Burgos, G.; Amoros, W.; Muñoa, L.; Sosa, P.; Cayhualla, E.; Sanchez, C.; Díaz, C.; Bonierbale, M. 2013. Total phenolic, total anthocyanin and phenolic acid

concentrations and antioxidant activity of purple-fleshed potatoes as affected by boiling. *Journal of Food Composition and Analysis*. 30, 6–12.

PROCEDURE 7
Analysis of ß-carotene in freeze-dried and milled sweetpotato roots

1. Sampling and sample preparation

As described in Annex 2.

2. Extraction

- Weigh a portion (0.1 to 0.3 g) of the freeze-dried and milled sample (the weight depends on the color of the sample) in a glass beaker and hydrate for 10 minutes with still water (1:5; sample: water).
- Place the hydrate sample in a mortar containing a small amount of Hyflosupercel, add 30 ml of cold acetone, mix with the help of the pestle and filter with suction through a sintered glass funnel, receiving the extract in a protected suction flask. Wash the mortar, pestle, and residue with a small amount of acetone.
- Repeat the extraction 3 to 4 times until the residue is devoid of color.

3. Partitioning to petroleum ether

- Put 20 ml of petroleum ether in a separatory funnel and add a small portion of the acetone extract.
- Add distilled water slowly, letting it flow along the walls of the funnel. To avoid formation of an emulsion, do not shake (once formed, an emulsion can be broken by adding acetone or sodium chloride).
- Let the two phases separate and discard the lower aqueous-acetone phase. Add another portion of the acetone extract and repeat the operation until all of the extract has been transferred to petroleum ether, then wash 4 to 5 times with water to remove residual acetone.

- Collect the petroleum ether phase in a 25 ml volumetric flask making the ethereal extract pass through a glass funnel containing an anhydrous sodium sulfate.

4. Spectrophotometric analysis of total carotenoid

- Read the carotenoid ethereal extract at 450 nm and calculate the total carotenoid concentration using the coefficient of absorption for β-carotene (2592).

5. HPLC analysis for individual carotenoids

- Place 15 ml of the extract in a tube. Place the tube in warm water (35 °C) and dry the extract with nitrogen gas (N2). Store at –20 °C protected from light and, immediately before injection, thaw and redissolve in 1 ml of HPLC grade acetone, sonicate for 30 seconds, filter through a 0.22 um PTFE syringe filter (Millipore) directly into sample vials and inject 10 µl into chromatograph.

  HPLC chromatographic conditions
  Polymeric C30 column: YMC C30, 3 um, 4.6 x 250 mm
  Mobile phase: methanol:methyl-tert-butyl ether
  Isocratic elution: 80:20 during 50 min.
  Flow rate: 0.8 ml/min.

- Calculate each individual carotenoid concentration using the formula:

$$Cx\ (\mu g/g) = \frac{As \times Cs\ (ug/ml) \times \text{total volume of extract (ml)}}{As \times \text{sample weight (g)}}$$

  where Cx = concentration of carotenoid X; Ax = peak area of carotenoid X; Cs = concentration of the standard; As = peak area of the standard.

7. References

- Kimura, M.; Kobori, C.K.; Rodriguez-Amaya, D.; Nestel, P. 2007. Screening and HPLC methods for carotenoids in sweetpotato, cassava and maize for plant breeding trials. *Food Chemistry*. 100, 1734–1746.
- Rodriguez-Amaya, D. 2001. A guide to carotenoid analysis in foods. ILSI Human Nutrition Institute. Washington D.C.

ANNEX 1

Procedure for sampling and sample preparation of freeze-dried and milled potato tuber samples

I. Field sampling of potato tubers

- Make sure the tubers are mature and the plants are ready for harvesting.
- Cut the above parts of the plants to be harvested.
- Identify the plants to be harvested and cut the foliage.
- Harvest the tubers carefully with a garden tool (for example a fork), avoiding any tuber damage.
- Separate marketable and non-marketable tubers on the field.
- Select a representative sample. For this purpose, 7 to 10 potato tubers per genotype, clone or variety in each replication from the field should be collected at random. The tubers should have a representative size for the genotype, clone or variety,
- Avoid selecting damaged or greened tubers, tubers with extreme sizes, and tubers from plants that grew in the borders.
- Place the collected tubers inside a paper bag previously labeled with the corresponding identification code of the field plot.
- Place the paper bags with the potato tubers in a box and store them in proper conditions (dark, ventilated and fresh room, protected from dust, etc.) until sample preparation. The potato samples should be processed for nutrient analysis as soon as they arrive at the laboratory (maximum 1 week after harvesting).

II. Sample preparation of potato tubers

- Wash the 7 to 10 potato tubers with abundant tap water (trying to remove any soil residue), rinse with distilled water, and dry the tubers with paper towel.
- Place the potato tubers in a clean labeled bag and store them at 5 ^{0}C.

- One day before processing, place the samples in white plastic trays, sorting in a correct order.
- Peel the tubers with a high-grade stainless steel or ceramic peeler, wash them again with distilled water, dry using paper towel, and cut each tuber longitudinally in 4 sections with a high-grade stainless steel or ceramic knife. Peeling should be done carefully, with minimum removal of the flesh.
- Obtain 3 to 4 slices of each of two opposite sections of each tuber to obtain a 50 g weighed sample. Use a high-grade stainless steel or ceramic slicer.
- Put the potato slices in polyethylene bags and take note of the exact weight.
- Store the samples in a freezer at -20 °C and freeze-dry them until the residual moisture is less than 3%. At CIP's Quality and Nutrition Laboratory, 72 hours freeze-drying is needed for drying 350 potato samples (50 g fresh material each) in an industrial freeze dryer.
- Weigh the dried samples and take note of the exact weight. Use the fresh and dried weights to calculate the dry matter content of the samples.
- Mill the dried sample in a stainless steel mill (40 mesh) and place the milled sample in Whirl-Pak plastic bags.
- Store the milled sample at -20 °C.

Note: Tubers can be stored at 5^{0}C for minerals, vitamin C, carotenoid and phenolic analysis, but not for sugar analysis.

ANNEX 2

Procedure for sampling and sample preparation of freeze-dried and milled sweetpotato root samples

I. Field sampling of sweetpotato roots

- Make sure the roots are mature and the plants are ready for harvesting.
- Some days before harvesting, identify the plants to be harvested and cut the foliage.
- Harvest the roots carefully with a garden tool (for example a fork), avoiding any root damage.
- Separate marketable and non-marketable roots on the field.
- Select a representative sample. For this purpose, 5 to 10 sweetpotato roots should be collected at random in each replication. The roots should have a representative size for the clone, variety or genotype.
- Avoid selecting damaged roots, roots with extreme sizes, and roots from plants that grew in the borders.
- Place the collected roots inside a paper bag previously labeled with the corresponding identification code of the field plot.
- Place the paper bags with the sweetpotato roots in a box and store them in proper conditions (dark, ventilated and fresh room, protected from dust, etc.) until sample preparation. The sweetpotato samples should be processed for nutrient analysis as soon as they arrive at the laboratory (maximum 1 week after harvest)

II. Sample preparation of sweetpotato roots

- Wash the 5 to 10 roots with abundant tap water (trying to remove all soil residue), rinse with distilled water, and dry the roots with paper towel.
- Put the washed roots in a clean labeled paper bag and store them under proper conditions (dark, ventilated and fresh room, protected from dust, etc).

- Place the samples in white plastic trays, sorting in a correct order.
- Peel the roots with a high-grade stainless steel or ceramic peeler, wash them again with distilled water, dry using paper towel and cut each root longitudinally in 4 sections with a high-grade stainless steel or ceramic knife. Peeling should be done carefully, with minimum removal of the flesh.
- Obtain 3 to 4 slices of each of two opposite sections of each root to obtain a 50 g weighed sample. Use a high-grade stainless steel or ceramic slicer.
- Put the sweetpotato slices in polyethylene bags and take note of the exact weight.
- Store the samples in a freezer at -20 °C and freeze-dry them until the residual moisture is less than 3%. (At CIP's Quality and Nutrition Laboratory, 72 hours freeze-drying is needed for drying 350 sweetpotato samples (50 g fresh material each) in an industrial freeze dryer.
- Weigh the dried samples and take note of the exact weight. Use the fresh and dried weights to calculate the dry matter content of the samples.
- Mill the dried samples in a stainless steel mill (40 mesh) and place the milled sample in Whirl-Pak plastic bags.
- Store the milled sample at -20 °C

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The International Potato Center (known by its Spanish acronym CIP) is a research-for-development organization with a focus on potato, sweetpotato, and Andean roots and tubers. CIP is dedicated to delivering sustainable science-based solutions to the pressing world issues of hunger, poverty, gender equity, climate change and the preservation of our Earth's fragile biodiversity and natural resources.
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